Thermoregulation Research and Clinical Applications

Editors: P. Lomax, E. Schönbaum

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THERMOREGULATION: RESEARCH AND CLINICAL APPLICATIONS

FINAL REPORT

PETER LOMAX

AUGUST 1, 1989

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Thermoregulation: Research and Clinical Applications

Thermoregulation: Research and Clinical Applications

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PREFACE

We are pleased to present the proceedings of the seventh symposium on the *Pharmacology of Thermoregulation: Research and Clinical Applications* in which leading experts describe the latest advances in the field and evaluate their application to the management of patients suffering from thermoregulatory disorders. To the many colleagues who shared the view that this constitutes an important area for discussion we extend our appreciation for their participation in the symposium. The volume contains primarily current research reports, together with reviews of several important areas which were presented at the meeting as thematic lectures. These proceedings are intended to be an assessment of recent developments and a guide to future research directions. By publishing the volume as expeditiously as possible following the symposium we hope to emphasize its value in these last respects and to point out the magnitude of the problems to be resolved.

As has been the case with the previous symposia we have received help from many quarters and we are most grateful for this. We wish to acknowledge the support of the Astra-Group A/S, Copenhagen, Behring, Copenhagen, Den Danske, Odense, Novo Industries, Copenhagen, the Lundbeck Foundation, Copenhagen, the Sven Petersen's Memorial Fund, Nakkebølle and by the United States Army Medical Research and Development Command. The collaboration of Odense University, Organon, Oss and the University of California at Los Angeles was invaluable in arranging the conference.

The outstanding success of the meeting was echoed repeatedly by those taking part and this was due primarily to the superb organization orchestrated by Dr. Ove Nedergaard and his associates at Odense University. Gitte Jensen and Margarethe Hansen made a beautiful job of the program booklet, tickets etcetera, and ran the secretariat with skill and good humor. We were also fortunate in having the services of so excellent a support staff to take care of the audiovisual equipment, with never a single technical problem. The social program was a notable feature of the conference and its first rate realization was the result, in large part, of the time and effort expended by Pamela Nedergaard who made everyone feel at home in Odense.

All of the participants extend their thanks to Karen Roberts for the meticulous preparation of the manuscripts and Virginia Brownscombe for the proof reading of these Proceedings. The editors hope that all contributors and participants will find that their efforts have been worthwhile. We also extend our thanks to Joseph Gerlock for his assistance with the financial planning.

Venhorst, The Netherlands, August 1988   **Peter Lomax**

Eduard Schönbaum

INTRODUCTION

The seventh symposium on the Pharmacology of Thermoregulation was held at Odense University, Odense, Denmark from August 22 to 26, 1988.

The theme of the meeting was *Research and Clinical Applications*, with the emphasis on current advances in the management of patients with disorders of thermoregulation.

There were 87 scientific participants present. The program consisted of four thematic review lectures, 26 oral presentations and 38 poster presentations. These Proceedings also include several papers which were submitted by authors who were subsequently unable to attend the meeting. Two of the thematic lecturers did not submit manuscripts and so, in this Introduction we review the major points raised in these presentations as well as highlighting additional information which arose during the extensive discussion of papers.

GRANBERG discussed the management of accidental hypothermia and cold-related injuries. In the Nordic countries these conditions continue to be a major clinical problem. History abounds with the serious of these problems, most notably in relation to military operations (during the second World War there were approximately 2,000 cold injuries per day). In terms of the management of such patients it is useful to classify them as acute, subacute and chronic, based on the duration of exposure to cold. With respect to rewarming of the hypothermic individual considerable controversy still exists. Although, under ideal circumstances, extracorporeal warming of the blood offers the most effective method and allows the tightest control of associated disturbances of acid-base and electrolyte balance, facilities for such management are not usually available under field conditions. Professor Granberg emphasized that experience and familiarity with a given technique are perhaps more important than attempting to apply the newest theoretical methods. Any form of rewarming can be effective provided the physician maintains complete physiological control of the patient. He noted that potassium balance is critical although it is unclear why the hypothermic patient requires so much potassium during rewarming. Coupled with this is the unexplained of the nature of the marked diuresis which appears to be an osmodiuresis, particularly a sodium loss, rather than a water diuresis; the possible role of antidiuretic hormone needs to be explored. Ingestion of ethanol is very commonly a factor in accidental hypothermia. It is an interesting observation that in cases of alcohol induced hypothermia in which the patient survives there is often a better outcome. Possible ethanol has a selective depressant action on the autonomic nervous system which seems to lessen the development of complications. Practical points to reduce the rate of heat loss include reduction in muscle activity, for example, during cold water immersion one should not attempt to swim to keep warm but rather adopt a heat escape lessening posture (H.E.L.P.). And the simple precaution of ensuring that one's shoe laces are not tied too firmly lessens the danger of frost bite to the feet! Also discussed was the paradoxical terminal sensation of warmth, frequently manifest by removal of clothing, and Professor Granberg suggested that this may be due to neurochemical changes.

SATINOFF reviewed her extensive studies of the relationship between temperature regulation, sleep and circadian rhythms. She demonstrated that the frequency and duration of episodes of rapid eye movement (REM) sleep are influenced by the ambient temperature; REM sleep periods increase steadily as the environmental temperature increases from 20°C to 30°C. The induction of fever also increases total REM sleep. Professor Satinoff also discussed the changes in the circadian rhythms as a function of the ambient temperature and the possible role of the autonomic nervous system in mediating the effects of the external temperature on sleep patterns and circadian rhythms.

Disturbances of temperature regulation during surgical anesthesia were discussed in papers by BERNARD, KRISTENSEN, RUBENSTEIN and SESSLER. The technique of intraesophageal warming using a circulating water double lumen tube connected to an automatic heating device was effective in preventing intraoperative hypothermia. There was discussion concerning the nature of the shivering associated with recovery from general anesthesia; is it a reflection of a thermoregulatory change or could it be due to a direct action of the anesthetic *per se*, for example, an effect on spinal disinhibition? Also, there was debate concerning the criteria for extubation of the patient - is it appropriate to rely on the return to a predetermined core temperature or should other clinical criteria be considered. On the other hand,

NIELSON, from a study of surgical patients over a period of twelve years, concluded that there was no evidence that active rewarming was superior to passive or spontaneous rewarming in reducing postoperative morbidity and mortality provided heat production mechanisms and the circulatory system were intact.

An area of investigation which up to now has received little attention is the possible use of pharmacological agents to improve resistance to cold or to prevent thermal injury. WANG has studied the ability of theophylline to increase mean body temperature during cold exposure, and WEI has investigated the effects of neuropeptides in protecting the skin against heat induced injury. These studies are of particular interest in relationship to the report of MACKENZIE on the management of patients with severe disorders of thermoregulation, primarily the result of hypothalamic lesions. Such individuals are unable to maintain their body temperature under normal ambient conditions. The use of drugs to correct this deficiency could offer a useful therapeutic approach.

The debate concerning the pathogenesis of hyperthermia as distinct from fever was addressed by LONG. Using antisera against IL-1 it was concluded that the endogenous pyrogen is involved in the genesis of fever but not of stress induced hyperthermia.

Undoubtedly, these complex questions will continue to be the subjects of further investigation. Although the time and place of the eighth symposium have yet to be decided upon, it can be expected that further elucidation of the complex interactions of the organism with the environment will emerge at that time.

Venhorst, The Netherlands, August 1988   **Peter Lomax**

Eduard Schönbaum

A SIMPLE DEVICE FOR ACTIVE CORE REWARMING IN DEEP HYPOTHERMIA

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Optimal treatment of deep accidental hypothermia has been under debate for years. At present it seems agreed that cases with a core temperature below 30°C, with no clinical evidence of shivering thermogenesis, should be treated by active, central rewarming. This is a potentially difficult task for ICU staff with lack of experience, since commonly recommended methods are invasive and can be used only in highly specialized units. Recently the problems for small units in the management of rewarming patients with dangerous hypothermia have been discussed [1].

As a conclusion the Danish Society of Anaesthesiologists has recommended that every ICU in the country possess facilities for central rewarming.

The oesophageal thermal tube designed by *Kristensen* [2] represents a method of true non-invasive central rewarming and seems to meet the needs for simplicity and safety.

In animal trials this tube has proved itself equal to peritoneal dialysis [2] and in humans it has been used successfully in preventing hypothermia during abdominal surgery [3].

The purpose of the present study is to describe the clinical use of the tube in patients suffering from deep accidental hypothermia.

METHODS: The equipment consists of a thermal tube connected with a thermostat controlled waterpump. The thermal tube has a double lumen, closed at its distal end, thus allowing circulation in a closed system. The tube is 45 cm long. The inner tube is made of PVC, diameter 15 mm. The outer one is non-elastic polyurethane, 30 mm in diameter, wall thickness 0.1 mm. It is inserted into the oesophagus after tracheal intubation.

The pump is an Exacon Thermal Therapy unit model TT 8200. It has a water reservoir of 3 litres from which hot water (42°C) circulates with a flow of about 3.8 l/min. The amount of heat conducted to the patient lies between 100 and 200 kcal/h depending on the patient's temperature. The pump is provided with temperature and leak alarms.

RESULTS: Trials with the double lumen tube have been under way since September 1986 in a multicenter study, including most hospitals in northern Sealand, Denmark. Rewarming is started within 1 h in all cases of accidental hypothermia with a core temperature below 30°C in which the patient is not shivering, has respiratory difficulty and is over 18 years old.

Operating temperature in the tube is 42°C. Under optimal conditions the main observations are: core temperature (tympanic membrane and rectal), skin temperature, ECG, central venous pressure, and BP (by arterial cannulation).
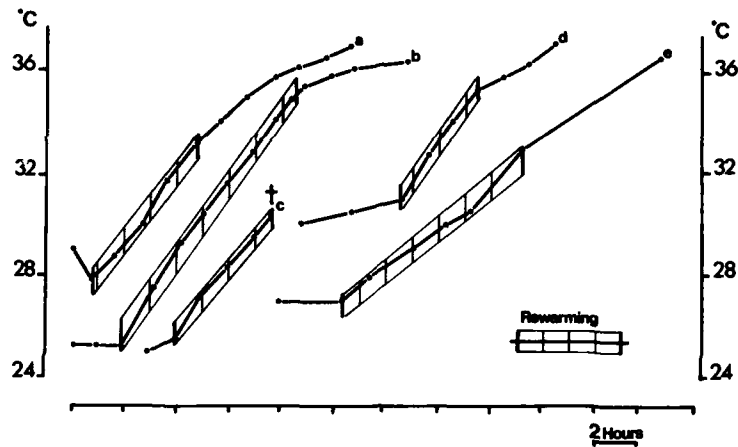
FIGURE 1: Rewarming curves from all five patients showing tympanic membrane temperatures. Curve C represents the patient with cardiac arrest (see text). The period of active rewarming is marked with hatched bars on each curve.

Five patients have been rewarmed from deep accidental hypothermia with the thermal tube as the only external source of heat (Fig. 1). None of the patients has demonstrated signs of shivering thermogenesis even though two of them showed slight spontaneous rises in rectal and tympanic temperatures. They exhibited a close to linear rise in core temperature of $1.5°C/h$. This occurred even in one patient with asystoly who underwent external cardiac massage for several hours. In all cases the temperature gradient body-core/surface remained steady and no cases of afterdrop were seen. Cardiac dysrythmias were not seen in any of the cases during rewarming with the thermal tube.

Two out of the five patients survived without complications. Three patients died - two of them a few days after rewarming because of sequelae to the accident. In the last case rewarming was interrupted because of cardiac failure occurring at $30.5°C$ (initial temperature $25°C$). In another two patients rewarming was unsuccessful. These patients had asystoly, and peritoneal irrigation and partial cardiac bypass were without effect on the core temperature. In the non-survivors, necropsy revealed no damage to the oesophagus or stomach.

DISCUSSION: The oesophageal thermal tube is a simple, safe and effective non-invasive method for active central rewarming in deep accidental hypothermia. The results equal those obtained with peritoneal irrigation in the treatment of hypothermia, however the thermal tube requires no specially trained personnel.

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HUMAN ACCLIMATION, BODY TEMPERATURE AND ENDURANCE PERFORMANCE

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One feature in thermal adaptation is the modification of the regulatory characteristics, i.e., changes in threshold and gain of the regulatory reactions [1]. Thus, it has been shown that prolonged or repeated cold as well as heat exposure [2] cause the threshold temperatures for both cold defence and heat dissipation to shift to a lower mean body temperature. As a consequence, resting body temperature is slightly decreased, i.e., the set point of the control system appears to be decreased. Similar changes have been found after physical training (marathon running; 3).

Physical exercise is usually accompanied by an increase in deep body temperature which is closely related to the intensity of work relative to maximum work rate [4], and relatively independent of environmental temperature. For some time it has been thought that this increase was due to an increased set point of the thermoregulatory system and it has been surmised that this increase facilitated in some way the performance of physical exercise, e.g., by optimizing enzyme activity. This concept cannot be reconciled, however, with the above described adaptive modifications, which allow the acclimated subject to maintain lower body temperatures during heat and exercise loading. Moreover, recent studies showed that not only extremely high body temperatures, as encountered with exhausting exercise in the heat, are limiting endurance factors [cf. 5], but that even temperature changes near the normal resting temperature may influence exercise performance. Thus, using a precooling maneuver (see below) mean body temperature could be decreased by about 1°C below resting level. Under these conditions work rate was increased by 6.8% in a 1 h exercise test on a cycle ergometer in comparison with control conditions in the same subjects [6]. Here we report on a subsequent study in which the effects of precooling on endurance time at 80% of maximum work rate and the possibly underlying basic physiological mechanisms were examined. Part of this study has been published elsewhere [7].

METHODS: Seven subjects dressed in shorts and tennis shoes exercised on a cycle ergometer (providing constant work rates independent of pedal revolution rate) placed in a climatic chamber. Work rate was increased at 4 min intervals from 0.1 to 0.25, 0.4, 0.5 and to $1.0 \cdot WR_{80\%}$; $WR_{80\%}$ (i.e., work rate corresponding to 80% of $\dot{V}O_{2max}$) was sustained up to exhaustion; pedal rate could be freely chosen by the subjects. On one of two experimental days the subjects began to work after a resting period at neutral temperature (28°C, 50% relative humidity). On the other one the test began again with a resting period at neutral temperature, but the exercise period was preceded by a precooling maneuver described below. Exercise was then carried out at 18°C in both protocols. The precooling test (PRET) and the control test (CONT) were assigned randomly to the sequence of experiments in each subject. In a preceding experiment $\dot{V}O_{2max}$ was determined employing an incremental performance test.

For the precooling maneuver, a 30 min period of rest at 28°C was followed by two consecutive cold exposures during which the chamber temperature dropped to about 5°C. The rationale behind the double cold exposure with an intermittent rewarming period is a previously described short term shivering threshold decrease [9], as a result of which the metabolic response to the second cooling period occurs at a lower mean body temperature.

Oxygen uptake ($\dot{V}O_2$, STPD) was measured by an open system. Heart rate (HR) was calculated from the R-R intervals of a bipolar chest wall ECG. Oxygen pulse (OP) was calculated according to $OP = \dot{V}O_2/HR$. Besides esophageal (T_{es}) and tympanic temperatures (T_{ty}) four skin temperatures were measured on the chest (A), upper arm (B), thigh (C) and lower leg (D). Mean skin temperature and mean body temperature were calculated as previously described [7,8]. Local sweat rate was measured by means of a ventilated capsule attached to the chest skin. The local heat clearance technique was employed for continuous estimation of skin blood flow at the lower arm. Impedance cardiography was used for the

determination of the cardiovascular variables. Cardiac output (CO) was calculated according to CO = HR·SV and arterio-venous oxygen difference ($AVDO_2$) was calculated according to $AVDO_2$ = $\dot{V}O_2$/CO.

All variables (except the impedance cardiography data) were sampled and processed by a computer and stored on a magnetic tape [for details see 7].

RESULTS: As shown in Fig. 1 body temperatures were significantly lower in PRET than in CONT and the time courses were different under the two conditions. The $\overline{T}_{sk}$ difference was most pronounced immediately after the start of exercise (-4°C), whereas the T_{es} difference reached its maximum at 20 min (-0.8°C). When the subjects gave up at the end of the exercise period the body temperatures were *still lower* in PRET. As a most global result, the average endurance time (± SEM) measured from the beginning of the heavy exercise period ($1.0 \cdot WR_{(80\%)}$) was increased from 18.5 ± 2.5 min in CONT to 20.8 ± 2.3 min in PRET (+12%; P < 0.05). The shortest run was terminated 12 min after the beginning of heavy exercise (= min 28 of exercise period; see Fig. 1 and 2). Thus, only the exercise period up to min 28 (phases I and II) could be subjected to an analysis of variance. The time elapsing between 28 min and the "end" (Fig. 1,2) of the exercise tests varied between 0 and 18 min in the group of seven subjects. With increasing body temperatures, sweat rate (SR) increased; its time course was changed in PRET and its level was significantly lower in phase II (Fig. 2). Forearm blood flow showed a similar trend. At the termination of the exercise test, i.e., when the subjects gave up due to exhaustion, the differences in sweat rate had almost disappeared (P = 0.24).
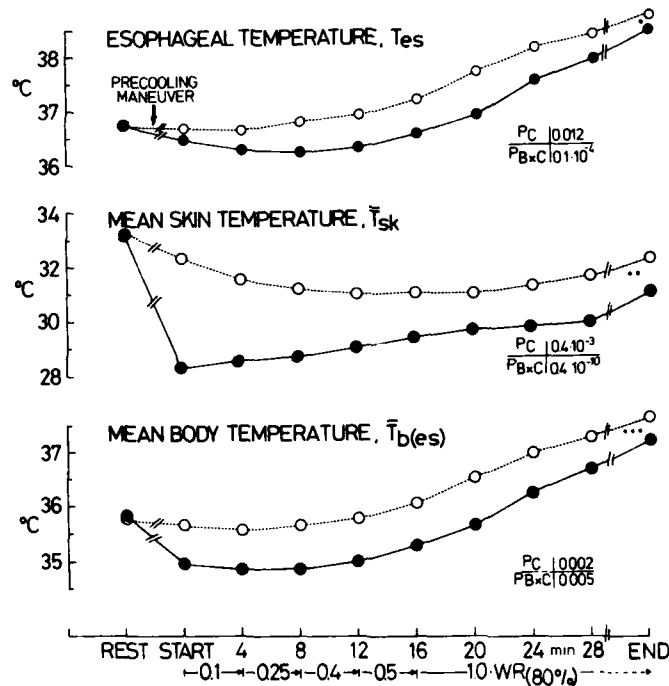
FIGURE 1: Body temperatures in relation to time and work rate; the work rate was increased in a stepwise manner from 0.1 to $0.5 \cdot WR_{80\%}$ (phase I) and then sustained at a level corresponding to 80% of $\dot{V}O_{2max}$. Min 16-28 is referred to as phase II. Analysis of variance was restricted to phases I and II. Mean values of n=7 subjects. p_C and p_{BxC}, probability of 1st type error for rejection of the corresponding null hypothesis ($H_{0(C)}$, no difference in level between PRET and CONT; $H_{0(BxC)}$, no difference in time course between PRET and CONT). PRET precooling test, CONT control test.
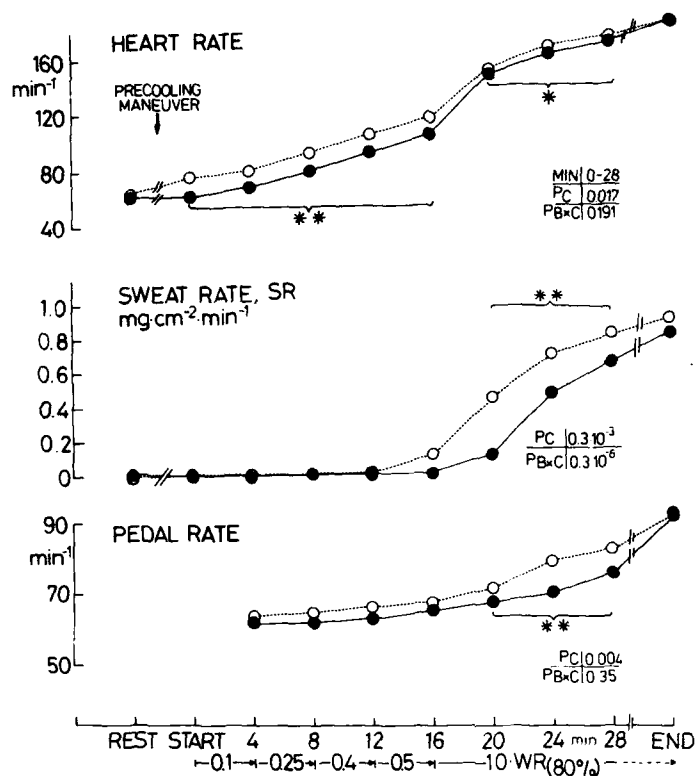
FIGURE 2: Responses of the three cardinal variables to exercise in control and precooling tests. Mean values of n=7 subjects. * and ** Scheffé test: $p < 0.05$ and $p < 0.01$, respectively. For further explanation see legend to Fig. 1.

Heart rate (HR) was significantly lower throughout phases I and II of the exercise period, but at the end of the exercise period (Fig. 2) the difference had disappeared. Oxygen uptake ($\dot{V}O_2$) and net efficiency were not significantly different between the conditions. Due to the lower HR in PRET oxygen pulse (OP) was considerably increased in PRET phase I (+2.2 ml = 18%, $P < 0.01$). The arterio-venous oxygen difference ($AVDO_2$) was increased by 15% in PRET phase I ($P < 0.05$; min 4-12); stroke volume (SV) was increased by 8%, but the latter difference was not statistically significant ($P = 0.087$). Nevertheless, the differences in both $AVDO_2$ and SV appeared to share in the marked increase of OP in PRET. During phase II (heavy exercise) the cardiovascular differences vanished except for that in HR (Fig. 2). At the end of the exercise tests, however, the HR difference had disappeared as well as that in sweat rate.

Preferred pedal rate was increasing throughout the exercise test, in particular during phase II; this increase was significantly smaller in PRET than CONT (Fig. 2). Again at the point of termination of the exercise period the PRET-CONT difference in PR had disappeared. The close relationship between body temperature and preferred pedal rate is demonstrated in Fig. 3.
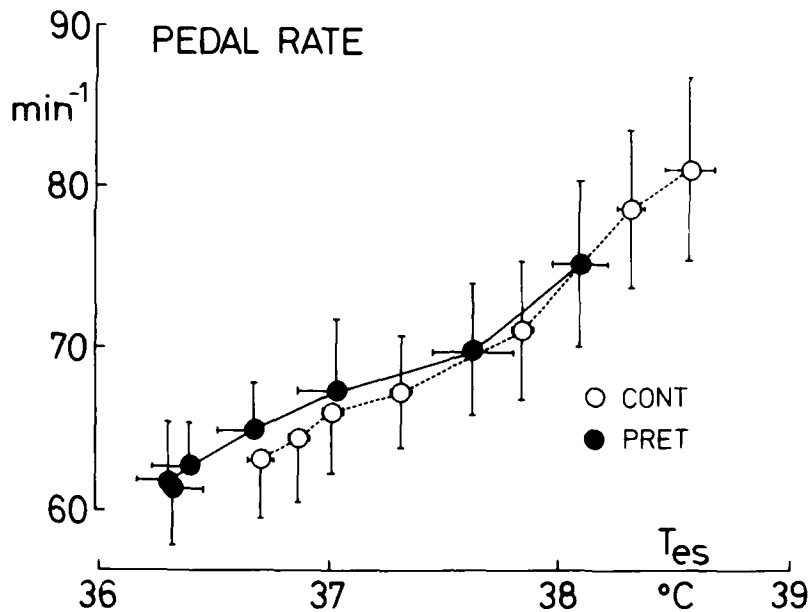
FIGURE 3: Preferred pedal rate vs. esophageal temperature. Data points represent mean values ± SE of pedal rates and T$_{es}$ of six subjects obtained from 4-min exercise bouts in control and precooling tests.

DISCUSSION: The demonstrated dependence of exercise performance on slight changes in body temperature could be related to three physiological parameters: heart rate; heat dissipation rate; preferred pedal rate. The increase of these parameters during the exercise bouts appears to indicate growing discomfort which would counteract, and finally, inhibit completely the drive to exercise. Since all three parameters have been shown to be dependent on body temperature precooling would be expected to prolong endurance time and this has, in fact, been shown in the present study.

While the physiological mechanisms underlying the temperature dependence of heart rate and heat dissipation during exercise are relatively well understood [for discussion see 7], the temperature dependence of the preferred pedal rate (PPR), and the way in which it is linked to exercise performance is still a matter of conjecture, although such interrelationship has not been shown for the first time. Thus, with increased body temperature during the luteal phase of the menstrual cycle PPR was increased and metabolic rate for the same work rate was increased; as a corollary net efficiency was significantly decreased [10].

As for the physiological basis of the interdependence of body temperature, PPR and exercise performance it might be assumed, firstly, that slight muscle temperature variations accompanying the body temperature changes have a direct effect on muscle metabolism [11] which would, in turn, determine the preferred pedal rate. Secondly, it might be assumed that body temperature influences the central nervous control of muscle activity and motor performance; this might, in turn, cause a change in the proportion of anaerobic/aerobic metabolism and consequently influence endurance time. A recent study would favour the second hypothesis [12]. Subjects were pedalling at prescribed pedal rates of either 60 or 90 rpm at identical work rates (75-90% of $\dot{V}O_{2max}$) for 15 min in each test. Moreover, body temperature was varied by a precooling maneuver (described above) preceding one of the two tests at 60 and 90 rpm, respectively. Exercising at the higher pedal rate resulted in a marked increase of the aerobic and anaerobic metabolism ($\dot{V}O_2$ + 11%; plasma lactate concentration at the end of the exercise bouts 14.2 vs. 8.7 mmol/l, P < 0.01). These results closely matched the subjective exertion ratings (RPE, Borg scale): The RPE scores were significantly higher at 90 rpm than to 60 rpm (P < 0.02) indicating increased discomfort at 90 rpm. Precooling had a significant effect on heart rate but neither on metabolic parameters nor on perceived exertion.

These data suggest that the heart rate is dependent on both body temperature and pedal rate whereas the rate of anaerobic metabolism is predominantly dependent on pedal rate. The mechanism of the presumed direct relation between body temperature and pedal rate remains to be studied.

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BODY AND SURFACE TEMPERATURES OF MAN EXPOSED TO SAUNA, SHOWER AND ICE WATER IMMERSION

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Winter swimmers are people who enjoy taking dips in ice water in natural waters throughout the winter season in Finland. Most of them alternate the ice water exposures with a hot sauna, but a minority prefers regular ice water immersions only. The reasons winter swimmers take up this peculiar form of self care are above all recreational with maintenance of health and prevention of any illnesses also ranking high on their list of reasons [1]. The current study was designed to elucidate, among other things, the influences of acute and extreme hot and cold exposure of the type common in Finland on the body and surface temperatures of man.

METHODS: The subjects were nine men, all winter swimmers, chosen from the health survey of winter swimmers [2]. Only men with no signs of cardio-respiratory illnesses were accepted. Characteristics of the subjects are shown in Table I. The subjects were informed volunteers. During the experiments the subjects' deep body temperature (and heart rate) were recorded using a thermistor probe inserted into the esophagus behind the atria of the heart [3]. Skin temperatures from six points were recorded with thin copper-constantan thermocouples attached to the skin with heat reflecting shields. The skin temperature probes were placed (a) in the center of the forehead; (b) in the interscapular region of upper back; (c) on the chest, about 5 cm above the left nipple; (d) on the lateral side of the left upper arm, about 7 cm above the elbow; (e) on the anterior aspect of the left thigh, about 25 cm above the knee; (f) in the middle of the dorsal side of the left foot. The mean skin temperatures were calculated as the arithmetic mean of points c to f.

After two trial sessions, the subjects were exposed to four different combinations of hot and/or cold. Experiment A involved sauna (84°C db, 47°C wb, 80°C bb) until the esophagus temperature reached 39°C, head-out immersion in ice water (17°C) for 10 sec [1], and the same sauna and ice water repeated, followed by a 30 min rest at room temperature (24°C) lying down, uncovered. Experiment B was an exact replica of experiment A, with the ice water immersion replaced with a 10 sec exposure to 15°C shower. Experiment C involved similar exposures to sauna as the other two, but the sauna exposures were followed by a 5 min rest sitting at room temperature (24°C). Experiment D comprised a head-out immersion in ice water (6°C) for 20 sec [1] followed by a 2 min rest sitting at room temperature (23°C) and finally a 30 min rest lying down under cover. The subjects were exposed to these experiment types at random orders.

The experiments were carried out between January 27 and April 29, and October 26 and November 3, 1987, i.e. during the winter swimming season [1]; and always between 16:30 and 18:30 h.

RESULTS: Upon entering the sauna in all three experiments the esophageal temperature rose from the beginning mean of 37.6°C to 38.0°C at a rate of 0.07°C/min. Then the rate changed to a rise of 0.3 to 0.4°C/min. At the same time the slopes of the skin temperatures fell from the initial forehead skin temperature increments of 0.69, 0.92 and 0.63°C/min, interscapular skin temperature increments of 0.96, 0.97 and 0.1.12°C/min, and mean skin temperature increments of 0.94, 0.87 and 1.02°C/min to 0.24, 0.24 and 0.31°C/min on the forehead, 0.42, 0.08 and 0.16°C/min in the interscapular region and 0.48, 0.32 and 0.31°C/min in the mean skin temperature in experiments A, B and C, respectively.

Upon the second entry into the sauna in experiments A and B, the esophageal temperature at first declined at the rate of 0.35 and 0.32°C/min, in experiment C, following room temperature, only at a rate of 0.08°C/min. When the esophageal temperature started to rise the rate of temperature increment repeated the pattern of the first exposure with the exception of experiment A, where the esophageal temperature rose from 38.0 to 39.0°C at a rate of 0.50°C/min.

TABLE 1   Characteristics of the subjects

Age (years)	Winter Swimming (years)	Height (cm)	Weight (kg)	Body Fat (%)
22	4	183	81.0	21.8
46	14	172	80.7	20.9
25	3	187	91.6	19.1
25	4	190	79.4	18.5
34	5	190	87.9	9.4
48	5	176	79.6	25.1
30	1	180	83.7	26.2
50	18	173	77.0	28.2
34	13	180	69.4	8.8
means: 34.9	7.4	181.2	81.1	24.7

The decrease in esophageal temperature during the cooling phases was most rapid and greatest in experiment A and the slowest and least in experiment C.

The temperatures of the forehead, interscapular region and mean skin were, for all practical purposes, the same throughout experiment C. In experiment A, alternating sauna and ice water immersion, the skin temperatures of the forehead and the interscapular region were clearly above the me..n skin temperature during ice water immersions and throughout the 30 min rest period, the difference remaining between 1 to 2°C. The skin temperature responses in experiment B fell intermediate between experiments A and C.

In experiment D, where no sauna was involved, the esophageal temperature remained virtually unchanged throughout the experiment. After the initial fall of 0.1°C in the esophagus, 0.3°C on the forehead (not immersed), 3.1°C in the interscapular region and 2.1°C in the mean skin temperatures, the forehead and mean skin temperatures continued to decline at room temperatures at the rate of 0.44 and 0.72°C/min, respectively, while the skin temperature in the interscapular region turned to a sharp rise at the rate of 0.78°C/min. The second immersion did not induce an immediate change in the interscapular skin temperature which at first remained on a plateau while the mean skin temperature declined, and then fell about 0.5°C within 1 min before turning to a slow rise. The forehead skin temperature changed to a slow decline and the mean skin temperature into a gradual rise. The difference between the mean skin temperature and the temperature in the interscapular region remained between 2.5 and 3.0°C throughout the recovery period.

DISCUSSION: The changes in esophageal temperature upon entering a medium hot sauna [4], where the subjects were allowed to adjust the humidity at will and no radiating heat caused discomfort, were rather rapid in reference to the 10 min which has been recommended as a suitable time to spend in the hot room. Some of the subjects took a full 10 min to raise their internal temperature to 39°C during the first exposure, but in most of them the internal temperature set as a limit for our experimental exposures was reached considerably faster. The second exposure always resulted in a faster warming than the first.

It was an interesting observation that many of the subjects spontaneously expressed their willingness to leave the hot room when their esophagus temperature reached 39°C, which they would have done in normal circumstances.

The body and skin cooling after the sauna complied well with the type of cold exposure, with the fastest and greatest cooling during head-out ice water immersion. The experiment with head-out ice water immersion and no sauna, simulating a type of winter swimming [1] demonstrates the activation of thermogenesis in the inter-scapular region (brown fat) [5,6] of these winter swimmers. Barely visible

shivering commenced shortly after the second ice water immersion and continued until the end of the recovery period. This was enough to keep the subjects awake, although the same men, as a rule, fell asleep during the recovery periods following the three experiments with a combination of hot and cold.

Summarizing, the results show that no exact time limits should be set for the people to stay in the sauna hot room. Instead, bathers are well advised to observe their feelings of comfort and leave the heat when so inclined. The results of the experiment involving only acute cold exposures suggest that non-shivering thermogenesis by interscapular brown fat is activated upon acute cold exposure in people who have practiced winter swimming. This may be one of the factors making winter swimming an enjoyable practice to people living in a cold climate.

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PERIANESTHETIC THERMOREGULATION

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Intraoperative hypothermia decreases respiratory drive, alters drug metabolism, increases *protein* catabolism, causes hemodynamic instability, and prolongs *recovery from surgery* [1,2]. Hypothermia during surgery *results from the interaction of three factors*: 1) decreased metabolic heat production secondary to central depression and peripheral muscle paralysis; 2) increased heat loss to the environment from cutaneous vasodilation, surgical exposure, dry respiratory gases, and unwarmed intravenous fluids; 3) reduced compensatory responses (vasoconstriction, nonshivering thermogenesis, and *shivering*) due to *thermoregulatory suppression and the effects of muscle relaxants.* The extent to which each factor contributes to intraoperative hypothermia is unknown.

Anesthetics are believed to inhibit thermoregulatory responses causing surgical *patients to be* poikilothermic. However, intraoperative thermoregulation may be active, manifest by nonshivering thermogenesis (increased metabolic heat production) [3] and cutaneous vasoconstriction (decreased environmental heat loss) [4]. Shivering during anesthesia is directly inhibited by anesthetics and muscle relaxants.

CLINICAL OBSERVATIONS AND DISCUSSION: To test the hypothesis that thermoregulatory *responses do occur during anesthesia, we studied five generally healthy adults undergoing abdominal* surgery, breast reconstruction, or ophthalmic surgery who were anesthetized with ≈1.2% isoflurane/air [5]. Rectal temperatures were monitored and cutaneous vasoconstriction was assessed with skin surface temperature gradients (forearm - finger tip temperature) and a laser Doppler perfusion monitor. The laser Doppler perfusion index and skin temperature gradients correlate well with other measures of vasoconstriction [6]. Oxygen consumption was calculated from inspired and expired oxygen concentration, expired carbon dioxide concentration, and expired gas volume.

Three of the five patients became hypothermic during surgery, reaching steady state core *temperatures of 33.3 - 34.5°C. In each case, steady state temperature* was associated with profound vasoconstriction (Fig. 1). The other two patients maintained near normal esophageal temperatures and showed no evidence of vasoconstriction (Fig. 2). These results suggest that steady state temperatures in hypothermic patients (core temperatures < 34.5°C) are maintained by active vasoconstriction, while steady state temperatures in warmer patients result from passive interaction with the environment. Non-shivering thermogenesis was less important in these anesthetized adults than has been reported in unanesthetized humans [3].

Although results during isoflurane anesthesia suggested that vasoconstriction was thermoregulatory, *the possibility remained that vasoconstriction was due to hypovolemia or surgical stress.* To test the effects of vascular volume and surgical stress, we evaluated vasoconstriction in patients undergoing similar surgery, but maintained at different temperatures [7].

Ten healthy, unpremedicated patients electively donating a kidney to a relative were anesthetized with ≈0.8% halothane in oxygen. Patients were randomly assigned to standard treatment (no hypothermia precautions) or warming measures including warm fluids, warming blankets, airway humidification, and a warm room. Esophageal temperature, skin-surface temperature gradients, and halothane concentrations were monitored. The thermoregulatory threshold was prospectively defined as the esophageal temperature associated with a skin temperature gradient ≥4°C. None of the warmed, normothermic patients [35.8 ± 0.5°C (SD)] demonstrated significant vasoconstriction. However, vasoconstriction was observed in each hypothermic patient at a mean esophageal temperature of 34.4 ± 0.2(SD)°C (Figs. 3,4). Thus, profound vasoconstriction was observed in hypothermic patients, but not in relatively normothermic patients, undergoing similar changes in vascular volume and surgical stress. These results indicate that vasoconstric-

tion in hypothermic patients results from active thermoregulation rather than hypovolemia or surgical stress. The thermoregulatory threshold in adults receiving ≈1.2 MAC halothane is ≈34.4°C.
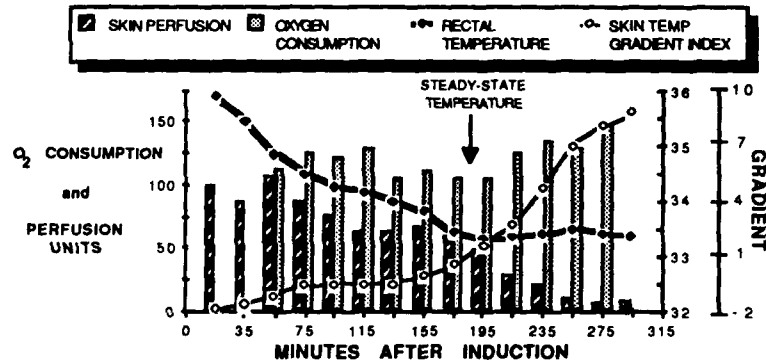
FIGURE 1: Rectal temperature, laser Doppler perfusion index, skin temperature gradients, and oxygen consumption in a patient undergoing abdominal surgery with isoflurane/air anesthesia. The vertical axis on the far right is skin-surface temperature gradient (forearm-finger tip temperature) in °C. Thermal steady state occurred at 33.3°C, 195 min after induction of anesthesia.
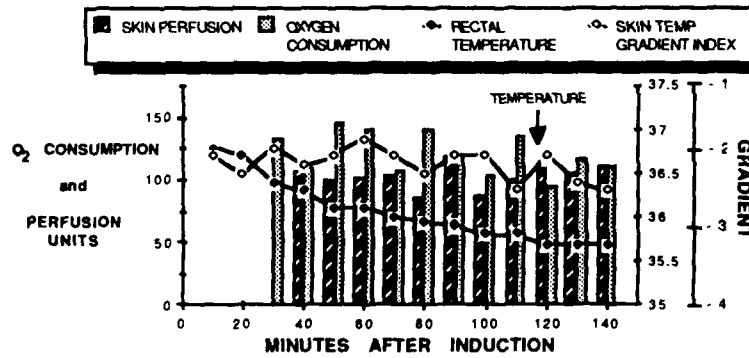
FIGURE 2: Rectal temperature, laser Doppler perfusion index, skin temperature gradients, and oxygen consumption in a patient undergoing ophthalmic surgery with isoflurane/air anesthesia. The vertical axis on the far right is skin-surface temperature gradient (forearm-finger tip temperature) in °C. Thermal steady state occurs at 35.7°C, 120 min after induction of anesthesia.
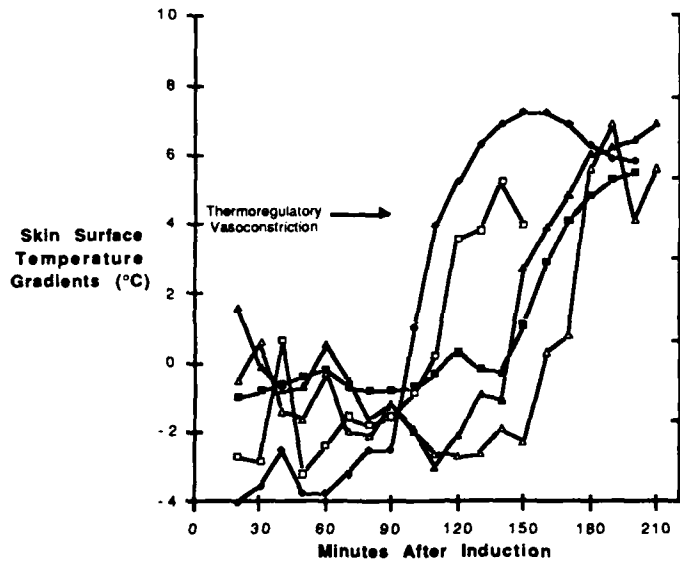
FIGURE 3: Skin-surface temperature gradients (forearm-fingertip temperature) in five kidney donors who became hypothermic during anesthesia with halothane 0.86% and oxygen. Each patient is represented by a different symbol. Thermoregulatory vasoconstriction (prospectively defined as a gradient $\geq 4°C$) was observed in each patient between 110 and 180 min following induction. The average esophageal temperature at the time of vasoconstriction was 34.4 ± 0.2°C (SD).
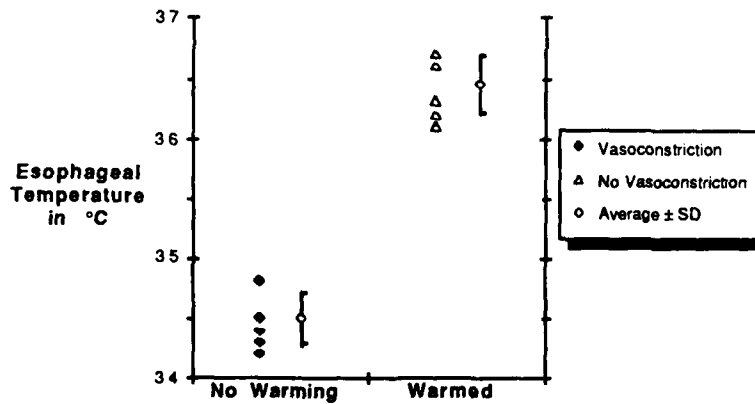
FIGURE 4: Significant vasoconstriction was observed in 5 patients who became hypothermic during donor nephrectomy surgery (left side of figure). Vasoconstriction did not occur in 5 other patients maintained normothermic (right side of figure). Thermoregulatory vasoconstriction was prospectively defined as a skin-surface temperature gradient (forearm-finger tip temperature) $\geq 4°C$. The thermoregulatory threshold during surgery with halothane 0.86% and oxygen is 34.4 ± 0.2°C (SD).
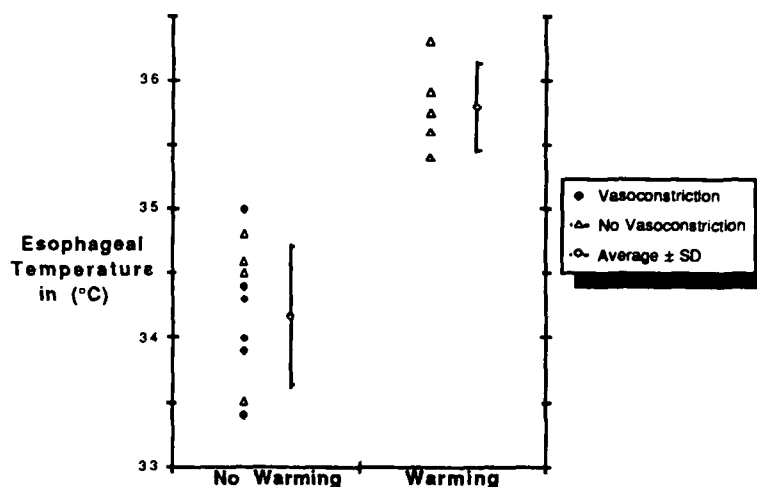
FIGURE 5: Significant vasoconstriction was observed in 6 of 10 elective kidney donors who became hypothermic during anesthesia and surgery with N_2O/fentanyl (left side of figure), but not in 5 others maintained normothermic (right side of figure). Thermoregulatory vasoconstriction was prospectively defined as a skin-surface temperature gradient (forearm-finger tip temperature) $\geq 4°C$. The thermoregulatory threshold during N_2O/fentanyl anesthesia is 34.2 $±0.5°C$ (SD).
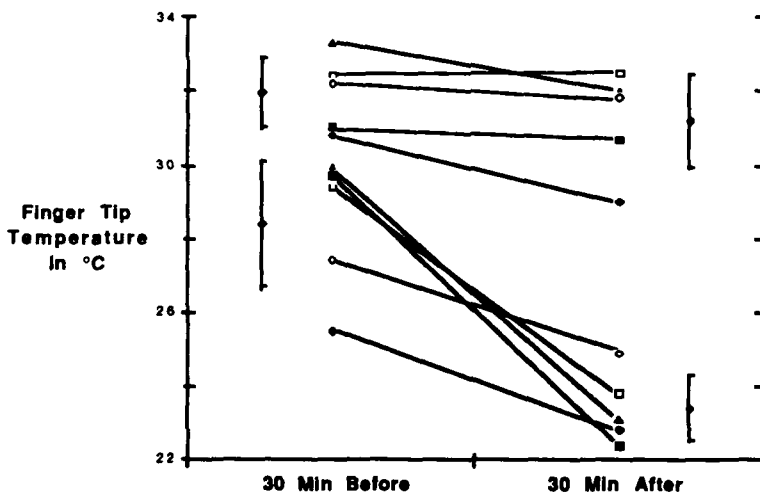
FIGURE 6: Changes in index- and fourth finger-temperatures from 30 min prior to vasoconstriction (forearm-index finger gradient $\geq 4°C$) to 30 min after constriction. Just after induction of anesthesia, bupivacaine (1.5 ml) was infiltrated subcutaneously into four sites around the base of the fourth finger to prevent neuronal transmission. Each symbol represents one patient. Temperatures of the index fingers are connected with thick lines, whereas those of the fourth fingers have thin lines. The averages and standard deviations for each group are indicated on the far right and far left sides of the figure. Both before and after vasoconstriction, fourth-finger temperatures were significantly higher than index-finger temperatures. Although the temperatures of the index and fourth fingers both decreased significantly during vasoconstriction, the decrease in fingers blocked with bupivacaine was seven-fold less.

Narcotics [8,9] and nitrous oxide (N_2O) [10,11] inhibit thermoregulatory responses in animals. We determined the extent to which N_2O/fentanyl anesthesia lowers the thermoregulatory threshold in humans [12].

Fifteen healthy, unpremedicated patients were anesthetized with N_2O (70%) and fentanyl (10 mg/kg iv bolus followed by 4 mg/kg/h infusion) during elective, donor nephrectomy. Patients were randomly assigned to receive additional warming (humidified respiratory gases and warmed intravenous fluids; (N, 5) or standard temperature management (no special warming measures; N, 10). Significant vasoconstriction was prospectively defined as a skin surface temperature gradient $\geq 4°C$. The base of the fourth finger in each patient was infiltrated with bupivacaine and its temperature measured. Near normothermic patients [35.8 ± 0.4°C (SD)] did not demonstrate vasoconstriction but six hypothermic patients vasoconstricted at a mean esophageal temperature of 34.2 ± 0.5°C. Four hypothermic patients developed a passive thermal steady state without becoming sufficiently cold to trigger vasoconstriction (Fig. 5). Neuronal blockade with bupivacaine prevented thermoregulatory vasoconstriction (Fig. 6). These data indicate that thermoregulatory vasoconstriction requires local neuronal control and is not mediated by circulating factors.

In summary, active thermoregulation occurs during N_2O/fentanyl anesthesia, but does not occur until core temperatures are ≈2.5°C lower than normal. The thermoregulatory threshold during N_2O/fentanyl anesthesia is similar to that during halothane (34.4 ± 0.2°C) and isoflurane anesthesia (≈34°C).

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PREVENTION OF INTRAOPERATIVE HYPOTHERMIA

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Intraoperative hypothermia (IH), defined as lowering of the central core temperature (CT) to values
below 36°C, is a very common problem [1]. Taking all surgical procedures performed under general
anaesthesia, some 60% of patients will end up with a core temperature below 36°C - and this percentage
increases if one considers only patients undergoing major surgery, especially if the body cavities (thorax
and/or abdomen) are exposed [2]. This results in a postoperative hypothermic phase which induces a
marked rise in both shivering and non-shivering thermogenesis, leading to a marked increase in metabo-
lism - up to 500% above resting level - thereby increasing oxygen demand [3]. This is of no significance
in young, healthy individuals, but for older people, and especially patients with restricted cardio-
pulmonary reserves, there is a risk of developing cardiac arrhythmias, heart failure and even myocardial
infarction [4] - thereby converting an otherwise successful operation into a catastrophe.

METHODS: In an attempt to avoid IH we have tried the Exacon Thermal Tube® which is placed
in the oesophagus [5]. It is a closed, double lumen tube, allowing closed circulation of warm water (42°C)
at a high flow - 3 to 4 litres/min - from a water bath controlled by a thermostat. The tube acts as a
radiator placed in mediastinum, providing heat to the large vessels and the heart surrounding the
oesophagus. In a prospective trial 40 patients with planned major abdominal surgery were randomized
into two groups, A and B, after preoperative informed consent. The study was approved by the local
ethics committee. Patients in group A were warmed with the thermal tube throughout the operation -
while patients in group B, the control group, did not receive active warming. 7 patients were excluded
during the study, 4 because of a too short a time under anaesthesia - 2 h were the preset minimum - one
because of a morning temperature above 37.5°C, one because of a procedure mistake and one because of
technical failure (thermostat breakdown). Thus, the study was reduced to 33 patients, 15 in group A, 18
in group B. The two groups were fully comparable (Table I). The morning temperature (T_M), was
measured with a mercury maximum thermometer while the core temperature was measured peroperatively
with a thermistor on the tympanic membrane, connected to an electronic thermometer (accuracy 0.1°C).
Neurolept-anaesthesia was used for all patients. Warming in group A was started within 20 min of in-
tubation.

TABLE I

No significant differences between groups A and B in respect to:

Morning temperature
Temperature immediately after intubation
Age
Sex
Transfusions and infusions given
Duration of anaesthesia

RESULTS: In both groups the first temperature, measured after intubation (T_o) showed a fall of about 1°C, with no significant differences between the groups. Already after 1 h of anaesthesia the temperature difference between the two groups was significant $(p<0.05)$, median temperatures in the two groups: A: 36.0°C; B: 35.5°C. The temperature difference became more pronounced with time, thus, after 2 h (T_2): Group A: 36.1°C; Group B: 35.0°C $(p<0.001)$. Temperature at the end of the operation, immediately before extubation - after a median anaesthesia-time of 3.5 h in both groups, (T_{END}) were: Group A: 36.8°C; Group B: 34.9°C $(p<0.001)$. No complications referable to the use of the thermal tube were observed, either intraoperatively or postoperatively.

DISCUSSION: The question is not should one prevent intraoperative hypothermia, but how to avoid it, since it is potentially dangerous, at least to older patients and to patients with restricted cardio-pulmonary reserves. Furthermore, in the hypothermic condition the peripheral circulation is reduced to a minimum, and the circulating blood volume is decreased. This makes the patient more vulnerable to, e.g., postoperative bleeding [6].

In this study we have demonstrated a simple, effective way to prevent IH. We therefore recommend this method in all abdominal operations with an expected duration of more than 1 h, at least in older patients and in known cases with restricted cardio-pulmonary reserves.

There are two potential risks to the use of this system: damage to the oesophagus if the temperature exceeds 42°C for a long period of time; in cases of leakage the stomach may fill with water at a rate of 3-4 litres/min. It is therefore important only to use thermostats with built-in alarm systems and automatic shut off if the temperature exceeds 42°C or if more than 500 ml of water escapes from the system. At present only one commercially available thermostat meets these needs: "Exacon Thermal Therapy Unit, model TT 8200 (manufactured and sold by "Exacon", Industrivej 21, DK-4000 Roskilde, Denmark).

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POSTOPERATIVE TREMOR

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Generalized postoperative tremor (which clinically resembles shivering) can cause complications including an increase in metabolic rate of up to 400%, hypoxemia, wound dehiscence, dental damage, and disruption of delicate surgical repairs [1-3]. Conventional theories of thermoregulation predict that patients with low central temperatures will feel cold, shiver, and undergo peripheral vasoconstriction [4]. However, in preliminary studies, we found that many hypothermic postoperative patients did not shiver. Anesthetic induced inhibition of normal thermoregulation could explain this observation, but probably would be associated with evidence of residual anesthetic effect, a feeling of warmth, and vasodilation. We also observed many normothermic postoperative patients who demonstrated vigorous tremor (without vasoconstriction), a finding difficult to reconcile with conventional theories of thermoregulation.

CLINICAL OBSERVATIONS: We evaluated two potential response patterns in hypothermic postoperative patients: 1) normal thermoregulatory responses in patients who felt cold, were vasoconstricted, and shivered, and 2) anesthetic induced inhibition of normal thermoregulation in patients who felt warm, were vasodilated, and did not shiver. Ninety-seven generally healthy adults were monitored at 15 min intervals during recovery from general anesthesia. Oral temperatures, sensation of cold, and clinically apparent tremor were evaluated at each time period. Anesthetic effect was evaluated by assessing pain and level of consciousness, and by comparing postoperative blood pressure and pulse rate to preoperative levels. Vasoconstriction was evaluated by measuring skin-surface temperature gradients (elbow-finger tip temperature), a sensitive index of thermoregulatory vasoconstriction [5,6]. The incidence of clinically apparent tremor was approximately 50% at all oral temperatures (Fig. 1) and was not significantly correlated with evidence of residual anesthetic effect or of vasoconstriction (Fig. 2). However, 16 fully conscious, hypothermic patients who felt warm, were not vasoconstricted (skin temperature gradients near 0°C), and did not shiver. From these data we conclude: 1) postoperative tremor frequently is not associated with vasoconstriction, even in hypothermic patients (as would be expected if this tremor were thermoregulatory shivering); and 2) normal thermoregulation is inhibited during recovery from anesthesia, even in patients with little residual anesthetic effect.

To determine whether postanesthetic tremor differs from thermoregulatory shivering, we studied the electromyographic (EMG) tremor patterns in 9 women recovering from isoflurane/oxygen anesthesia [7]. EMG of 8 muscles were recorded continuously and analyzed for frequency, amplitude, and power spectra [8]. These spectra were compared with those obtained during our previous studies of pathologic clonus in unanesthetized patients with spinal cord transection [9] and of cold induced shivering in normal control subjects [10]. "Spontaneous EMG clonus" was defined by regular 5-7-Hz bursts superimposed on a low voltage baseline spectra and "tonic EMG activity" by rapid tonic, signals having irregular (5-15 Hz) bursts superimposed on an active baseline spectra.

Clinically assessed data were collected at 10 min intervals by evaluating the following responses: 1) rectal and average skin temperatures; 2) end-tidal isoflurane concentration; 3) clinically visible clonic activity in response to rapid plantar flexion, rated 0 = no clonus, 1 = clonus < 5 sec, and 2 = clonus > 5 sec; and 4) clinically visible tremor, rated 0 = no activity, 1 = intermittent muscular activity, and 2 = vigorous, continuous activity. Fig. 3 provides typical EMG signals from the soleus muscle during A) normal cold induced shivering [10]; B) pathologic clonus due to spinal cord transection [9]; C) postanesthetic spontaneous EMG clonus; D) postanesthetic tonic EMG activity; and E) postanesthetic plantar flexion-induced clonus. The traces shown in panels C, D, and E are from the same patient. Pathologic clonus,
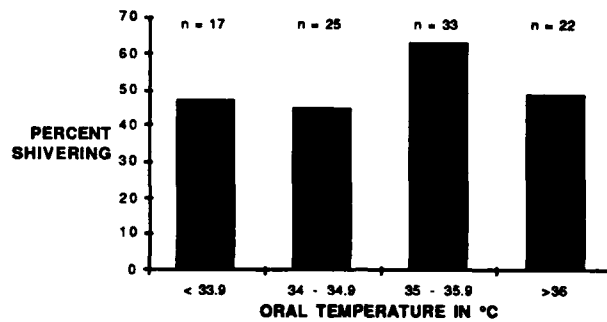
FIGURE 1: The incidence of clinically apparent tremor plotted against oral temperature in 97 patients recovering from general anesthesia. The incidence of postoperative tremor was ≈50% in all temperature ranges.
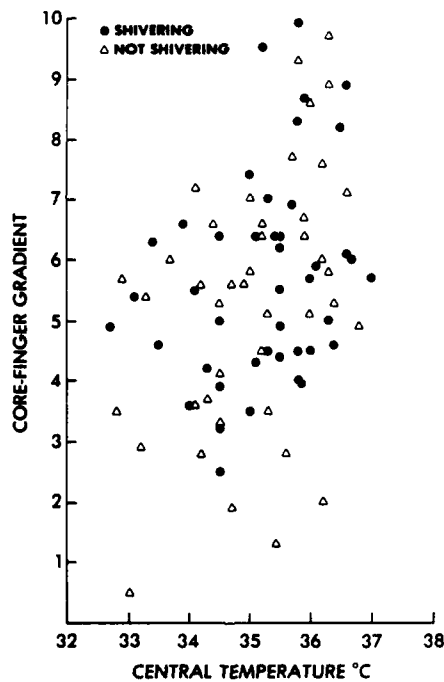
FIGURE 2: Skin-surface temperature gradients (elbow-fingertip temperature) in 97 patients with and without clinically apparent tremor during recovery from general anesthesia. There was no correlation between the incidence of tremor and skin-surface temperature gradients (as would be expected if tremor were all thermoregulatory).

Normal shivering   5 uV

Pathologic clonus   20 uV

Spontaneous EMG clonus   10 uV

Tonic EMG activity   2 uV

Flexion-induced clonus   10 uV

I sec
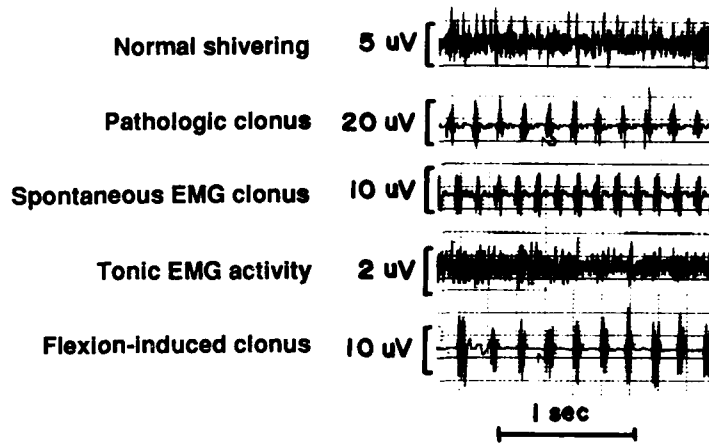
FIGURE 3: Typical EMG signals from the soleus muscle in: A) normal cold-induced shivering, B) pathologic clonus in a patient with spinal cord transection, C) postanesthetic spontaneous EMG clonus, D) postanesthetic tonic EMG activity, and E) postanesthetic clonus induced by plantar flexion. The traces shown in panels C, D and E are from the same patient. Pathologic clonus, spontaneous EMG clonus, and flexion induced clonus have a similar, rhythmic, 5-7-Hz "on-off" bursting pattern. Although raw EMG signals produced by tonic EMG activity and normal shivering resemble each other, their power density spectra may be quite different (see Fig. 4).

EMG POWER

NORMAL SHIVERING
TONIC EMG ACTIVITY
SPONTANEOUS EMG CLONUS

FREQUENCY IN Hz
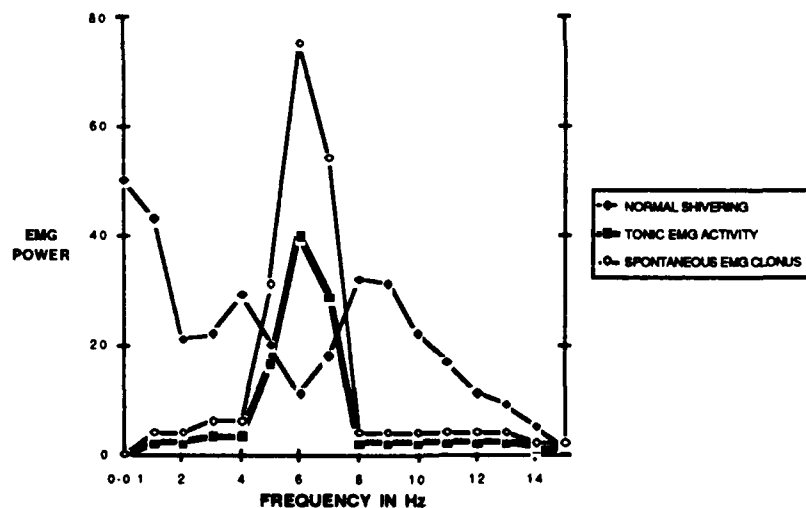
FIGURE 4: Power density spectra of normal thermoregulatory shivering in an unanesthetized subject vs. spectra from postanesthetic tonic EMG activity and postanesthetic spontaneous EMG clonus in a subject from this study. All signals were from the soleus muscle; power is expressed in arbitrary units. The raw EMG signals produced by tonic EMG activity and normal shivering resemble each other, but have different power density spectra. Normal shivering contains little power between 5-7 Hz, whereas much of the signal power from tonic EMG activity is concentrated in this frequency. The power spectra of both postanesthetic tonic EMG activity and spontaneous EMG clonus have patterns similar to that produced by pathologic clonus in patients with spinal cord transections.
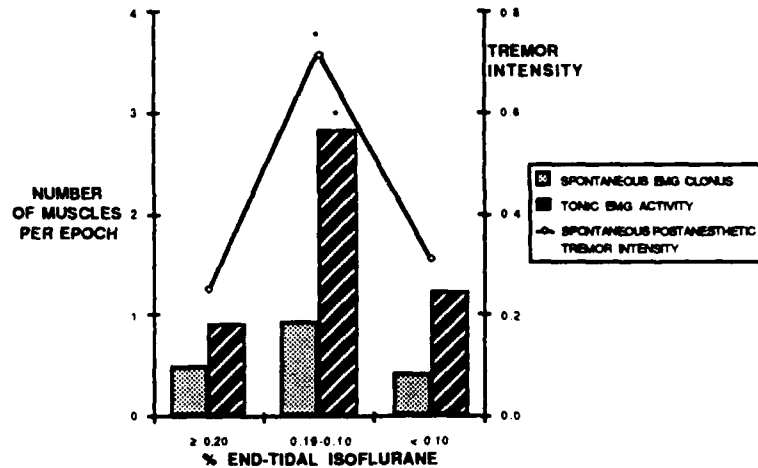
FIGURE 5: The number of muscles per 10-min epoch that demonstrated spontaneous EMG clonus and tonic EMG activity is plotted against end-tidal isoflurane concentration, and against clinical tremor intensity. The latter was derived by dividing the sum of the clinically-assigned intensity scores by the number of epochs in each isoflurane concentration range; these data are plotted using the vertical axis on the right side of the figure. There were 12 epochs during which the isoflurane concentration was $\geq$ 0.2%, 24 epochs between 0.1 and 0.19%, and 54 epochs at a concentration $\leq$ 0.1%. Both EMG patterns were observed most often when end-tidal isoflurane concentrations were between 0.1 and 0.19%, but only the incidence of tonic EMG activity was significantly greater (*, $P < 0.05$). The maximal clinical tremor intensity also occurred in this concentration range (*, $P < 0.05$).

spontaneous EMG clonus, and flexion-induced clonus have a similar, rhythmic, 5-7-Hz frequency and an "on-off" bursting pattern. The EMG pattern generated by postanesthetic tonic EMG activity differs from that of normal thermogenic shivering because it: 1) does not occur in all muscles simultaneously, 2) does not have the slow 4-6 cycle/min synchronous waxing and waning pattern typical of thermogenic shivering, and 3) often has an underlying 5-7-Hz clonic pattern (Fig. 4). Fig. 5 shows the average number of muscles demonstrating spontaneous EMG clonus, tonic EMG activity, and clinically observed tremor intensity, plotted against anesthetic concentration. Spontaneous EMG clonus and tonic EMG activity were most common at isoflurane concentrations between 0.10-0.19%, as was clinically observed peak tremor intensity. Clinical grade 2 tremor and grade 2 flexion-induced clonus correlated significantly with spontaneous EMG clonus. Spontaneous EMG clonus was twice as common when average skin temperature was less than 33.0°C than at higher temperatures (38% vs. 19%, $P < 0.05$). Neither the incidence of tremor, tonic EMG activity, nor spontaneous EMG clonus correlated with rectal temperature. No clinical tremor or EMG activity was detected in 23/35 (66%) of the epochs during which rectal temperatures were < 36.5°C and isoflurane concentrations were < 0.10% ($\approx$7% of a fully anesthetic concentration).

DISCUSSION: The postoperative period represents a continuum that begins with patients anesthetized and ends when they are fully awake. We propose the following stages which are qualitatively illustrated in Fig. 6: 1) Early recovery (isoflurane concentration $\geq$ 0.2%), when little muscular activity occurs; 2) Middle recovery (isoflurane concentration 0.1-0.19%) during which thermoregulatory responses to cold are inhibited, but spinal reflex activation causes a clonic spontaneous tremor (spontaneous EMG clonus and tonic EMG activity with underlying clonus); 3) Late recovery (isoflurane concentration < 0.1%) during which thermoregulatory responses are no longer inhibited and spinal reflexes are no longer activated. If patients with low isoflurane concentrations remain hypothermic, they will demonstrate normal shivering.
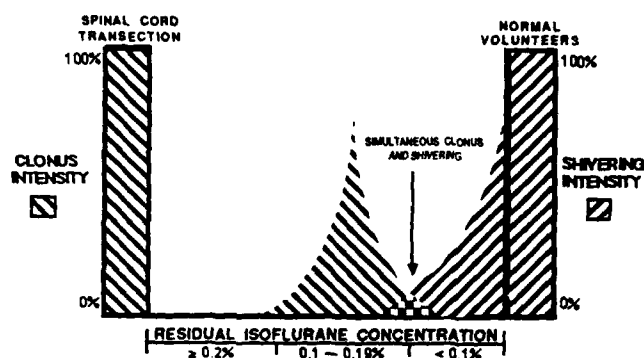
FIGURE 6: A qualitative illustration suggesting possible recovery stages in patients who remain hypothermic during recovery from isoflurane anesthesia. The vertical axis on the left indicates clonic tremor intensity and includes a control panel; the vertical axis on the right indicates the intensity of normal shivering and includes a similar panel. The left control panel indicates that tremor in patients with spinal cord transections is completely clonic; the panel to the right indicates that spontaneous tremor in unanesthetized volunteers is normal shivering. Recovery, which is divided into three stages, is indicated on the horizontal axis. At isoflurane concentrations $\geq$ 0.2%, there is little muscular activity (both brain and spinal cord are "asleep"). At isoflurane concentrations between 0.1 and 0.19%, tremor is mostly clonic (brain is "asleep" and spinal cord is "awake," causing a functional cord transection). When the isoflurane concentration decreases to < 0.1%, normal shivering predominates (both brain and spinal cord are "awake"). Both clonic tremor and normal shivering may occur simultaneously when isoflurane concentrations are near 0.1%.

In summary, our studies indicate that thermoregulatory vasoconstriction and normal shivering do not occur during recovery from general anesthesia in moderately hypothermic patients (core temperature 35-37°C) and that the electromyographic characteristics of postoperative tremor resembles pathologic clonus, not those of thermoregulatory shivering. These observations cannot be reconciled with existing theories of anesthetic influence on thermoregulation, nor with the conventional explanation for postanesthetic tremors.

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THE COMPARATIVE EFFECTS OF BUPIVACAINE EPIDURAL ANALGESIA ON POSTOPERATIVE REWARMING

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Recovery from general anaesthesia is known to be associated with marked hemodynamic and metabolic changes related to rewarming. On the first three postoperative days following total hip replacement continuous bupivacaine epidural analgesia blunts the stress response to surgery as measured by oxygen consumption ($\dot{V}O_2$) [1]. The influence of epidural blockade on postoperative rewarming is unknown. The aim of this study was to evaluate during this period the hemodynamic and metabolic effects of continuous epidural infusion of bupivacaine at two concentrations (0.10% and 0.25%).

After approval by the institutional human subject protection committee, 14 patients undergoing total hip replacement were studied. At the end of the procedure, they were allocated randomly to one of two groups for postoperative epidural infusion: group I (n, 7) received bupivacaine (0.10%) 20 ml/h; group II (n, 7) received bupivacaine (0.25%) 8 ml/h, i.e., the same cumulative dose. Data were taken using a radial artery catheter and a thermodilution Swan Ganz pulmonary artery catheter upon arrival of patient in the recovery ward, when pulmonary artery temperature reached 35°C, 36°C and 37°C. Statistical analysis was by ANOVA.

The main hemodynamic and metabolic results are summed up in the figures. In group I, there was a significant decrease in systemic vascular resistance (SVRI) as soon as patients warmed. In this group, the mean blood pressure and the SVRI were significantly lower than in group II. Excepted at 37°C, $\dot{V}O_2$ and oxygen content arterio-venous difference C (a-v)O_2 were lower in group II than in group I.

The use of greater infusion volume of the epidural space while maintaining the identical local anaesthetic dose is accompanied by a more extending sympathetic blockade. During recovery, this effect does not appear to be useful, because $\dot{V}O_2$ is greater whereas C (a-v)O_2 widens.

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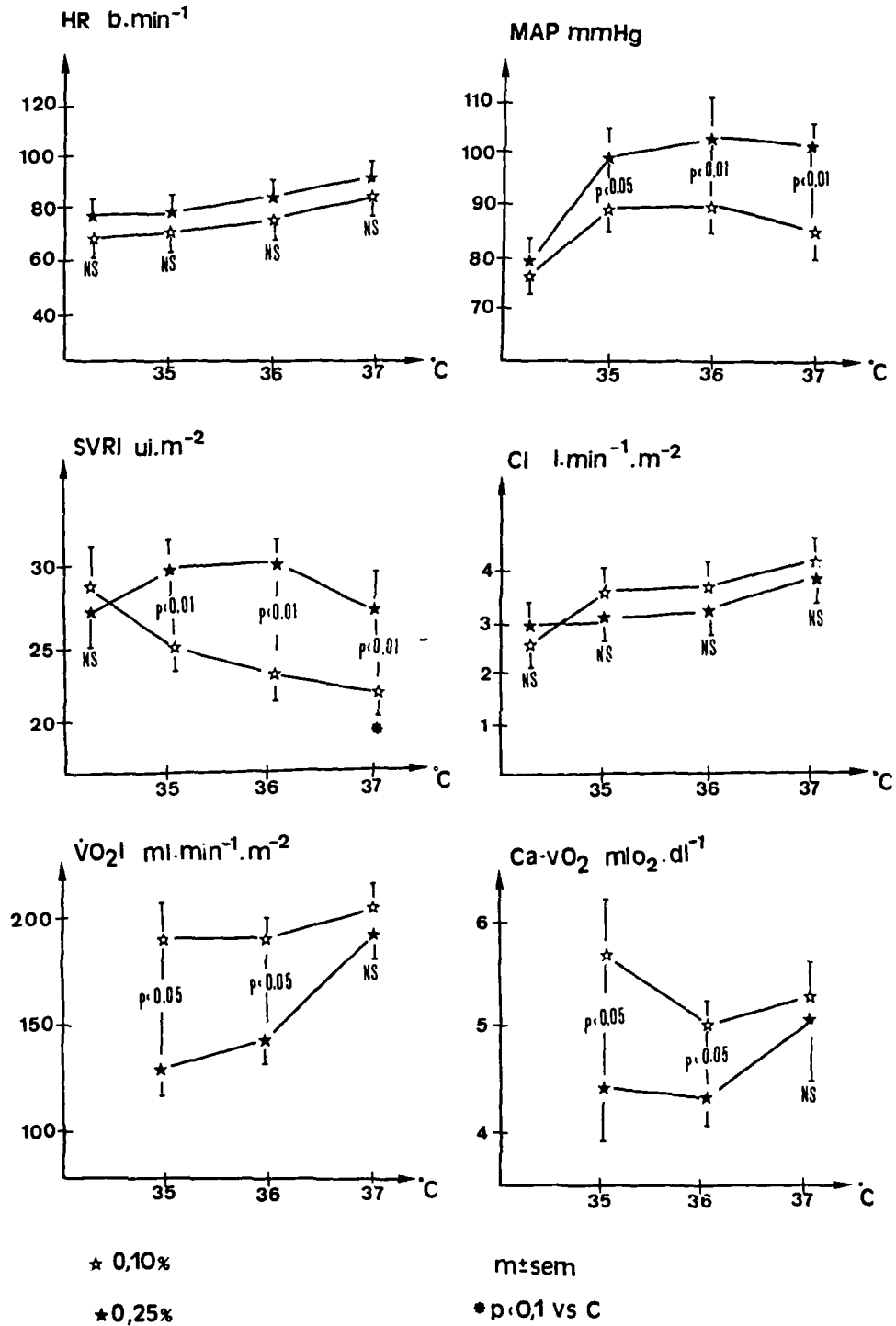
FIGURE 1: Effects of bupivacaine during recovery from anesthesia.

HUMAN OBESITY AND BROWN FAT METABOLISM

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NONSHIVERING THERMOGENESIS AND FACULTATIVE THERMOGENESIS: In *rodents*
brown adipose tissue (BAT) is the major site of cold induced nonshivering *thermogenesis* (NST), and it
may account for as much as 70% of the whole body *response* [1]. The remaining *part of the heat genera-*
tion seems to originate from skeletal muscle and the liver [2]. NST *is* mediated through an activation of
the adrenomedullary system, which *releases catecholamines* acting on β-adrenoceptors on the target cells
[3]. BAT is known to have an abundant sympathetic *innervation* and to respond to chronic sympathetic
stimulation by hypertrophia and hyperplasia, thereby increasing the thermogenic responsiveness to
catecholamines [3]. This trophic effect can be imitated by infusion with catecholamines or by treatment
with β-adrenergic agents [4]. Also skeletal muscle shows an increased sensitivity to the thermogenic effect
of a sustained β-adrenergic stimulation, as observed in rodents after cold acclimation [5] and endurance
training [6].

Also food intake is accompanied by a thermogenic response mediated by the sympathetic nervous
system (SNS) and unrelated to the obligatory processing of nutrients. This component is named facultative
thermogenesis, and may share a common mediation and target tissue with NST. Rodents often exhibit an
enhanced facultative thermogenesis (diet induced thermogenesis) when overfed with palatable food [7].
Evidence exists to indicate that an important part of this thermogenesis may be mediated by SNS and take
place in BAT [7]. Recent findings, however, cast doubt on the concept of SNS and BAT being involved
in diet induced thermogenesis in rodents, and suggests that the increased energy expenditure during over-
feeding is due merely to alterations in body composition [8]. Also, dogs possess a substantial capacity for
cold induced activation of SNS leading to NST [9]. They increase NST during cold acclimation, but do not
show any evidence of an increased facultative diet induced thermogenesis [9]. Thus, the alleged identity
of NST and facultative thermogenesis is highly questionable, particularly in non-rodents.

THERMOGENESIS IN MAN: There is compelling evidence that adult man responds with an in-
creased energy expenditure during stimulation with nutrients, cold exposure, hypoglycemia and
catecholamines (Fig. 1), and that the responses can be reduced by the β-blocking agent propranolol [10].
The magnitude of the increase in thermogenesis during cold exposure or catecholamine infusion is, how-
ever, considerably more modest than in rodents (Fig. 2). While unambiguous evidence has been presented
that the sympatho-adrenal system plays a major role in the mediation of thermogenesis, there is conflict-
ing opinion on the importance of the thermogenic target tissues involved [2,11,12].

DOES ADULT MAN POSSESS FUNCTIONAL BAT?: First of all, the alleged connection between
BAT and obesity in humans implies that functional BAT is present in adult man. In contrast to the large
number of papers speculating on an etiologic, defective BAT thermogenesis in human obesity, in only very
few *in vivo* studies has the quantitative importance of BAT for the thermogenic response to catecholamines
in adult man been investigated.

Human BAT has been demonstrated to receive sympathetic innervation [13], but the amount of tis-
sue is small [14].

The warm interscapular areas localized by thermography during sympathomimetic stimulation was
suspected to be the site of thermogenetically active subcutaneous BAT [11,15]. A closer examination,
however, revealed that the increased cutaneous and subcutaneous temperatures and subcutaneous blood
flow during stimulation was due to a vasodilatatory effect [16]. In addition, no BAT was present in biop-
sies taken from the warm areas [16]. Brundin *et al.* [17] have confirmed these results by demonstrating
that the oxygen consumption and heat production from the tissue drained by the azygos vein were modest
and uninfluenced by norepinephrine infusion.

PHYSIOLOGIC STIMULI FOR FACULTATIVE THERMOGENESIS

FOOD

COLD

HEAT

STRESS

EXERCISE

FIGURE 1: Different physiological stimuli causing increased facultative thermogenesis via activation of the sympatho-adrenal system.

THERMOGENIC RESPONSE TO NOREPINEPHRINE
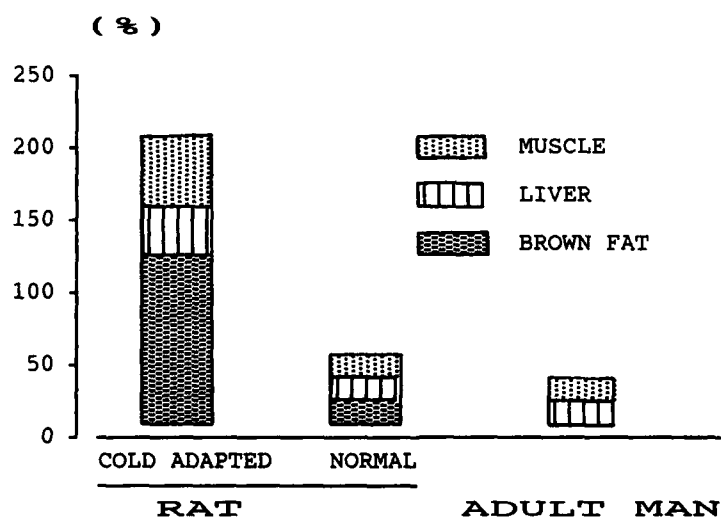
FIGURE 2: An estimate of the contribution of skeletal muscle, the liver and brown fat to norepinephrine-induced thermogenesis in cold adapted and normal rats, and in adult man.

Biopsy studies have shown that BAT is very rare in adult man in the interscapular area, but it can be found more regularly in the perirenal fat depot. Astrup et al. [18] found no function of the perirenal BAT in 4 of 5 subjects after ephedrine stimulation. A single subject had a very modest increase in BAT thermogenesis which, together with other BAT depots, in the most favourable view may contribute with 14% of the increase in whole body oxygen produced by ephedrine [18]. Cunningham et al. [14] concluded

that the perirenal BAT depot could account for 0.2% of the thermic response to infused norepinephrine. This is in accordance with a previous finding that the perirenal fat behaves more like white adipocytes with increased lipolysis during exercise [19].

From different BAT depots Lean's group [20] have performed a number of *post mortem* measurements of the concentration of the uncoupling mitochondrial protein, thermogenin, which is unique to BAT. Thermogenin could not be detected in all subjects, and in the positive cases the concentration was generally very low [20]. On the whole, the above mentioned researchers agree that there is only a quantitatively unimportant role for BAT in the normal adult human.

Chronic treatment with β-adrenergic agents mimicks cold acclimation and induces BAT hypertrophia and enhanced thermogenic responsiveness to catecholamines in rodents [3], dogs [21] and monkeys [22]. Also man reactivates BAT during conditions with excessive levels of catecholamines [3], and responds with an increased thermogenic response after treatment with β_1-agonists [23]. However, facultative thermogenesis does not seem to increase [24]. Bearing in mind that SNS is highly differentiated, the most likely explanation is that different stimuli, such as food and cold, may activate separate branches and thus different thermogenic target organs.

THE THERMOGENIC TARGET ORGAN FOR CATECHOLAMINES IN MAN: The cardiac and respiratory work induced by catecholamines can only explain a small part of the thermic whole body response. The contribution of the liver to catecholamine-induced thermogenesis has only been examined in a few studies, but they convincingly demonstrate increases in splanchnic oxygen consumption during infusion of epinephrine as well as norepinephrine [see 2]. Others have measured splanchnic oxygen consumption during cold exposure or during low dose epinephrine infusion, and found only modest, insignificant increases. Bendtsen *et al.* [25] have reported recently that propranolol decreases splanchnic oxygen consumption in patients with cirrhosis.

The reviewed studies on the splanchnic contribution to facultative thermogenesis are clearly insufficient to draw any quantitative conclusion.

In rodents, cats and dogs resting skeletal muscle has for many years been known to respond to infused catecholamines with an increased oxygen consumption. However, as a consequence of the enormous thermogenic capacity of BAT in rodents, skeletal muscle does not account for more than 10-20% of thermogenesis induced by norepinephrine infusion. In contrast, adult man, like the dog and the pig, possesses a much lower thermogenic capacity, and skeletal muscle might therefore play a more significant role. Based on determination of forearm or leg oxygen consumption and by the simultaneous measurement of whole body oxygen consumption it is possible to estimate the contribution of the entire body muscle mass to the whole body thermogenic response. Such studies show that skeletal muscle has an important role, as it may account for as much as 25-50% of the whole body response [2,3]. However, the lack of dose-response studies and proper control experiments makes any exact estimation speculative.

Recent studies have shown that skeletal muscle participates in carbohydrate induced thermogenesis in man, and this tissue may account for the major part of the facultative thermogenesis [26].

DEFECTIVE THERMOGENESIS IN OBESE AND POST-OBESE PATIENTS: It is well known that the obese organism not only has larger lipid stores but also an increased lean body mass, which causes a higher RMR. The search for a thermogenic defect in obesity has focused on the thermic effect of food, and on RMR in the post-obese. A number of studies have demonstrated that obese and post-obese patients have a blunted thermogenic response to food compared with lean controls [see 10,27]. Obese patients with marked insulin resistance, however, have an elevated fasting splanchnic gluconeogenesis which contributes to their increased resting metabolic rate [28]. This energy dissipating process is suppressed by a meal due to the increase in plasma insulin. Thus, the whole body thermogenic response is made up of at least three components: 1) obligatory thermogenesis; 2) facultative thermogenesis of sympatho-adrenal mediation; 3) suppression of hepatic thermogenesis.

To avoid the confounding effect of the third component, glucose induced thermogenesis (GIT) has also been studied selectively in skeletal muscle in severely obese patients separated into two groups with normal (NGT) and impaired glucose tolerance (IGT). Whereas the whole body GIT of the lean control group was 11.5%, that of the NGT obese was 5.3%, which was significantly more than the -4.2% exhibited by the IGT obese group [29]. The negative GIT value is attributed to suppression of a substantial hepatic gluconeogenesis. The plasma norepinephrine response was diminished in both obese groups compared with the lean group, a finding that points to a defective SNS activity in the patients becoming severely obese [29]. The IGT group also had a lower increase in skeletal muscle thermogenesis after oral glucose [29]. Interestingly, these abnormalities did not improve after a weight reduction of 30 kg [30]. This suggests

that the defective SNS may be etiologic in the development of obesity. Also, a defective activity in the obese of SNS to other stimuli has been reported [10].

Despite a certain residual overweight after the weight loss, the resting metabolic rate had decreased to a level comparable with that of the lean controls. Similar results have been reported by other groups using post-obese patients compared with weight matched lean controls [31]. Segal *et al.* [32] did not find higher resting metabolic rate in an obese group than in the lean controls, despite the fact that the obese were 16 kg heavier. Also meal induced thermogenesis was significantly lower in obese patients, regardless of whether the meal was administrated before, during or after exercise [32]. In another elegant study Segal *et al.* [33] compared two groups of subjects with the same degree of overweight, but differences in LBM (obese vs. body builders). They found that the RMR and meal induced thermogenesis were lower in the obese patients [33]. Nielsen *et al.* [34] have demonstrated recently that aerobic endurance training increases the thermogenic response to cold exposure and sympathomimetic stimulation. These studies emphasize the importance of LBM, probably skeletal muscle as a determinant of RMR and the thermogenic response.

The cellular mechanisms responsible for facultative thermogenesis in skeletal muscle are not well understood. Different processes, such as stimulation of K^+-Na^+-pumps, increased contractile tone [3], and substrate cycles may be of importance [35]. We have measured energy rich phosphorous compounds in skeletal muscle using ^{31}P nuclear magnetic resonance spectroscopy, and have found a significant increase in inorganic phosphate after glucose ingestion in lean young adults. These results warrant further investigations by this non-invasive technique in order to separate the changes into obligatory and facultative components and to study obese and post-obese patients.

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ADRENERGIC REGULATION OF ION FLUXES IN BROWN ADIPOCYTES

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Since the demonstration of the quantitative significance of brown adipose tissue mediated non-shivering thermogenesis in the mammal [1] there has been great interest in gaining a full understanding of the hormonal mechanisms which lead to thermogenesis in this tissue. The earliest observable events after adrenergic stimulation of brown adipose tissue include a series of changes in the plasma membrane potential [2,3]. Norepinephrine first induces a rapid (within 2 sec) and large (25 mV) depolarization of the membrane after which there is a full repolarization. After this, if the stimulation is maintained, the membrane again slowly depolarizes. The rapid depolarization occurs specifically in response to stimulation of the α-adrenergic receptor, whilst the repolarization and the second, slower depolarization are essentially β-adrenergically mediated responses [2,3]. It has been our aim to investigate the significance of these fluctuations in membrane potential and to identify the ion fluxes that are responsible for eliciting these changes. Below we present a summary of our investigations of the fluxes of Ca^{2+}, K^+, Na^+ and Cl^- in these cells.

AN UNUSUAL Ca^{2+} METABOLISM: We were initially interested in the effect of α_1-adrenergic stimulation on the Ca^{2+} metabolism of the brown adipocyte and found that such stimulation led to the mobilization of Ca^{2+} from an intracellular Ca^{2+} pool [4]. This concurred with the observations that α_1-adrenergic stimulation of the brown adipocyte led to both the so-called "PI response" [5] and the production of intracellular inositol-trisphosphate [6], which, in a wide range of cells, releases intracellularly stored Ca^{2+}. Our observations suggested that the hormone sensitive Ca^{2+} pool in the brown adipocyte was of mitochondrial origin [4], which conflicted with the emerging view that the hormone releasable Ca^{2+} pool of the mammalian cell is located in the endoplasmic reticulum. Further experiments are needed to define unequivocally the location of the hormone sensitive Ca^{2+} pool in this cell, and if the mitochondrial location is confirmed, the brown adipocyte, which has a great abundance of mitochondria, will provide a very interesting and unusual model for studying the control of cellular Ca^{2+} after hormone stimulation.

An indication that the Ca^{2+} metabolism of the brown adipocyte may be unusual has come from recent studies of rapid $^{45}Ca^{2+}$ uptake by these cells [7]. Quite unexpectedly, specific stimulation of the cell via the β-adrenergic receptor led to a marked inhibition of cellular Ca^{2+} uptake and this inhibitory effect was mediated by cyclic-AMP. Such a cyclic-AMP mediated effect on Ca^{2+} uptake is not seen in other cell types. Interestingly, free fatty acids which - acting as both substrate and mitochondrial uncouplers within the cell - are potent stimulators of brown adipocyte respiration, could also inhibit Ca^{2+} uptake. Further, in the presence of the artificial mitochondrial uncoupler FCCP, the inhibitory effect of β-stimulation was significantly reduced, indicating that at least a large part of the response to the hormone could be ascribed to a reduced flux of $^{45}Ca^{2+}$ into the mitochondrial Ca^{2+} pool [7]. In several other cell types, Ca^{2+} mobilized from the endoplasmic reticulum after hormone stimulation elevates the cytosolic Ca^{2+} concentration, which leads to an uptake of Ca^{2+} into the mitochondrial pool [for references see e.g., 7]; the mitochondria thus buffering the cytosolic Ca^{2+} level. The reduced capacity of the mitochondria within the stimulated (thermogenically active) brown adipocyte to take up Ca^{2+} is therefore unusual and probably arises as a consequence of *in vivo* mitochondrial uncoupling through the uncoupling protein thermogenin, which is unique to brown adipocytes (Fig. 1A).

In many cell types, stimulation with α_1-adrenergic agonists (or other hormones) induces a rapid influx of Ca^{2+} over the plasma membrane. This Ca^{2+} is thought to maintain the elevated cytosolic Ca^{2+} levels when the limited amount of Ca^{2+} found in the intracellular Ca^{2+} stores becomes depleted during persistent hormone stimulation. However, in isolated brown adipocytes no such α_1-stimulation of Ca^{2+} uptake can be observed [7], making Ca^{2+} influx an unlikely cause of the α-adrenergic depolarization and implying that some other mechanism of maintaining an elevated cytosolic Ca^{2+} level must be operating. In

this context it should be noted that norepinephrine acts simultaneously on the α_1- and β-adrenergic receptors of the brown adipocyte. Since α_1-stimulation leads to intracellular Ca^{2+} mobilization [4] and since β-stimulation inhibits Ca^{2+} uptake into the mitochondria [7], then the β-stimulus would be expected to potentiate and sustain the α_1 induced rise in cytosolic Ca^{2+}. This may be an explanation at the subcellular level for some of the observations of co-operativity between α_1- and β-stimulations in brown adipose tissue that have been described recently [8-11].

Thus, Ca^{2+} metabolism in the brown adipocyte seems to be very different from that of other cell types studied, the origin of this difference perhaps lying in the mitochondrial adaptations necessary for this cell to perform its highly specialized thermogenic function (Fig. 1A).
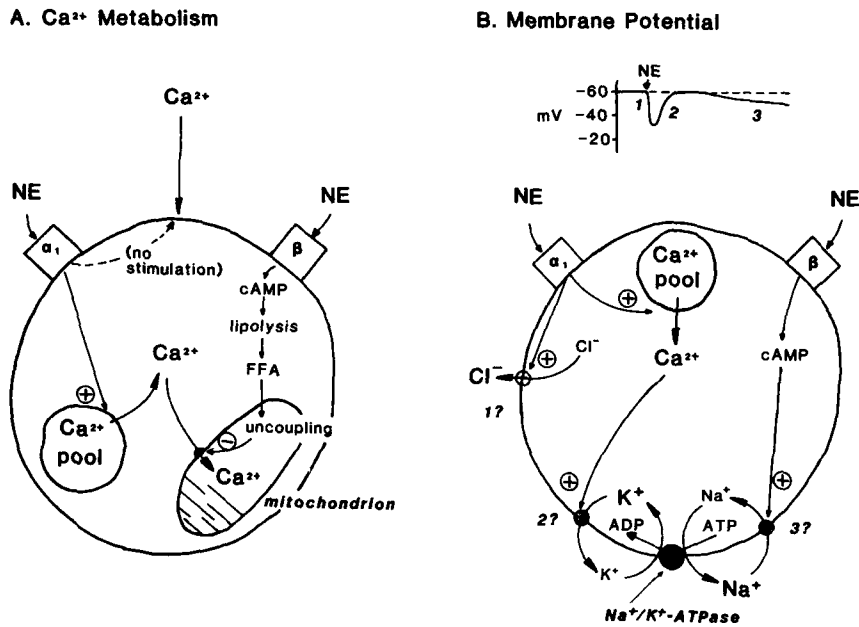
FIGURE 1: A summary of adrenergic effects on ion fluxes in the brown adipocyte. Stimulatory and inhibitory influences are indicated by "$\oplus$" and "$\ominus$" respectively. A. Ca^{2+} metabolism. B. Possible basis of the alterations in membrane potential after stimulation. The inset is a schematic representation of the changes in membrane potential induced by norepinephrine as reported in [1,2]. The ion fluxes that might explain the numbered phases of the response (1,2,3) are indicated as 1?, 2?, 3?.

K^+ MOVEMENTS THROUGH A Ca^{2+} DEPENDENT K^+ CHANNEL: Specific stimulation of the α_1-adrenergic receptor induces an efflux of K^+ from brown adipocytes [12]. We have demonstrated that this K^+ efflux occurred through a Ca^{2+} dependent K^+ channel which was characterized by its sensitivity to the bee venom toxin apamin [13]. This response was an indication that α_1-adrenergically stimulated Ca^{2+} mobilization led to a functionally significant elevation of the cytosolic Ca^{2+} level. Theoretically, the K^+ efflux could be responsible for the repolarization of the membrane potential of the stimulated cell (Fig. 1B), but the fact that this repolarization is mainly a response to β-adrenergic stimulation [2,3] does not support this idea. The K^+ efflux might, however, be the basis of the increased activity of the Na^+/K^+ ATPase which occurs after α_1-adrenergic stimulation [14] (Fig. 1B).
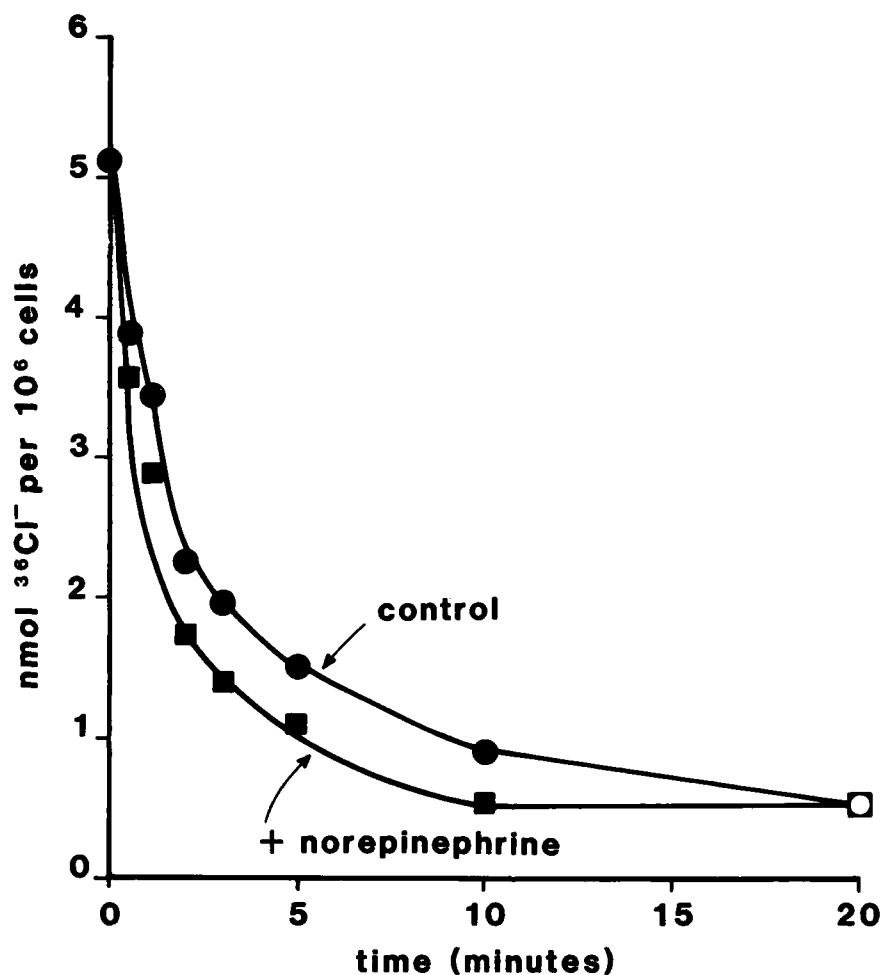
FIGURE 2: Norepinephrine-stimulated Cl⁻ efflux from isolated brown adipocytes. The curve shows the amount of $^{36}Cl^-$ remaining after incubation of preloaded cells in radioactively unlabelled but Cl⁻ containing medium for the indicated times. For details see ref. 16.

HORMONE STIMULATED Na⁺ INFLUX: The observation that α_1-adrenergic Ca^{2+} mobilization in the brown adipocyte was dependent upon the presence of extracellular Na^+ [4] suggested that a Na^+ influx into the cytosol might cause the release of intracellular Ca^{2+}. Using $^{22}Na^+$, we examined Na^+ fluxes in these cells and, as predicted, adrenergic stimulation led to a Na^+ influx [15]. However, this Na^+ influx was clearly mediated through the β-adrenergic receptor and via cyclic-AMP [15]. Furthermore, an artificial elevation of the intracellular Na^+ levels by exposure of the cells to the Na^+ ionophore monensin could not elicit a Ca^{2+} mobilization (unpublished observations). Thus, Na^+ influx was not responsible for the release of intracellular Ca^{2+} stores. The physiological relevance of the Na^+ influx remains unknown but the resultant elevation in the cytosolic Na^+ concentration probably underlies the stimulation of the Na^+/K^+ ATPase seen after β-stimulation in these cells [14] (Fig. 1B).

COULD Cl⁻ EFFLUX CAUSE THE α-MEDIATED DEPOLARIZATION? We have questioned whether a Cl⁻ flux could explain the α-adrenergic depolarization and have examined the efflux of ^{36}Cl⁻ from preloaded brown adipocytes. Norepinephrine elicited an enhanced rate of Cl⁻ efflux from the cells (Fig. 2), a response which could be blocked by the α_1-adrenergic antagonist prazosin but not by the β-antagonist propranolol and which was not dependent upon the elevation of cytosolic Ca^{2+} levels [16]. Thus, a stimulated Cl⁻ efflux may be the molecular basis for the α-adrenergic membrane depolarization in the brown adipocyte.

CONCLUSIONS: The brown adipocyte shows a highly unusual Ca^{2+} metabolism (Fig. 1A) which is linked to the efflux of K^+ from these cells. The sequential changes in membrane potential of the stimulated brown adipocyte may be due to first Cl⁻ efflux (α-adrenergic depolarization), then K^+ efflux (repolarization) and finally Na^+ influx (β-adrenergic depolarization) (Fig. 1B). The metabolic roles of the various ion fluxes as well as the significance of the changes in the membrane potential remain to be elucidated.

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PHARMACOLOGICAL CHARACTERIZATION OF α_1 - AND β -ADRENERGIC RECEPTORS IN BROWN ADIPOSE TISSUE: A COMPARATIVE STUDY WITH THE RADIOLIGANDS (^{3}H)PRAZOSIN AND (^{3}H)CGP-12177

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Heat production in brown adipose tissue (BAT) is triggered by norepinephrine released from sympathetic nerves innervating individual brown adipocytes. Norepinephrine binding to adrenergic receptors on brown adipocytes initiates a series of biochemical processes that results in increased oxygen consumption, i.e., heat production [see 1].

The main types of adrenergic receptors in BAT are the β- and the α_1-adrenergic receptor, as demonstrated by both physiological and radioligand binding studies [see 1,2]. While the direct thermogenic effect of activation of β-adrenergic pathways (including cAMP-mediated stimulation of lipolysis) in BAT is well documented, the physiological significance of α_1-adrenergic receptors and responses is still somewhat obscure. However, it has been suggested that α_1-adrenergic receptors play an important role in long-term regulation of BAT metabolism [2,3]. In good agreement with what is known about the α_1-adrenergic responses in other tissues, the α_1-adrenergic effects in brown fat cells are, at least in part, mediated by changes in phosphoinositide metabolism and Ca^{2+} homeostasis [4-6].

There has been considerable debate concerning various aspects of adrenergic receptors and responses in BAT. As previously pointed out [2,7], some of this discussion is due to the important, but often overlooked, fact that catecholamines show only relative selectivity for β- and α_1-adrenergic receptors. Not only the natural neurotransmitter norepinephrine, but also phenylephrine (often used as a "selective" α_1-agonist) show poor selectivity between α_1- and β-adrenergic receptors in brown fat cells, as judged by the inability to stimulate selectively α_1-adrenergic responses in brown fat cells [7]. The present experiments were designed to elucidate this further using direct radioligand binding techniques.

METHODS: A crude membrane fraction of brown adipose tissue was prepared from hamsters and radioligand binding assays were performed as described previously [8] with (^{3}H)CGP-121.7 and (^{3}H)prazosin to label β- and α_1-adrenergic receptors, respectively, with (-)alprenolol (1 μM) or phentolamine (10 μM) present in parallel incubations for determination of nonspecific binding. The IC_{50} values were determined by competition experiments and the K_i values were estimated according to the equation [9]: $K_i = IC_{50}/1 + (L)/K_D$, where K_D is the affinity and (L) the concentration of the radioligand.

RESULTS AND DISCUSSION: The nature of the β-receptor in brown (and white) adipose tissue is presently under discussion [10]. Thus, it is not certain that radioligands, developed to bind to traditional β-receptors, are adequate for investigations in adipose tissue. In order to examine whether radioligand binding sites in adipose tissue nevertheless fulfill accepted criteria for adrenergic receptor binding, we used the relatively new hydrophilic radioligand (^{3}H)CGP-12177 [11] in hamster BAT. Scatchard [12] analysis of equilibrium binding data (not shown) revealed a dissociation constant (K_D) of 0.7 ± 0.2 nM and a maximum binding capacity (B_{max}) of 33 ± 8 fmol/mg protein (n, 4). Thus, the receptor density identified by (^{3}H)CGP-12177 was exactly the same as that previously demonstrated in hamster crude membrane preparations with (^{3}H)DHA (dihydroalprenolol) (34 fmol/mg protein) [13]. This indicates that the radioligands (^{3}H)DHA and (^{3}H)CGP-12177 labelled the same receptor population, as has been shown in other tissues [11,14]. Agonist and antagonist competition curves for (^{3}H)CGP-12177 binding sites are shown in Fig. 1A. As can be seen, the potency of agonists and antagonists in displacing specific (^{3}H)CGP-12177 binding revealed β-specificity, with isoprenaline > norepinephrine > phenylephrine $\geq$ oxymetazoline for agonists, and propranolol >> prazosin for antagonists. The K_i values are presented in Table I. A similar K_i value for norepinephrine for (^{3}H)CGP-12177 sites in rat BAT membrane preparations was reported by Levin and Sullivan [15].
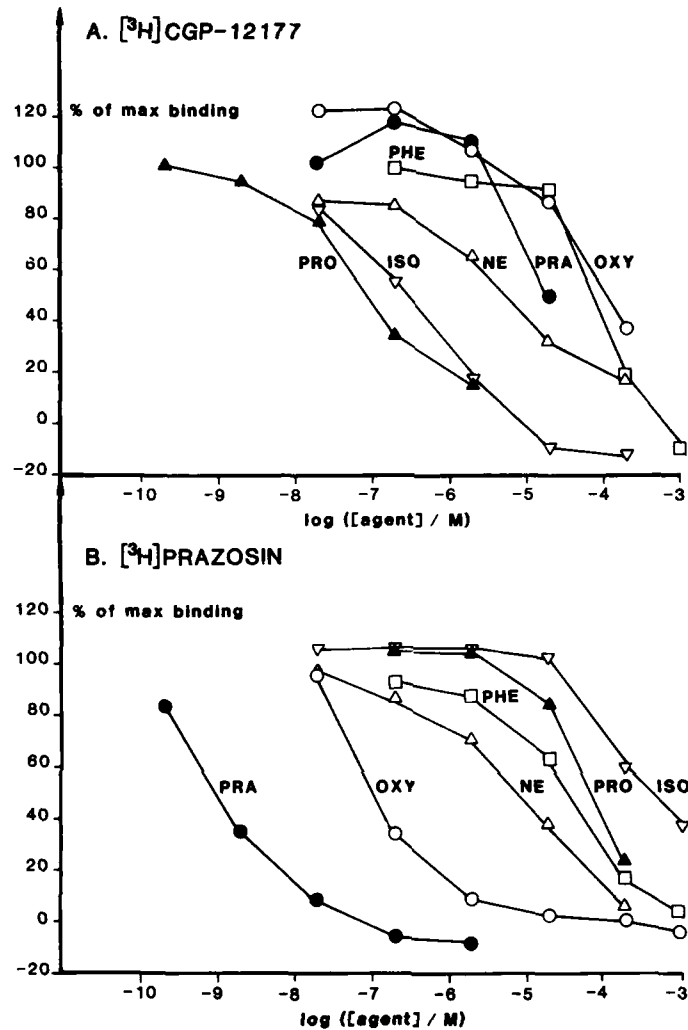
FIGURE 1: The results are the means of duplicate determinations performed on 3 different crude membrane preparations. Agonists: ISO, (-)isoprenaline; NE, (-)norepinephrine; PHE, (-)phenylephrine; OXY, oxymetazoline. Antagonists: PRO, (±)propranolol; PRA, prazosin.

(^{3}H)Prazosin binding to crude membranes of hamster BAT was a high affinity, saturable process with a K_D value of about 0.4 nM and a B_{max} of 70 fmol/mg protein (not shown), in good agreement with previous results [8,16]. The rank order of agonists and antagonists in displacing (^{3}H)prazosin binding was norepinephrine ≥ phenylephrine > isoprenaline, and prazosin >> propranolol (Fig. 1B). This is fully what would be expected of the α_1-receptor. The K_i values (Table I) were in fair agreement with a previous report [8].

One of the major problems in investigating α_1- and β-adrenergic responses in brown fat cells has been the difficulty in selectively stimulating them [3,7]. When norepinephrine or phenylephrine are used in order to stimulate α_1-responses, propranolol has always to be included in order to block the β-adrenergic processes [2,3,7]. However, propranolol shows an unexpectedly low potency in inhibiting β-adrenergic responses in intact brown fat cells [7,10].

TABLE I   Comparisons of inhibitor constants (K_i values) of adrenergic agonists and antagonists for (^{3}H)CGP-12177 and (^{3}H)prazosin binding sites

Agent	(^{3}H)CGP-12177 (nM)	(^{3}H)Prazosin (nM)	α_1/β
(-)isoprenaline	55	150 000	0.0004
(-)norepinephrine	1 200	2 100	0.57
(-)phenylephrine	14 200	8 600	1.65
oxymetazoline	20 800	25	832.
(±)propranolol	17	16 000	0.0010
prazosin	>20 000	0.2	>100 000.

The K_i values are derived from the results presented in Fig. 1.

We have therefore compared directly the affinities of different adrenergic agents for the (^{3}H)prazosin and (^{3}H)CGP-12177 binding sites and calculated the ratio between their affinities. Thus, provided that with these radioligands we are identifying the functional α_1- and β-receptors of brown fat, and from knowing which agents can be classified as antagonists and agonists, the following conclusions can be drawn: 1) Norepinephrine functions as a non-selective α_1/β-agonist in brown fat with only medium affinity; 2) isoprenaline is a high affinity, selective β-agonist; 3) phenylephrine is a non-selective low affinity agonist for α_1-adrenergic receptors. It has only 1.5-times higher affinity for the (^{3}H)prazosin sites than for the (^{3}H)CGP-12177 sites; 4) in contrast, oxymetazoline is a selective high affinity ligand for α_1-receptors with a nanomolar affinity range. Our preliminary experiments indicate that it can also be used as a potent agonist in stimulating α_1-adrenergic respiration (not shown); 5) propranolol is a selective, high affinity β-antagonist. However, in micromolar concentrations it may interfere with α_1-adrenergic receptors; 6) prazosin is a selective, high affinity α_1-antagonist. Thus, it would be the prediction from these studies that oxymetazoline would be a much preferred agent for functional studies of α_1-adrenergic effects in brown adipocytes.

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MORPHOLOGICAL CHARACTERISTICS OF EPIDIDYMAL WHITE ADIPOSE TISSUE AFTER INTERMITTENT INTENSIVE COLD STRESS IN RATS

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On exposure to cold, mammals increase their heat production to maintain normal body temperature. One organ recognized as being involved in the maintenance of body temperature is brown adipose tissue (BAT) [1-3]. During adaptation of rats to cold, the cellular morphology of their BAT changes markedly: lipid droplets diminish in size, small lipid droplets are formed in increasing numbers, mitochondrial hypertrophy leads to crowding of mitochondria, and mitochondrial cristae are remodelled into a more irregular pattern. Subsequently, the cristae become straight and more numerous [4-5]. The effect of cold on the structure of white adipose tissue (WAT) has not been examined in detail. However, Lončar et al. [6] have shown that perirenal ('white') adipose tissue in cats after intermittent intensive cold stress morphologically resembles brown adipose tissue in newborn animals.

In the current study, the effect of cold stress on the structure of epididymal adipose tissue in rats was investigated, because this tissue is considered as 'pure' WAT with a structure and function different from BAT [7-12].

METHODS: Male Sprague-Dawley rats, aged 3 weeks, were placed in individual cages. The control group remained at room temperature (22°C). The experimental group was kept at a constant cold room temperature (4°C) for 1 week. The following week the animals from that group were exposed twice a day to a temperature of -20°C, for 1 h. On day 14, all animals were anesthetised and transcardially perfused with glutaraldehyde (2%). Small pieces of epididymal adipose tissue were prepared for electron microscopic examination.

RESULTS: The ultrastructural characteristics of the epididymal WAT in control rats were as earlier described [8-12]. The cells appeared as spherical or polygonal structures. The cytoplasm of these cells characteristically surrounded the large lipid droplet as a thin, inconspicuous rim (Fig. 1). A flattened nucleus was disposed at the periphery of the cell. Within the cytoplasm mitochondria of a filamentous and ovoid shape were found (Fig. 3). The internal structure of these organelles was simple, with evenly spaced cristae embedded in a dense matrix (Fig. 3). Micropinocytotic invaginations, vesicles and glycogen granules were also evident.

Fig. 2 illustrates epididymal adipose tissue after cold stress at the same magnification as Fig. 1. A large proportion of the adipocytes now had the nucleus situated in the center of the cell, and the cells had a very small lipid content. In the cytoplasm, numerous large mitochondria (Fig. 4) were present. They were more irregular and, in contrast to those from control animals, their matrix was lighter. Most oblong mitochondria displayed a transverse orientation of the cristae, and blind ending cristae forming incomplete septa had formed (Fig. 4). Numerous small vesicles were present in the cytoplasm, and even more in cytoplasm of endothelial cells (not shown).

DISCUSSION: Ultrastructural studies of white adipocytes in animals exposed to drastic physiological changes such as fasting and diabetes have been reported by several authors [7,9-15]. Such changes involve the transformation of the large unilocular, spherical adipocyte through a reduced multilocular stage to that of a lipid depleted, highly stellate form. Metabolism of lipid in white adipocytes in these cases involves hydrolysis of triacylglycerol to fatty acids and glycerol. The products of hydrolysis leave the cell. Generally, our morphological results in cats [6] and here with cold stressed rats confirm these studies. However, in addition, we found marked changes in the structure of the mitochondria.
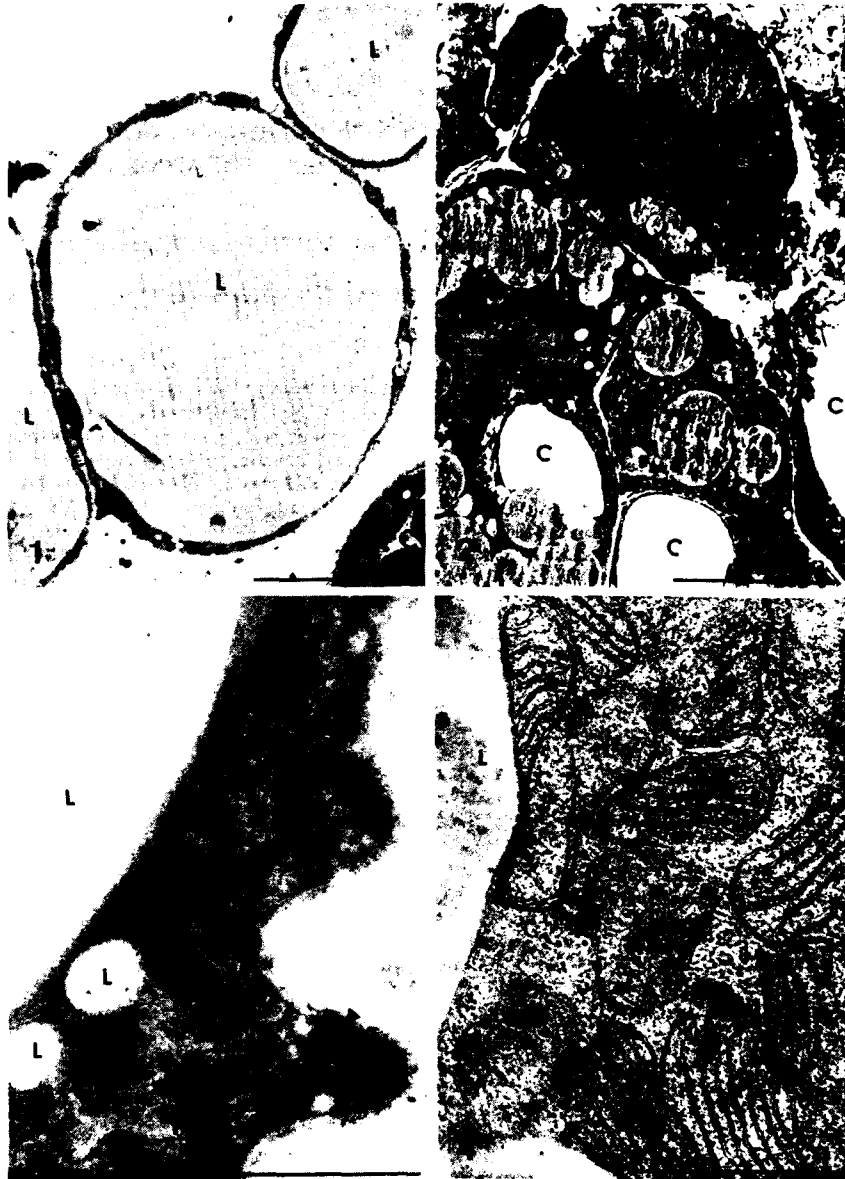
FIGURES 1-4: Epididymal adipose tissue of rats. Figs. 1 and 3 show results from the control group, and Figs. 2 and 4 from cold-stressed rats. FIGURE 1: Adipose cells from the control group have a central large lipid droplet (L), and a peripherally located thin rim of cytoplasm. Bar: 10 μm. FIGURE 2: Adipose cells from the cold-stressed rats are smaller and have an altered shape and a reduced lipid-droplet content. Between adipocytes are numerous capillaries (C). Same magnification as Fig. 1. FIGURE 3: In the control group, the thin rim of cytoplasm of the adipocytes contains mitochondria (M) and glycogen particles (G). The arrowhead points to the basal membrane on the surface of the cell. Bar: 1 μm. FIGURE 4: In the cold-stressed rats, there are big and numerous mitochondria (M) with light matrix, with or without cristae. Note that the number and orientation of the cristae resembles that found in mitochondria of stimulated brown adipose tissue. Same magnification as Fig. 3.

The appearance and structure of these organelles resembled mitochondria in BAT of cold exposed animals [4,5,16-18]. It is generally accepted that in BAT mitochondria with a very similar structure produce heat due to the presence of thermogenin (the 32 kDa uncoupling protein) [19,20]. Further investigations are required to understand the function of the mitochondria of WAT after cold stress, and to answer the very interesting question: can cold stress change typical fat storing adipose tissue (WAT) into heat producing adipose tissue (BAT)?

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DRUG ABUSE AND HEAT STROKE

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The major factors associated with the onset of stress induced heat stroke are strenuous exercise, high environmental temperatures and inadequate fluid intake. In addition to these necessary conditions there are certain contributing factors which are frequently implicated in the pathogenesis of the syndrome in otherwise healthy individuals. These are listed in Table I.

The role played by drugs in the incidence of classical heat stroke has long been recognized and the literature has been reviewed in detail by Clark & Lipton [1]. Attention has been focussed recently on the involvement of drugs in the occurrence of heat stroke in individuals exercising at high ambient temperatures and/or humidity [2] because of the rising number of cases among younger people, particularly professional athletes and military personnel. These last groups are especially prone to problems of substance abuse in many countries today.

In the management of such heat stroke victims the first priority is to lower the core temperature while supporting vital functions. For the treatment to be effective it is important that the mechanism(s) by which the drug or chemical modifies normal thermoregulatory function be identified.

TABLE I   Factors which may contribute to the onset of stress induced heat stroke in healthy individuals[1]

Acute infections
Allergic reactions
Chemical agents
Prescribed drugs
Substance abuse
Environmental contaminants
Fatigue, lack of sleep
Lack of acclimatization to the ambient conditions
Obesity
Salt and water depletion

[1]The listing is in alphabetical order, not order of importance or frequency

THERMOREGULATORY MECHANISMS: Body temperature is maintained by a control system which is typical of autonomic control systems in general. The model proposed by Hardy and his colleagues [3], which is based on engineering control theory, seems to be the most useful for explaining the effects of drugs on thermoregulation. In this system the hypothalamic temperature (T_{hyp}) is compared to an intrinsic set temperature (T_{set}); the offset between these values determines the direction and degree of thermoregulatory adjustment to be made so as to prevent any change in brain (rostral hypothalamic) temperature in the face of variations in the ambient heat load. The set temperature is adjusted appropriately

according to afferent inputs from peripheral thermosensors. The effector systems for maintaining thermal balance are vasomotor (regulating radiant heat loss by adjusting cutaneous blood flow), sweating, shivering, endocrine and behavioral changes. In man the physiological effectors have only a limited capacity to protect core temperature. Manipulation of the environment, and protection of the body with appropriate clothing, are the major mechanisms by which survival is possible under extreme climatic conditions. Thus, behavioral thermoregulation is the predominant factor in the protection of body temperature.

THERMOREGULATORY DISTURBANCES INDUCED BY DRUGS: The sites and mechanisms of action of drugs on the thermoregulatory system of animals have been studied extensively [for review see 4] but there have been relatively few detailed reports in humans. The role of drugs in the pathogenesis of classical heat stroke has received some attention [5] and the prescription drugs commonly involved have been identified.

A drug may interfere with normal thermoregulatory function by two general mechanisms: by changing T_{set} and/or disturbing the effector systems. In stress and exercise induced heat stroke a drug may have raised T_{set} and/or disrupted the heat loss functions (vasodilation, sweating, appropriate behavior) so that the system is not able to protect the core temperature in the face of the high ambient temperature and increased heat production.

A shift in T_{set} in animals can be detected relatively easily using behavioral paradigms [5], providing the drugs does not impair the ability of the animal to respond. Similar methods can be devised for human studies, usually, however, the subjective feelings are sufficient to confirm a shift, e.g., as occurs at the onset, and during deffervescence, of a fever. It would seem that changes in T_{set} have been overlooked in considering the etiology of drug related heat stroke [6].

With a few exceptions (such as malignant hyperpyrexia) the rise in body temperature induced by drugs does not endanger life at normal ambient temperatures. However, when the drug is administered to an individual undertaking strenuous exercise at high ambient temperatures the added effects of the drug may prove to be fatal.

DRUG ABUSE: Serious or fatal disorders of thermoregulation can result from the administration of a number of drugs of abuse. Many of these, especially ethanol [7] have been associated more with accidental hypothermia than with heat stroke. Amphetamines are included in the category as there are probably no supportable indications for the clinical use of this group of compounds.

Most frequently abused substances are taken in scenarios in which exertion can hardly be considered a risk factor; or else the drug may so incapacitate the individual (e.g., hyperthermia during delirium tremens [1]) that its consideration is inappropriate to the present discussion.

Amphetamines: Amphetamine and its congeners have been extensively abused both by the medical profession and by the lay public. The pharmacological activity of amphetamine is due primarily to its ability to release catecholamines from adrenergic neurons; it is this action in the central nervous system which accounts for the psychic stimulant effect. The drug increases muscle activity and heat production and causes cutaneous vasoconstriction which decreases radiant heat loss. It may also raise T_{set} due to increased adrenergic activity in the rostral hypothalamus [8].

Sellers et al. [9] have reviewed the clinical histories of 15 patients who developed heat stroke following ingestion of amphetamines. Ten of these died. Maximum core temperatures of over 40°C were recorded and the manifestations included agitation, restlessness, increased muscle tone and seizures.

Marihuana: In surroundings at a normal ambient temperature marihuana smoking does not have a significant effect on body temperature, and only slight rise occurs following intravenous injection of tetrahydrocannabinols (THC). In subjects smoking 'standard' marihuana cigarettes at high ambient temperatures (40.6°C) there was a significant rise in brain temperature (measured at the tympanic membrane) which persisted for 2 h or more [10]. During this study the subjects reported feeling "less hot", and sweating ceased, during smoking and this indicates a rise in T_{set} and the threshold for sweating. Behavioral studies in mice [11], however, indicate that Δ^9-THC lowers T_{set} and causes hypothermia in this species. These phenomena warrant further investigation since marihuana is so widely used by the population at risk and by those residing in hot climates.

Cocaine: Nasal or intravenous administration of cocaine to humans has been reported not to alter body temperature under normothermic conditions (Resnick et al., 1977, cited by Clark & Lipton [1]). Terminal hyperthermia was noted in a review of street deaths following recreational use of the drug [12]. Recently, Loghmanee & Tobak [13] have reported the death of a 20 yr old male with a rectal temperature

of 109°F (42.8°C) following an evening of cocaine and ethanol abuse. Blood ethanol on admission to hospital was 15 mg/dl and urine cocaine 9.3 μg/ml. This case is complicated, however, by the fact that the victim had a family history of susceptibility to malignant hyperpyrexia, although neither cocaine nor ethanol are recognized as triggering drugs for this syndrome. National attention in the USA has been drawn to the case of a young collegiate basketball player who died during a game in a hot humid gymnasium after having used cocaine. In this case death was ascribed to "acute cardiac failure". It should be noted that this is almost always the initial, and often the final, recorded reason for death in heat stroke victims. The correct diagnosis is commonly missed because the normal clinical thermometer registers up to only 42°C and core temperatures can reach 46°C; also, measuring oral temperature is completely useless in such cases. As was noted above heat stroke deaths have occurred after abuse of amphetamines [9], which share the neuronal reuptake blocking properties of cocaine.

Cocaine blocks neuronal reuptake of catecholamines and the euphoric effect of the drug may be a consequence of the enhanced central noradrenergic activity. Since catecholamines injected into the rostral hypothalamus can cause a rise in core temperature in the rat, which is due to a rise in the set point [14], it might be predicted that hyperthermia could readily occur after use of cocaine, especially during exercise at normal or high ambient temperatures.

In the Rhesus monkey intravenous injection of cocaine (5.0 mg/kg) caused a significant increase in heart and respiratory rates and core temperature; lower doses were without effect. Rectal temperature rose ~1.2°C within 30 min when the animals were resting at 23°C [15]. The onset of the temperature change was delayed for some 15 min after injection and the hyperthermia persisted for up to 2 h; this perhaps raises the question as to the involvement of a metabolite (e.g., ecognine methyl ester). Under similar environmental conditions (ambient temperature not specifically stated) Gonzalez & Byrd [16] found significant rises in rectal temperature in squirrel monkeys treated with cocaine (0.1-3.0 mg/kg iv).

With respect to the doses it has been estimated that cocaine abusers mainline 10-120 mg (about 0.07-0.85 mg/kg; a remarkably wide range). In man 160 mg iv (1.14 mg/kg) is usually fatal. Blood levels at necropsy from cocaine fatalities have ranged from 9-210 mg/dl with a mean of 62 mg/dl [12]. At 2 h after intramuscular injection of cocaine (15 mg) blood levels were 0.3 mg/dl and after oral ingestion 0.1-1.5 mg/dl. Clearly, poisemetric data in humans are imprecise, but it would appear that the monkey doses are in a similar range to those experienced during abuse of the drug.

Cocaine abuse by highly paid professional athletes has risen to endemic proportions in several countries, most visibly in the USA. Since many of these individuals are undertaking severe exertion, frequently in hot, humid ambient conditions, an awareness of the danger of heat stroke by attending medical staff is imperative. Additional research into this problem should certainly be carried out since effective treatment regimens could result.

Phencyclidine (PCP): Rectal temperatures exceeding 42°C have been reported in patients who were intoxicated with PCP [17] and Jan *et al.* [18] found that moderate hyperthermia was not uncommon in such cases. Patients who ingest large doses of the drug are usually agitated or hyperactive and seizures can occur. Rhabdomyolysis, with a raised serum creatine phosphokinase level, is not unusual These observations suggest that augmented thermogenesis concomitant with the muscular spasms may underlie the rise in body temperature.

Itoh and his colleagues [19] found that in mice pretreated with a histidine decarboxylase inhibitor PCP induced a significant rise in core temperature. In rats under normal ambient conditions PCP (1-20 mg/kg sc) was injected daily for 14 days. The first dose caused a dose dependent fall in body temperature. In contrast, on days 7 and 14 a dose dependent rise in core temperature followed the injections [20].

CONCLUSIONS: The primary factors leading to the onset of exertion induced heat stroke are a high ambient temperature and increased heat production from the muscular activity. Most normal, healthy individuals, in whom the thermoregulatory system is unimpaired, would not be expected to experience any serious increase in body temperature under such circumstances provided an adequate level of hydration to preserve the cardiovascular response is maintained. Many drugs may, however, interfere with these normal temperature regulatory responses by raising the set point and/or disrupting the thermoregulatory effector systems in such a manner that heat production is augmented and heat loss diminished. In the majority of cases the adverse thermoregulatory effects of drugs are side effects, divorced from their usual pharmacological actions, so that neither the physician who prescribes the drug, nor the individual who uses them would suspect their potentially serious consequences. Since the morbidity and mortality rates are so high in victims of drug related heat stroke education of the medical profession and the lay public is clearly needed if the risks are to be reduced.

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CHLORPROMAZINE/U-50,488H HYPOTHERMIA: ELECTROCARDIOGRAPHIC RESPONSES AND REPEATED DRUG ADMINISTRATION

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Previous reports from this laboratory [1-3] have demonstrated that the combination of a kappa opioid receptor agonist such as U-50,488H and a neuroleptic such as chlorpromazine produces profound hypothermia and poikilothermia in rats that is partially reversible by naloxone. The drop in body temperature (T_b) can amount to as much as 12°C at an ambient temperature of 20°C and all animals recover completely in 24-36 h. Each of the drugs, administered separately, produces a small change in T_b, but the combination results in a true potentiation (superadditive effect). Since one of the major problems associated with recovery from marked hypothermia is cardiac irritability with accompanying arrhythmias and an increased chance of ventricular fibrillation [4], it was of interest to determine whether such arrhythmias accompany recovery from this drug combination. A second question was whether tolerance develops to the hypothermic effect of the combination following repeated administration. This paper is concerned with these two issues.

METHODS: Young adult male Sprague-Dawley rats (Zivic Miller) were housed in groups of 6 to a cage in animal quarters maintained at 22 ± 2°C and approximately 50% relative humidity for at least 1 week. Lights were on from 07:00 h to 19:00 h. Food and water were available *ad libitum* except during testing.

For studies of T_b rats were kept in individual cages in an environmental room at 20 ± 0.3°C and 55 ± 5% relative humidity. After a 20 min adaptation period temperatures were taken by inserting a thermistor probe approximately 7 cm into the rectum. The rat was held lightly at the base of the tail but was otherwise allowed free movement. Temperatures were read from a digital display thermometer. The first three readings were taken at 30 min intervals, with the first disregarded to allow for adaptation, and the second and third averaged for a baseline. Immediately after the third reading rats were given dorsal injections of chlorpromazine hydrochloride (CPZ; 5.0 mg/kg, sc) and/or U-50,488H (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate hydrate; 80 mg/kg, sc). Subsequent readings were taken as indicated in the text. Drugs were dissolved in 0.9% saline on the day of testing.

For electrocardiographic (ECG) studies wire electrodes were implanted under ketamine/ acepromazine anesthesia. One multi-stranded wire was anchored through the cartilaginous zyphoid process; the other was anchored on the sternum, in the area of the manubrium, through a 5 cm abdominal incision. Wires were threaded subcutaneously and connected to a headmount secured to the rat's skull with dental cement. Rats were allowed 5-7 days of postoperative recovery prior to testing. A baseline recording was then taken for each animal. Testing began 24 h later with administration of the drugs. ECG recordings were made for 5-10 min periods, following rectal temperature readings, over a period of 13 h at intervals of 1-3 h. In some rats an additional ECG recording was made at about 24 h postinjection, when behavior and T_b returned to baseline.

RESULTS: Pre-injection ECG recordings displayed an occasional ectopic beat (not considered unusual or significant) and a heart rate of approximately 420/min. Generally, the postinjection recordings differed little from the baseline records, showing stability of heart rate and a lack of arrhythmias. This was true as the temperature dropped and reached a low of 30-32°C, as well as in the actual recovery period. There was, however, an increase in the amplitude of the recording, but no change in heart rate as T_b dropped. Amplitude returned to baseline along with T_b. The scattered ectopic beats (ventricular or nodal) did not appear to be drug related. Two of the 11 rats receiving the combination of drugs had brief episodes of bradycardia or atrio-ventricular nodal rhythms with complete recovery. These episodes were

not repeated in later recordings. There was surprisingly little variability in the heart rate during the period of the marked hypothermia and there were no trains of ectopic beats. One rat received only CPZ and one only U-50,488H, rather than the drug combination. They also showed only an occasional ectopic beat.

Administering the combination of CPZ and U-50,488H once a day for 4 days resulted in what appears to be a slightly diminished response to the hypothermic effect of the drugs, but the difference is not statistically significant (Dunnett's). Data on all 4 days at various times postinjection are shown in Table 1. As can be seen, there was little difference on successive days in T_b at 60 min postinjection, but the 120 and 180 min readings showed an increasing trend towards a diminished response. Comparison with results from animals administered CPZ or U-50,488H alone showed that there still appeared to be a potentiation of the hypothermic effect on day 4.

TABLE I   Effect on T_b of four daily administrations of U50,488H (80 mg/kg) + CPZ (5 mg/kg)[1]

	Pre-injection Control	+60 min	Post-injection ΔT_b(°C) ± S.E.M.[2] +120 min	+180 min
Day 1	37.73±0.09	-3.38±0.26	-5.53±0.44	-6.40±0.54
Day 2	37.51±0.10	-4.07±0.27	-5.62±0.34	-5.24±0.33
Day 3	38.03±0.11	-3.83±0.54	-5.18±0.79	-4.94±1.00
Day 4	38.06±0.14	-3.64±0.34	-4.98±0.49	-4.46±0.56

[1]N, 8
[2]Change from control period prior to each injection.

DISCUSS.ON: The results of the present study expand the information available about the effects of the combination of CPZ and U-50,488H on T_b. Previous reports from this laboratory [1,2] have implicated the kappa opioid receptor in the phenomenon. In the present study there appears to be some development of tolerance to the hypothermic effect after 4 daily administrations of the drug combination, but the degree is small and potentiation between the individual drugs still occurs. What tolerance does develop seems to be mainly in the duration of the response, indicating that pharmacokinetic factors may play a role. Further studies with increased numbers of animals, different doses, and longer periods of drug administration and temperature recording are needed before final conclusions can be drawn. In addition, monitoring of food and water intake, activities that may be affected by repeated periods of sedation and hypothermia, could be useful since altered ionic balances can influence the final temperature and electrocardiographic responses.

With regard to the electrocardiographic effects, it is important to note that, despite drops in T_b of up to 8°C, no significant changes were noted in any of the rats tested. In non-hibernating mammals the incidence of cardiac fibrillation begins to increase as falling T_b approaches 30°C [5]. Ventricular fibrillation is even more common during recovery from deep hypothermia, possibly because of temperature gradients set up across the myocardium upon rapid rewarming [4,6]. Because none of the animals in this study had a T_b below 30°C, additional ECG recordings need to be made in rats given higher doses of the combination to lower T_b further. Nevertheless, the lack of change in heart rate is important. One could reasonably have expected to see some arrhythmia development and more variability in heart rate. CPZ, although an antiarrhythmic, can cause arrhythmias under certain conditions [7]. Although we do not know of any reports on the subject, it is possible that the U-50,488H may have some antiarrhythmic activity. In any case, thus far it appears that the recovery from the drug combination is gradual, complete, and without cardiac complications.

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THERMOREGULATORY EFFECTS OF PROGESTERONE AND ESTRADIOL IN LONG TERM OVARIECTOMIZED RATS DURING PROESTRUS LIKE PHASES

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In female mammals the preovulatory phase is characterized by surges of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and a shift in the basal body temperature [1]. The stimulation of gonadotropin release is triggered primarily by estradiol via the central nervous system thus enhancing the release of LH releasing hormone (LHRH) from the hypothalamus [2,3]. On the other hand, the elevation of body temperature seems to be due to increased production of progesterone in the ovary [1]. It is a matter of debate as to whether the thermogenic property of progesterone results from stimulation of thermosensitive neurones in the central nervous system or indirectly from hormonally induced stimulation of running activity [3,4].

The present investigation was performed using long term ovariectomized rats that were submitted to a regimen of estradiol and progesterone injections to imitate the preovulatory phase of intact cyclic rats [5]. The study was intended to examine possible changes in body temperature and heat production threshold during proestrus like phases according to that experimental model.

METHODS: Adult female rats of the strain Han:SPRD were maintained at room temperature (ca. 21°C) on a cycle of 12 h light : 12 h darkness (lights on from 07:00 to 19:00 h; food and water were available *ad libitum*). The animals were ovariectomized under pentobarbital sodium anaesthesia at the age of 50-60 days. Unless otherwise indicated they were given consecutive s.c. injections between 11:30 and 12:30 h 4 to 8 weeks after surgery as follows: Control group, sham injection on day 6; EPSS group, E on day 0, P on day 3, S on days 5 and 6; EPES group, E on days 0 and 5, P on day 3, S on day 6; EPEP group, E on days 0 and 5, P on days 3 and 6 [E, estradiol benzoate (20 µg/0.2 ml sesame oil); P, progesterone (2.5 mg/0.25 ml benzyl benzoate-sesame oil, 1:4, v/v); S, sesame oil (0.2 or 0.25 ml, respectively)].

Blood was collected from the trunk after decapitation. Serum was prepared by centrifugation and stored frozen (-20°C) for subsequent determination of LH.

The concentrations of LH were measured using specific radioimmunoassays for rat LH [6]. Values are given in terms of the respective NIDDK-rat RP-3 preparation. Determinations were performed in triplicate.

On the day of the first experiment (8 days before 'day 0') the animals weighed 302-344 g. A thermocouple was introduced into the colon (8-10 cm from the anus), using halothane anaesthesia. After the effect of the anaesthetic had worn off, the rat was fixed to a plexiglass plate with strings bound gently around the lower end of the four extremities as well as with tape around the back. The subsequent measurements were made with the animal in a fully awake state.

The course of the experiment was as follows. The animal was placed into a metabolic chamber with wall and air temperature held at 28.5-29.5°C for more than 1.5 h, during which time body temperature and metabolic rate stabilized at their normal resting levels [7]. Cold exposure was then applied. A 20 min rewarming period of exposure to 28-29°C concluded the experiment leading to recovery from the cold induced hypothermia.

After the treatment with drugs the above described procedure was repeated on day 6. The following physiological variables were measured during the whole course of the experiments: 1. Colonic temperature; 2. Metabolic rate (MR) by measuring oxygen consumption (open system) using a paramagnetic oxygen analyser. The variables listed and the ambient temperature were measured consecutively by a digital voltmeter and sampled every 30 sec and stored on magnetic tape. Colon temperature thresholds for rise in metabolic rate were used to characterize the heat production response during acute cold exposure.

Significant differences between mean values were determined by Student's t-test.

RESULTS and DISCUSSION: After consecutive injections of estradiol and progesterone proestrus like surges of LH were induced in accordance with previously published results [5]. The increased release of LH was observed in the afternoon on day 6 evoked by estradiol injected on day 5 (Fig. 1). Animals that had received vehicle on day 5 instead of estrogen did not exhibit gonadotropin surges (data not shown). Progesterone enhanced and advanced the estradiol induced secretion of LH when given at 11:00 h on day 6 (Fig. 1) whereas no measurable changes in LH concentrations were observed in blood samples collected 5 h after injection if progesterone was administered at about 16:00 or 22:00 h on day 6.
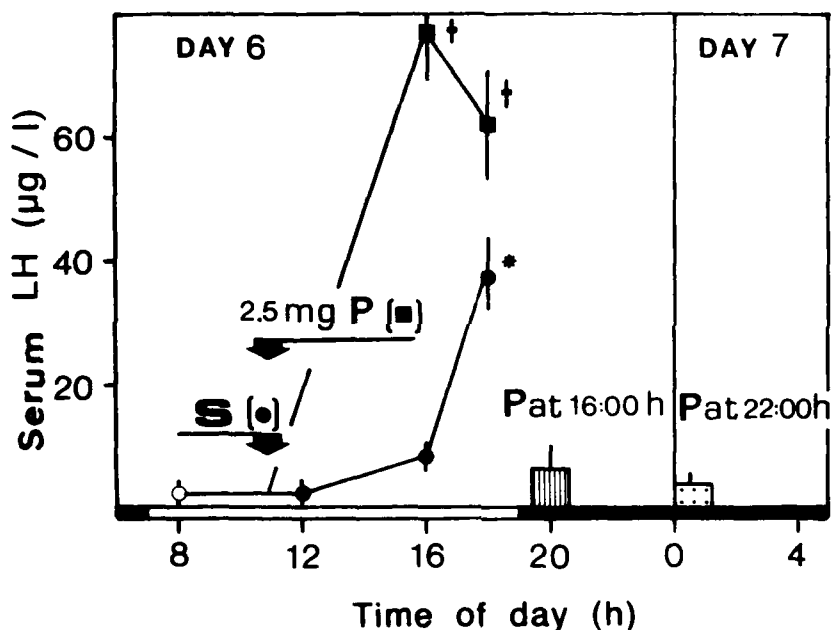
FIGURE 1: Serum concentrations of luteinizing hormone (LH) in long term (4-8 weeks) ovariectomized rats pretreated with estradiol benzoate (E, 20 μg sc) on day 0 and 5 and progesterone (P, 2.5 mg sc) on day 3. On day 6 the animals received further injections (arrows) of either sesame oil vehicle (S, closed circles) or P (closed squares) at 11:00 h. Some animals were injected with P in the afternoon between 15:30 and 17:15 h (hatched bar) or during the night between 21:00 and 22:30 h (stippled bar). Values represent the mean ± S.E. obtained from 3-8 animals.
*P < 0.01 compared with morning or noon levels on day 6,
+P < 0.05 compared with afternoon levels of E primed animals injected with S at 11:00 h.

The group EPSS of long term ovariectomized rats received estradiol benzoate (E, 20 μg sc, day 0) and progesterone (P, 2.5 mg sc, day 3). These animals exhibited heat production responses during the first cooling experiment before treatment as well as during the second cold exposure after treatment very similar to those in the control group (Fig. 2). No significant displacements of heat production threshold

between first and second cooling were seen in the control and EPSS groups (Fig. 3). The EPSS schedule of hormone treatment [8] is widely used to imitate proestrus surges of LH and FSH. The model, however, is insufficient as far as progesterone is necessary, in addition to estradiol, to trigger the gonadotropin surge, while in intact rats the surge is initiated by estradiol itself [3,5,9].
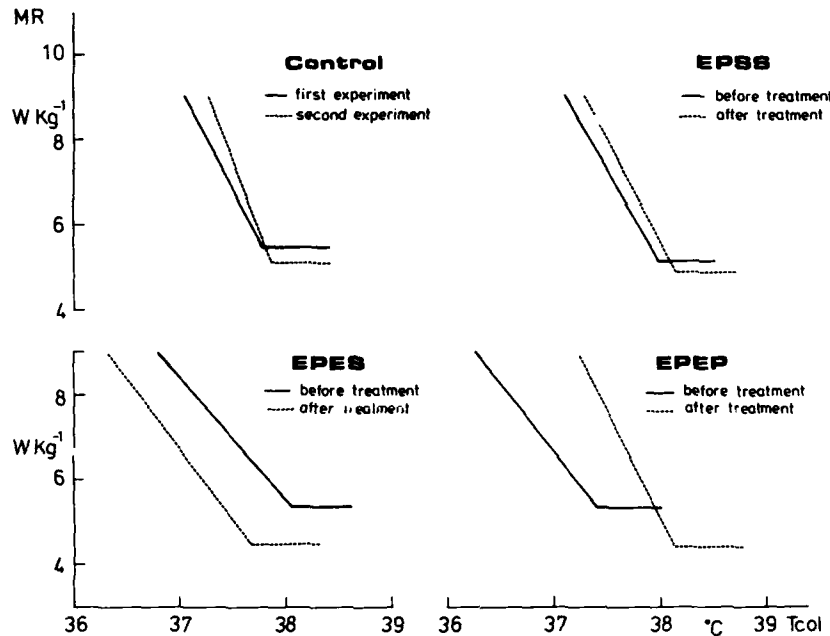
FIGURE 2: Mean values of thermogenic responses in relation to colonic temperature (T_{col}) for the different groups of ovariectomized rats acclimated to normal room temperature of ca. 21°C during an acute external cooling period before (———) and after (---) treatment with female sex hormones. Heat production shows significant threshold displacements in the groups EPES and EPEP.
(MR, Metabolic rate; for explanation of animal groups see 'Methods').

A second dose of estradiol benzoate (20 µg) given at noon on day 5 induced proestrus like surges of LH in the afternoon on day 6. In this group (EPES) the mean cold defence threshold was shifted to a lower core temperature level after treatment (Figs. 2,3). It seems that the thermal set point of these animals was slightly decreased.

Additional administration of P (2.5 mg) enhanced the stimulatory effect when given about 11:00 h on day 6. Progesterone did not affect the LH secretory system when injected in the afternoon or during the night. In this group (EPEP) the average heat production threshold was shifted to a higher colonic temperature after hormone treatment (Figs. 2,3). It appears that the thermal set point of these animals was raised.

In both the EPES and the EPEP groups the resting metabolic rate (basic rate) was reduced after the hormone treatment, whereas in the EPSS group no significant change was observed (Fig. 2). Thermoregulatory modifications during the estrus cycle have been discussed previously [10,11]. The present study demonstrates specific heat production threshold displacements in two different phases of the proestrus.
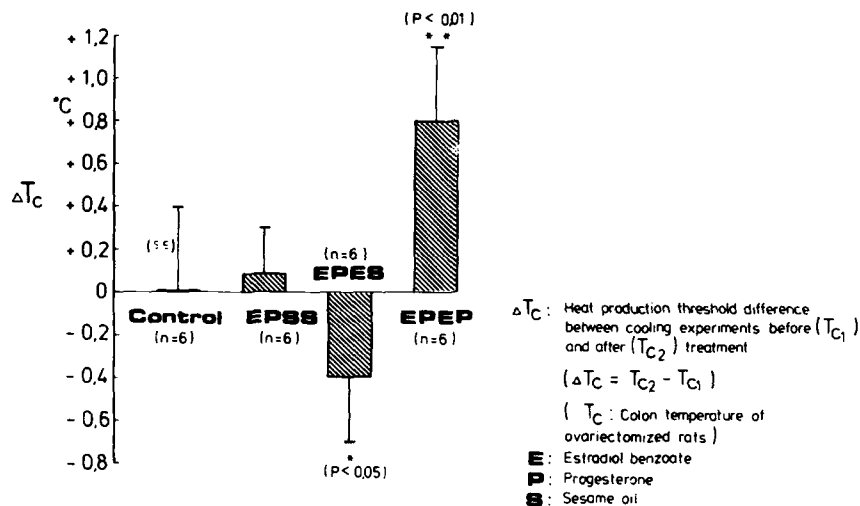
FIGURE 3: Average heat production threshold (n,6) shows a significant displacement to a lower body core temperature for the EPES group and a significant increase for the EPEP group in comparison to the control group (for explanation of different groups see 'Methods').

In summary, the long term ovariectomized rats treated with the EPEP schedule showed proestrus like phases indicated by surges of LH in the afternoon on day 6. In this animal group the heat production threshold was significantly shifted to a higher body temperature.

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SINGLE, OPTIMAL ORAL DOSAGE OF THEOPHYLLINE FOR IMPROVING COLD RESISTANCE IN MAN

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In humans the maintenance of a constant body temperature involves a balance between heat production and heat loss. In severe cold, heat loss may exceed heat production and hypothermia results. Since mechanisms for minimizing heat loss are fully activated (e.g., peripheral vasoconstriction and insulative adjustments by clothing, shelter, and posture) before heat production is fully deployed, it is reasonable to predict that the magnitude of heat production dictates the occurrence and severity of hypothermia.

It has been shown in animal studies that the timely supply of metabolizable fuels can significantly limit the full expression of maximum thermogenic capacity long before the saturation of the respiratory-cardiovascular capability for gas transport and exhaustion of the mitochondrial oxidative capacity for oxygen turnover [1,2]. It was shown in rats and in humans that by increasing the fuel supply, either by feeding prior to or during cold exposure [2,3] or by enhancing endogenous substrate mobilization with aminophylline (85% theophylline, 15% ethylenediamine) [3,4], the onset of hypothermia can be retarded or even prevented. However, previous human studies have used acute intravenous infusion of aminophylline [3] or oral pre-loading of theophylline for 2 days [5,6] prior to cold exposure. To determine whether an acute, single, oral dose of theophylline immediately prior to cold exposure may be effective in improving cold resistance, the present study was undertaken.

METHODS: Our experimental protocol has been approved by the Ethical Review Committee of the Faculty of Medicine, University of Alberta. Healthy male volunteers gave written consent prior to the study. A pilot run was conducted to familiarize the subject with the experimental protocol and to establish an appropriate ambient temperature (tailored for each individual; range: -8 to -4°C; mean, -6.7 ± 0.47°C) such that after 3 h of cold exposure the rectal temperature would drop approximately 1.5°C. Each individual was tested weekly in a random sequence, receiving one of the four treatments: placebo (corn starch); 125 mg, 250 mg or 375 mg of fast releasing theophylline ground and repacked in a gelatin capsule. In all, ten individuals were tested. Their anthropometric data were (mean ± S.E.M.; range): age (21.2 ± 0.7 years; 19-26); height (181 ± 3.1 cm; 165-198); weight (73.4 ± 3.8 kg; 63.0-92.3); body fat by hydrostatic determination (11.3 ± 1.09%; 6.22-15.30) and VO_2max (3.78 ± 0.16 l/min; 3.0-4.6).

The subjects refrained from ingesting xanthine containing beverages during the experimental period and fasted the night prior to the experiment. Prior to cold exposure, while the subject was sitting at 23°C and wearing only shorts and running shoes in the environmental chamber, a blood sample (20 ml) was taken via an indwelling venous catheter in the arm. While blindfolded, the gelatin capsule was taken with water. The temperature in the room was then lowered to the desired level (taking approximately 30 min) and the air was circulated at 0.9 km/h with fans facing the walls of the cold room. The individual's temperature was measured continuously from five different sites (rectal, chest, upper arm, upper leg and lower leg) and his electrocardiogram and metabolic rate (oxygen consumption and carbon dioxide production via an open flow system) were monitored for 50 min/h. The subject had a repetitive schedule of 20 min rest (sitting on a webbed lawn chair) with a 10 min exercise (riding an ergometer at his 50% VO_2max) for the full 3 h of cold exposure. At the end of each hour of cold exposure a blood sample was taken. A substrate mixture (ENSURE PLUS; 235 ml, 250 kcal) was drunk at the end of the first hour and a similar volume of water was given at the end of the second hour; both were warmed to the prevailing rectal temperature. These procedures consumed approximately 10 min or less. Blood was collected in glass tubes with EDTA (0.1 ml of 0.5 M EDTA/10 ml blood), centrifuged immediately at 2°C. The hematocrit was determined and the plasma stored in proper aliquots at -70°C for later analysis of plasma concentrations of substrates and hormones (to be reported elsewhere). Plasma theophylline concentration

was determined using a HPLC with UV detection [7]. Data were analysed by covariance for time dependent changes; post-hoc analysis with Student Neuman Keuls and paired T tests and linear regressions were performed.
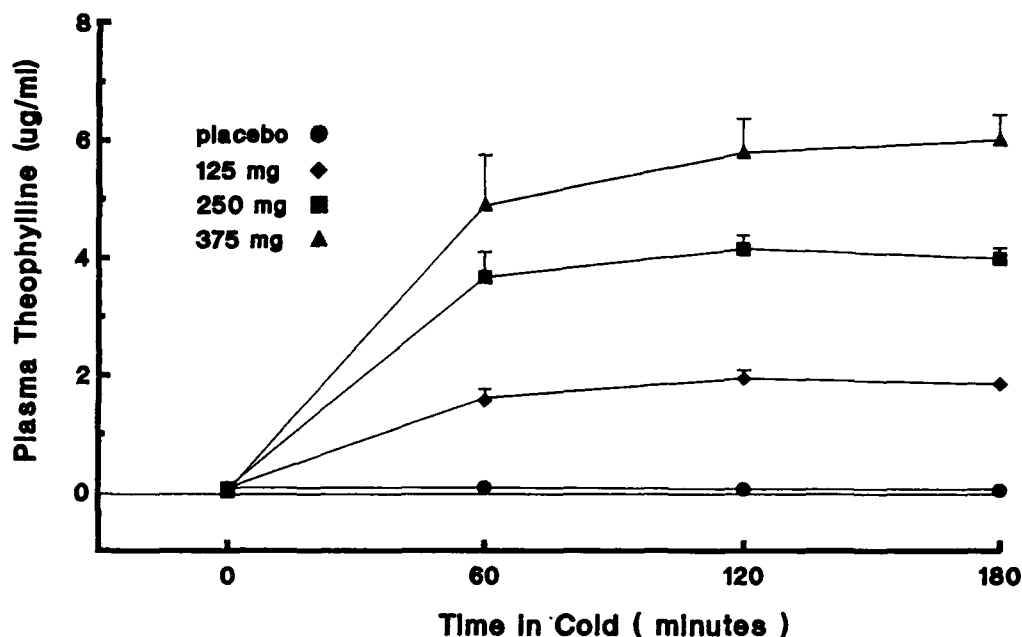
FIGURE 1: Plasma theophylline concentration (μg/ml; mean ± s.e.m.) after a single oral ingestion of Theolair® in males (n, 10) during 3 h of cold exposure.

RESULTS: Plasma theophylline levels showed a corresponding increase with dosage; the placebo treatment remained at pre-treatment levels (ca. 0.04 μg/ml - background level) and the 125 mg, 250 mg and 375 mg theophylline treatment reached maximum concentrations of 1.94, 4.15, and 6.01 μg/ml, respectively (Fig. 1). All doses of theophylline reached a plateau at the first hour and were significantly different (p < 0.05) at all sampling times. The first hour of cold exposure resulted in no significant (p > 0.05) change in rectal temperature from the initial value in any of the treatments (Fig. 2). During the second hour, rectal temperature began to decrease. Linear regression between rectal temperature and time for the second and third hour yielded slopes of -0.00776, -0.00439, -0.00433 and -0.00563 for placebo, 125 mg, 250 mg and 375 mg theophylline, respectively. The placebo slope was significantly greater than the 375 mg slope and both were significantly greater than the 125 mg and 250 mg slopes. At the end of the second hour, the rectal temperature after placebo was significantly lower than those after theophylline but no difference was found among different theophylline doses. At the end of the third hour the rectal temperature was 35.9°C ± 0.1 in the placebo treatment and was significantly (p < 0.05) lower than those of the 125 mg (36.5 ± 0.1°C) and 250 mg (36.4 ± 0.1°C) dosages of theophylline but not significantly different from that of the 375 mg dose due to a greater variance (36.4 ± 0.2°C). These dose-dependent changes are illustrated in Fig. 3. A similar trend in changes of mean body temperature (87% of rectal + 13% of the skin temperatures) was also evident (Fig. 3). The heat production during the resting periods (excluding the exercise bouts because our protocol maintained a relatively constant $\dot{V}O_2$ through variation of exercise load under all treatment conditions; (see ref 5) showed a significant increase at hours 2 and 3 as compared to hour 1. However, no difference was found among the doses in any hour. The total resting heat production for the three hours were 1223 ± 102, 1199 ± 72, 1283 ± 91 and 1252 ± 87 kJ for the respective dosages.
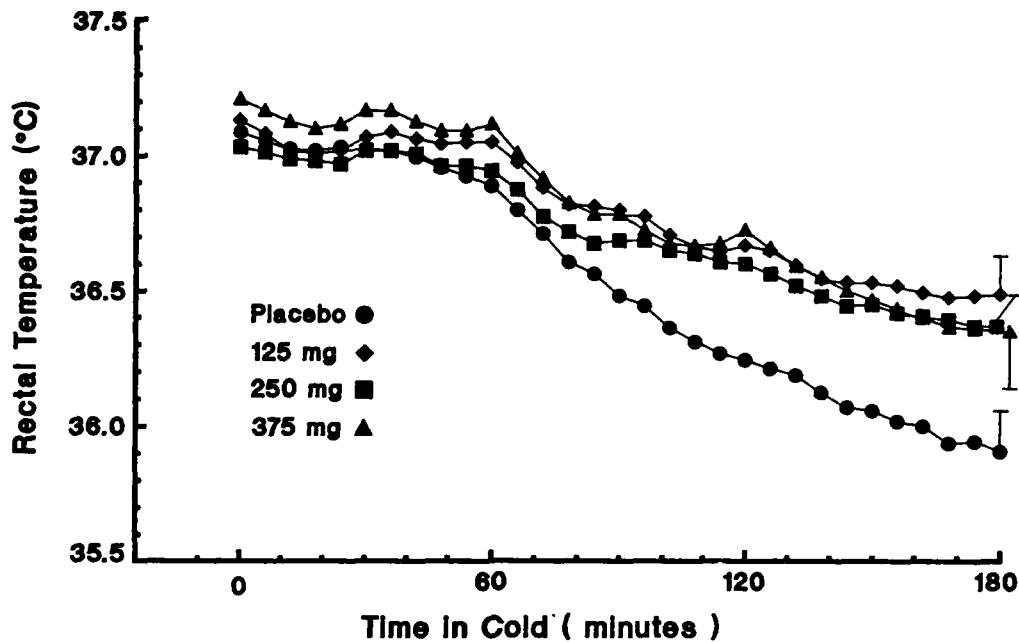
FIGURE 2: Rectal temperature (mean ± s.e.m. only at final temperature) of men (n, 10) receiving a single oral dose of theophylline during 3 h of cold exposure.

DISCUSSION: The present study has demonstrated that a single oral dose of theophylline is effective in improving cold resistance in males under acute, severe cold exposure. The time course of normally occurring hypothermia can be retarded by approximately 50% when an optimal dose of theophylline is used. That is to say, the same male has gained approximately 3 h before reaching the same degree of hypothermia if he took a single dose of theophylline (125 mg) prior to cold exposure. The optimal dose is likely to be between 125-250 mg; we have tried, in preliminary experiments, using 62.5 mg but with little or no significant improvement in cold resistance. At the optimal oral doses of theophylline, the plasma theophylline level in humans is approximately 1.8 - 4.1 μg/ml (0.01 - 0.023 mM). This concentration is significantly less than that required for the inhibition of phosphodiesterase (ca. 0.1 mM) but lies within the range for antagonism of adenosine receptors. Since adenosine is anti-lipolytic, its inhibition results in increased fatty acid mobilization [8]. Our previous studies have shown that an enhanced substrate mobilization is critical in sustaining an elevated thermogenic demand in severe cold [4,5], an increased substrate mobilization is likely to exert a beneficial effect in cold resistance. Although we have demonstrated a thermal benefit by 125 - 250 mg Theolair®, how this benefit is accomplished cannot be stated with certainty. Since the amount of net increase in total heat production to account for the change in body temperature under the present experimental protocol is very small (ca. 3.5%; see ref. 5) and total heat loss was not directly measured, we can only assume that at these doses of theophylline, a net heat gain can be realized. In view of the potentiation effect of adenosine inhibition on sympathetic activity [8], it is not unlikely that both heat conservation (e.g., peripheral vasoconstriction) and heat production (e.g., substrate mobilization, re-distribution of blood flow to muscles for shivering) could be affected by theophylline.
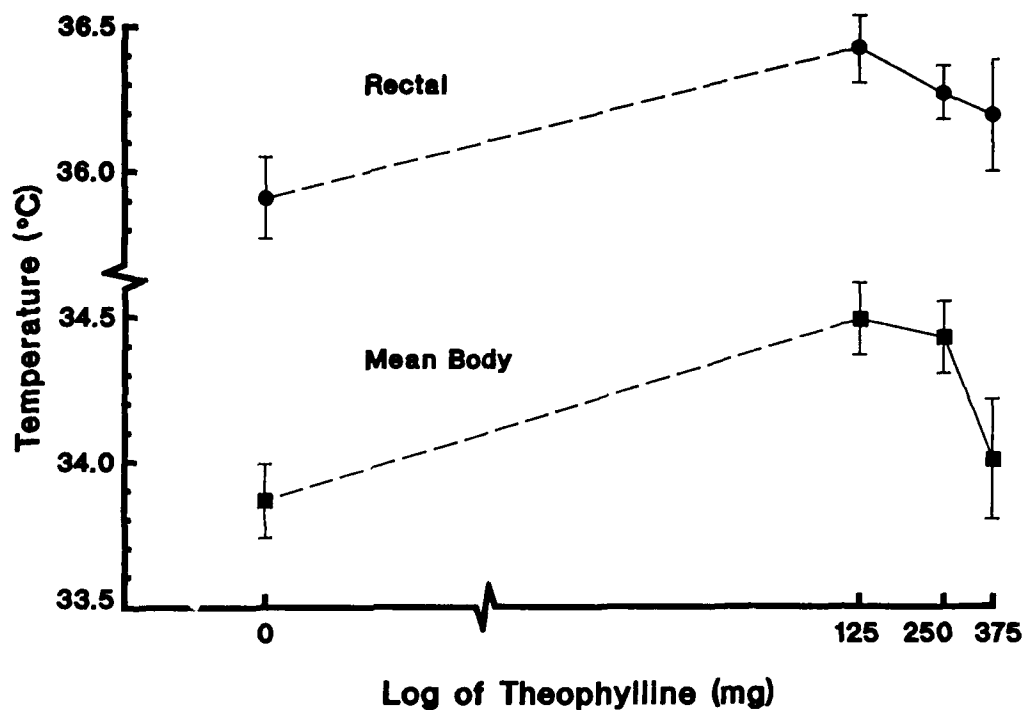
FIGURE 3: Final rectal and mean body temperature (mean ± s.e.m.) of men (n, 10) receiving a single oral dose of theophylline (expressed as log dose) and subjected to 3 h of cold exposure

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EFFECTS OF IRON DEFICIENCY AND BRAIN LESIONS ON THE BODY TEMPERATURE AND MOTOR ACTIVITY CIRCADIAN CYCLES

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Iron deficiency is the most prevalent nutritional disorder in man. Rats made nutritionally iron deficient (ID) have significantly lower brain iron (40-60%) and dopamine D_2 receptor number without significant effects on other brain neurotransmitter systems [1]. At the behavioral level, the ID rats exhibit a clear deficit in learning [2] and reversal of motor activity, body temperature [3], and pain circadian cycles.

Body temperature circadian cycles are also modified by specific brain lesions. Previous studies showed that the body temperature circadian cycle was changed in pinealectomized and hypophysectomized rats [4].

Dopaminergic neurons had been implicated in mediating the thermoregulatory system [5], and in the responses to ID [1]. Therefore, it is interesting to examine the combined effects of ID and brain lesions on the circadian cycle of body temperature. Measurements of motor activity circadian cycle were added in order to examine the relationships between the two cycles.

METHODS: Male Sprague-Dawley rats weighing 90-120 g were housed 6 per cage in a well ventilated room at an ambient temperature of 20°-22°C. Lights were on from 06:00 h to 18:00 h daily. Colonic temperature was measured as described previously [3]. Motor activity level was measured by an activity meter over 24 h. Colonic (T°) was measured every 3 h. Brain lesions (pinealectomy, hypophysectomy and area postrema lesion) were made as described [4]. There were 30 rats in each group; 15 were fed the control diet and 15 the ID diet [2].

RESULTS: Among intact ID rats, both circadian cycles were reversed (Table I). ID pinealectomized rats showed a reverse of body T°C cycle but not of the motor activity cycle, while ID hypophysectomized rats behaved in the opposite manner--their motor activity was reversed but not the body T° cycle. Area postrema lesioned rats exhibited shifted phase cycle and ID AP lesioned rats also showed a somewhat reversed shifted phase cycle. Recent preliminary studies showed that caudate nucleus lesioned rats (on the control diet) behaved like control intact rats, while nucleus accumbens lesioned rats (on the control diet) behaved like ID intact rats.

DISCUSSION: It is clear that ID has differential effects on the two circadian cycles among various lesioned groups. It is also clear that the mechanisms of the two circadian cycles are different. The findings that only NA lesioned (but not the caudate lesioned) rats exhibited reversal of the cycles, similar to ID rats indicated a role for DA in mediating ID effects. More studies are needed to clarify the neuropharmacological basis of the body T° circadian cycle changes.

TABLE I   *Effects of brain lesions on ID induced reversal of circadian cycles*

Diet	No lesion		Pinealec-tomized		Hypophysec-tomized		Area Postrema	
	Control	ID	Control	ID	Control	ID	Control	ID
Colonic T (°C)								
Peak	24:00	09:00	03:00	09:00	24:00	24:00	24:00	12:00
Trough	12:00	03:00	12:00	24:00	12:00	12:00	09:00	03:00
Motor Activity								
Peak	03:00	09:00	03:00	03:00	03:00	09:00	24:00	09:00
Trough	09:00	03:00	09:00	09:00	09:00	03:00	06:00	24:00

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ELECTROPHYSIOLOGICAL EVIDENCE FOR INVOLVEMENT OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN FEVER

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A growing body of evidence supports the view that arginine vasopressin (AVP) acts as a neuro-transmitter within the ventral septal area (VSA) as part of an endogenous antipyretic mechanism to affect a reduction in febrile hyperthermia. Infusion of AVP into the VSA attenuates pyrogen and prostaglandin induced fever in several animal species [cf. 10,11,14]. Release of AVP from nerve terminals in the VSA, measured by push-pull perfusion techniques, is inversely correlated with the change in core temperatures during fever [2]. Moreover, fever is enhanced when V_1 antagonists to vasopressin are injected into the VSA but not when injected into surrounding septal or hypothalamic sites [3].

Immunohistochemical staining techniques suggest that a major source of endogenous AVP to the VSA may be the bed nucleus of the stria terminalis (BST), an area rich in vasopressin positive neurons [16]. In support of these data are single unit recordings from VSA neurons which identify electrical connectivity between the BST and VSA which may mediate endogenous AVP release during fever [5]. However, very little is known about the electrophysiological properties of BST neurons [4] and no data are available specifically related to the activity of BST neurons during fever.

The experiments presented here use an electrophysiological approach to characterize further the connectivity between BST and VSA neurons and provide evidence that putative vasopressinergic cells in the BST are involved in the transduction of febrile information to the VSA.

METHODS: Male Sprague-Dawley rats (250-350 g) anesthetized with urethane (1.6 g/kg ip) were used. Body temperature was recorded continuously with a thermistor probe inserted into the rectum and maintained constant, with a radiant heat lamp, at 37°C. Arterial blood pressure was monitored by a polyethylene catheter inserted into a femoral artery and coupled to a pressure transducer.

A bipolar stimulating electrode (nichrome wire, tip separation <0.1 mm) was surgically implanted within the VSA using the stereotaxic coordinates of Paxinos and Watson [13] and connected to isolated stimulation units which delivered monophasic current pulses (0.2 msec duration, <1 mA) at 1 Hz. A stainless steel guide cannula was positioned over a lateral cerebral ventricle for injection of prostaglandin E_1 (120-200 ng/3µl/2 min). Extracellular recordings of neuronal activity were obtained from single units within the BST using glass micropipettes (4-10 Mohm) filled with Pontamine sky blue-sodium acetate. A variable voltage gate selected spontaneous action potentials to be displayed as rate meter records, or stimulus evoked potentials to be analyzed by computer as post-stimulus histograms. BST units were classified as being orthodromically excited or inhibited by VSA stimulation if they responded with a >50% increase or decrease in spike frequency, respectively. Antidromically activated cells responded with constant latency evoked potentials, were capable of following high frequency stimulation (200 Hz) and, in spontaneously firing cells, showed collision cancellation between spontaneous and evoked action potentials.

The location of recording sites was marked by iontophoresis of Pontamine sky blue dye at the termination of the electrode penetration. Histological verification was made of all stimulating and recording electrode sites.

RESULTS: Nearly 50% of the BST neurons examined (173/350) demonstrated electrical connectivity with the VSA. Within this group, a majority of cells (92/173) projected axons to the VSA; antidromic spike activation was recorded following VSA stimulation. The remaining fraction of units demonstrated orthodromic excitation (26/173), inhibition (26/173) or both (13/173), indicative of synaptic input from VSA neuron. (Fig. 1). None of the BST projection neurons (antidromic units) received orthodromic feedback from the VSA.
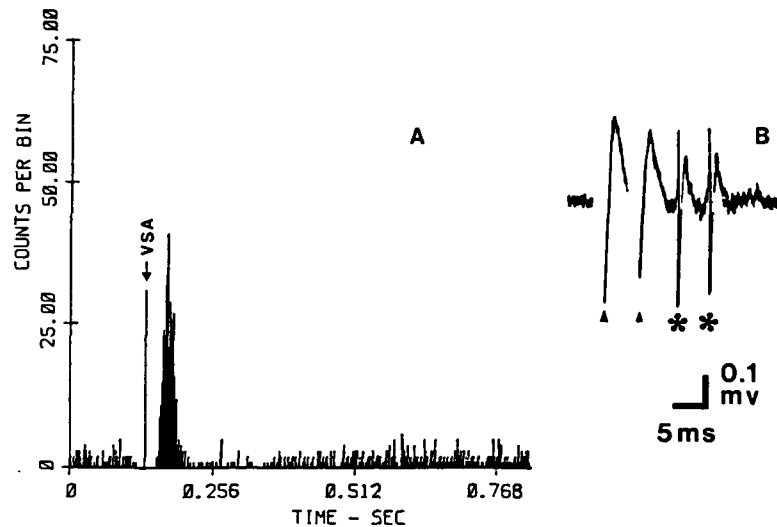
FIGURE 1: A) Post stimulus histogram of 0.8 sec duration illustrating the response of an orthodromically excited BST neuron following VSA stimulation. Arrow indicates VSA stimulus artifact. B) Extracellular voltage record of antidromic activation of a different BST neuron. The antidromic spikes (asterisks) follow 200 Hz stimulation (arrows) with a latency of approximately 6 msec.

To study the potential role of BST/VSA pathways in regulating the febrile state, the effects of PGE fever on BST sponaneous activity was measured. Baseline extracellular recordings were made from BST neurons and their connectivity with the VSA was determined. PGE was injected into a lateral cerebral ventricle and changes in the spontaneous spike firing were noted throughout the time course of the PGE fever. A total of 8 orthodromically and 10 antidromically activated cells were recorded successfully for the duration of a fever trial. The results indicated that approximately 40% of BST cells tested (7/18) responded to PGE fever with an increase in spike firing which typically occurred during the later rising phase of the temperature curve and persisted well into defervescence (Fig. 2). These responses could not be attributed to PGE induced changes in blood pressure (data not shown) and were seen in both the antidromically and orthodromically activated cell population.

One orthodromically activated neuron displayed a prominent inhibition of firing which began at the onset of the temperature rise and returned abruptly to baseline firing level at the peak of the fever curve. In the remaining 10/18 fever trials, the spontaneous activity of the neurons was unaltered by the PGE hyperthermia.

Histological verification of the location of each cell tested revealed a lack of regional topography in the BST with regards to the disribution of antidromic versus orthodromic activation from the VSA, although the distribution of cells responding to fever was limited to within the medial and intermediate parts of the BST.

DISCUSSION: Numerous studies have demonstrated the presence of vasopressin containing cell bodies within the BST [16,8]. The axons of these cells have been visualized in projections to various forebrain and brainstem regions [15,7]. Although a vasopressinergic projection from the BST specifically to the VSA has been difficult to identify with conventional neuroanatomical techniques, recent studies have recognized vasopressin positive nerve terminals in the VSA which may be derived from BST neurons [9]. The large proportion of BST neurons projecting to the VSA, as reported in the present study, confirms earlier reports that BST stimulation can evoke electrophysiological responses in VSA neurons [5] and that vasopressinergic neurons in the BST may relay inhibitory input to the VSA along this pathway [6]. Evidence that this vasopressin pathway is involved as part of an endogenous antipyretic mechanism was most convincing in a recent report by Naylor *et al.* [12] whereby electrical stimulation of the BST attenuated PGE fever in conscious rats, an effect that was blocked by localized injections of a vasopressin V_1 antagonist into the VSA. The results of these studies illustrate the potential importance for the BST in modifying the activity of the VSA under febrile conditions.
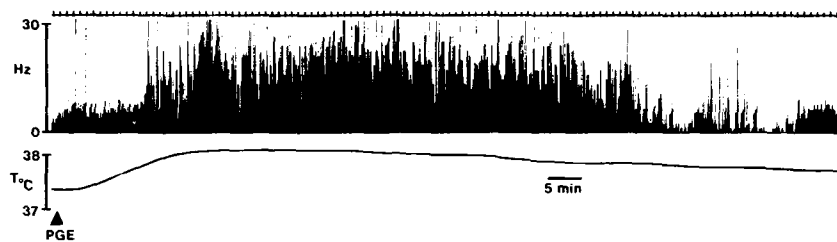
FIGURE 2: Continuous rate meter record of spontaneous spike discharge from a BST neuron during PGE fever (upper trace). Approximately 3 min following injection of PGE (200 ng) into a lateral ventricle, the animal's rectal temperature began to rise (T; lower trace) reaching a ΔT_{max} of 0.7°C. During the rising phase of the fever, this cell responded with an increase in spike firing which persisted throughout the plateau and initial decline in body temperature.

In the present study, single unit recordings were obtained from putative vasopressinergic neurons, as identified by antidromic activation from the VSA. However, the BST is known to project to widely diverse target areas [15] and hence, may contain a heterogeneous population of cells; only a fraction of the population may be vasopressinergic and therefore involved in the transduction of febrile information to the VSA. This idea is consistent with the present observation that a majority of antidromic units tested was unaffected by PGE fever. Furthermore, the observation that BST neurons which receive reciprocal input from the VSA are also responsive to fever raises questions as to the complexity of the circuitry within the BST/VSA pathway. Precisely how the vasopressin containing neurons fit into this circuit remains to be elucidated.

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ANTIPYRETIC ACTION OF VASOPRESSIN IN THE VENTRAL SEPTAL AREA OF THE GUINEA PIG'S BRAIN

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Evidence supporting the existence of endogenous peptidergic antipyretic systems in the brain has been accumulated in recent studies (cf. 1-6). It seems that the elevation of body temperature during fever is regulated, and sometimes even prevented, by an activation of peptidergic neuronal projections to the septum. In experiments in guinea pigs, such permanent activation could be demonstrated im-munocytochemically in central vasopressinergic pathways ascending to the septal area and to the amygdala for the pregnant animals at term and neonates several hours after birth. Both exhibited reduced febrile responses to the administration of bacterial pyrogen [7,8]. A similar, but short term, activation of these pathways is found in nonpregnant animals during the stage of fever rise [9] and during the reduction of the febrile response due to development of tolerance [10].

Here the influence of intraseptal application of exogenous arginine vasopressin (AVP) on febrile responses of guinea pigs to bacterial pyrogen has been studied.

METHODS: The experiments were performed in 6 guinea pigs (4 males, 2 females, body weight at the beginning of the study 320-365 g) The febrile response to intramuscular injection of bacterial endotoxin (E. coli, 20 μg/kg) has been compared several times in each animal, at 1 week intervals. The control test was done before the implantation of cannulae into the septum. The substances, or the solvent alone (sterile pyrogen free 0.9% sodium chloride solution) were applied bilaterally to conscious, freely moving animals by means of continuous microinfusion at 0.1 μl/min, which corresponds to the flow of in-terstitial fluid in the brain [11]. At the beginning of the experiment the infusion cannulae were lowered into the septum through guide cannulae permanently implanted according to a stereotaxic atlas for the guinea pig [12]. The infusion cannulae (length 8 mm) were connected with an infusion pump by means of polyethylene tubing (PE 10), filled with an infusing solution, and introduced into the septum. During the 8 h experiment colon temperature was measured every 30 min with a thin plastic coated thermocouple in-serted 6 cm beyond the anus, with the animal gently restrained. The animals rapidly became accustomed to the procedure which then did not cause excitement or any apparent change in their temperatures. AVP, its agonists, or solvent were microinfused bilaterally into the septum for 6 h, starting 1 h before the administration of bacterial pyrogen. The animals were quiet during the experiment and tolerated the infu-sions well. Regularly, only one experiment a week was performed in each animal, but the experiments could be repeated up to 8 times without any loss of response. Each animal thus served as its own control. At the end of the experiments the animals were anaesthetized with pentobarbital (40 mg/kg) and killed by perfusing their brains with saline and formaline for histological localisation of the infusion sites.

The fever responses were characterized as temperature-time curves for 6 h after pyrogen injection, and fever indices were expressed in °C.h (for 6 h).

RESULTS: Intraseptal bilateral microinfusion with AVP (9 μg/6 h) reduced the febrile response to bacterial endotoxin in all experimental animals. In the upper part of Fig. 1, the mean response to endotoxin during intraseptal AVP infusion (dots) is compared with the response of the animals to endotoxin during similar infusion of a solvent (circles). AVP infusion into the septal site prevented the febrile response since the small increase in temperature during the course of infusion (17% if we set the response to the solvent as 100%) can be seen when AVP is infused alone (lower part of Fig. 1). This reac-tion demonstrates that AVP does not reduce colon temperature in the normothermic state.
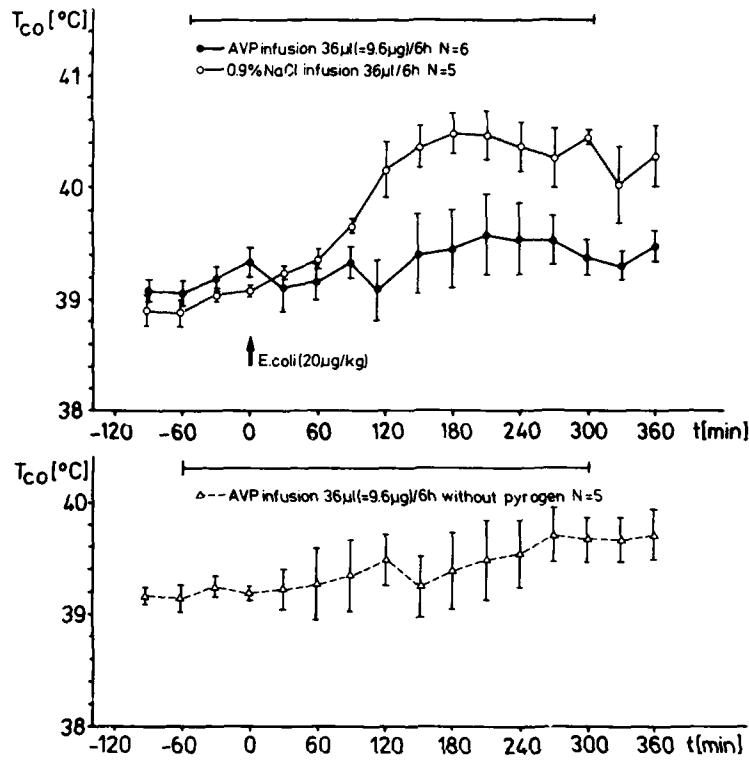
FIGURE 1: Comparison of mean febrile responses in 6 guinea pigs to bacterial pyrogen during bilateral microinfusion of the brain septum with AVP (9 μg/6 h) and with the solvent (saline). Below, the effect of the intraseptal AVP infusion on the colon temperature without pyrogen is shown. Bars indicate SEMs.

The febrile response could also be reduced, but to a lesser degree than with AVP, by intraseptal microinfusion of the AVP agonists acting at peripheral V_1 and V_2 receptor subtypes: AVP-V_1 agonist (Phe[2], Orn[8] VT-14.4 μg/6 h) reduced the febrile response by 42% and the AVP-V_2 agonist (dVDAVP-14.4 μg/6 h) by 58%, in comparison to febrile responses during infusion with the solvent (set as 100%). All febrile responses are compared in the upper part of Fig. 2.

All of the injection sites were distributed within the ventral septal area (lower part of Fig. 2).
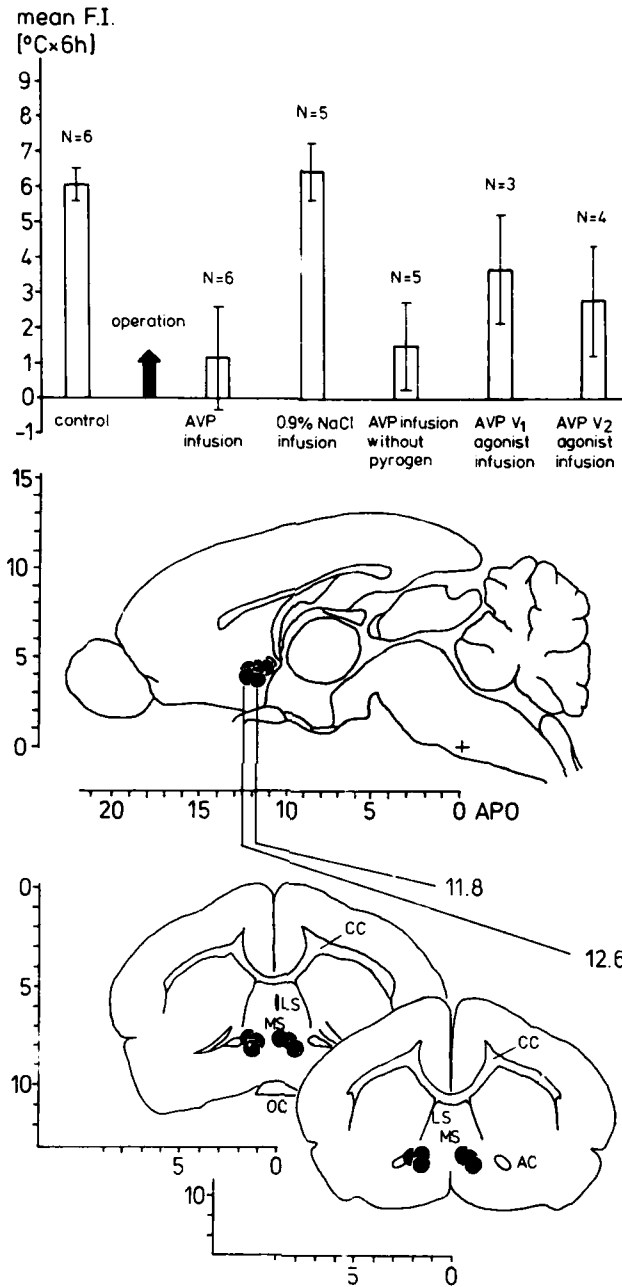
FIGURE 2: Above, the mean fever indices (columns) as a measure of responses to bacterial pyrogen (E. coli, 20 µg/kg) in guinea pigs without or with microinfusion of AVP, the solvent (saline), AVP without pyrogen, AVP-V_1 and AVP-V_2 agonists into the septum of the brain, respectively. The columns are in the time order of experiments made at 1 week ir.·ervals. Below, the sites of the tips of infusion cannulae in the 6 animals indicating their localisation within the ventral septum.

DISCUSSION: The suppression of the febrile response to bacterial pyrogen by infusion of AVP, or its a$_g$onists, into the septum has been described in a number of species [5,6] and, in the last year, in the guinea pig [13]. New in our study is the finding that the peripheral AVP-V$_2$ agonist is able to suppress fever when it is infused into the septal area. The technique of AVP application we have used is different. In comparison, the push-pull perfusion technique, due to its high speed, raises the possibility of destruction of brain tissue. The microinfusion technique that we used is slower and the dose infused is lower. Thus, it may be calculated that the dose applied per hour in our experiments was 8 times less than that used by Naylor [13]. In another experimental series we have found that intraseptal infusion of AVP (0.13 μg/6 h) still suppressed the febrile response to bacterial pyrogen. This dose is less than the amount of AVP found naturally in the pituitary tissue of the guinea pig (about 6.2 μg/pit), but higher than the levels of AVP found in the cerebrospinal fluid (16.3 pg/ml), which again seem to be about 5 times higher than the levels of AVP found in the blood [14]. A 5-10 times higher concentration of AVP in cerebrospinal fluid than in blood plasma has been described also in the dog [15].

In conclusion, these results support the concept that AVP may function within the septum as an endogenous antipyretic. The central AVP receptors have not been characterized as yet, but seem to be activated by both peripheral V$_1$ and V$_2$ agonists.

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PYROGENIC, INFLAMMATORY, AND SOMNOGENIC RESPONSES TO CYTOKINES: DIFFERENTIAL MODES OF ACTION

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It is generally accepted that fever results from the action of an endogenous mediator, interleukin-1 (IL1) [1] produced by activated macrophages and other cell types, which ultimately elicits some of its biological actions through the preoptic anterior hypothalamus (POA) [2]. The prevalent view is that IL1 initiates fever by stimulating prostaglandin E_2 (PGE_2) synthesis in the POA [3]. In addition to causing fever, systemically or intracerebrally administered IL1 induces hepatic acute-phase protein synthesis [4] and promotes slow wave sleep (SWS) [5]. Interferon α_2 ($IFN\alpha_2$) and tumor necrosis factor α ($TNF\alpha$, cachectin) are pyrogenic [6,7] and somnogenic [8,9]. Both evoke prompt thermal rises following intravenous or intracerebroventricular (icv) injection into rabbits, cats, and mice and enhance SWS in rabbits. Both stimulate PGE_2 synthesis in rabbit hypothalamic homogenates [6,7]. These results suggest that these substances may be endogenous pyrogens and somnogens, with many of the same host defense activities as IL1.

This study was undertaken to assess whether $IFN\alpha_2$ and $TNF\alpha$ indeed possess the same properties as IL1; in particular, whether they can induce fever and acute-phase responses when microinjected directly into the POA. Recombinant (r) IL1α and β and rIL2, a T cell-derived IL1-induced lymphokine, were also tested. In addition, the effects of these cytokines on the firing rates (FR) of temperature sensitive neurons in slices of the POA were evaluated.

METHODS: To assess pyrogenic and acute-phase effects, conscious, male, Hartley guinea pigs (300-350 g) with guide cannulae implanted into the POA, were injected (1 μl, bilaterally) with rIL1β, rIL2, rIFNα_2, or rTNFα. To assess somnogenicity, rIL1α and β were injected icv into male adult New Zealand White rabbits. The cytokines were diluted immediately before use in artificial cerebrospinal fluid (ACSF), control animals were injected with ACSF. The limulus amebocyte lysate test was performed and was negative. Core temperature (T_c) was measured with a thermistor probe inserted into the colon. Blood was collected by cardiac puncture 24 h after injection to determine plasma Cu level (atomic absorption spectrophotometry), as an indicator of the acute-phase glycoprotein ceruloplasmin. States of vigilance were determined by visual scoring of EEG and motor activity, as described previously [5]. The ambient temperature (T_a) was 25 ± 1°C. The positions of the cannulae tips were confirmed histologically at the conclusion of the experiments.

Male, Hartley guinea pigs (300-350 g) were decapitated and the brains quickly removed. Coronal slices, 350 μm thick, containing the POA were cut from each brain. A slice was selected and transferred to a temperature controlled recording chamber perfused with carboxygenated (95% O_2, 5% CO_2) guinea pig ACSF at a flow rate of 1.0 ml/min. Extracellular single unit activities were recorded from the medial POA with a glass microelectrode, and thermal responsiveness was determined by changing the slice temperature between 32 and 42°C, as described previously [12]. The responses of these units to rIL1β, rIFNα_2, rTNFα or their vehicles were then measured at chamber temperature 37°C.

RESULTS: Both rIFNα_2 (5×10^2 - 5×10^5 U/μl) and rTNFα (150-500 ng/μl) caused dose-dependent rises T_c when injected into the POA of conscious guinea pigs (n, 6-7/dose). However, the courses of these responses were distinct from those of rIL1β. Whereas rIL1β (10-100 ng/μl; n, 9-11/dose) elicited rapid onset, unimodal fevers of relatively short durations, the fevers after rIFNα_2 were more delayed in onset and longer in duration, and those after rTNFα were bimodal (Fig. 1). rIL1β also provoked a 67% elevation of plasma Cu levels 24 h after injection, while neither rIFNα_2 nor rTNFα did so. rIL2 had no effect on T_c or plasma Cu levels when administered into the POA.

TIME, h
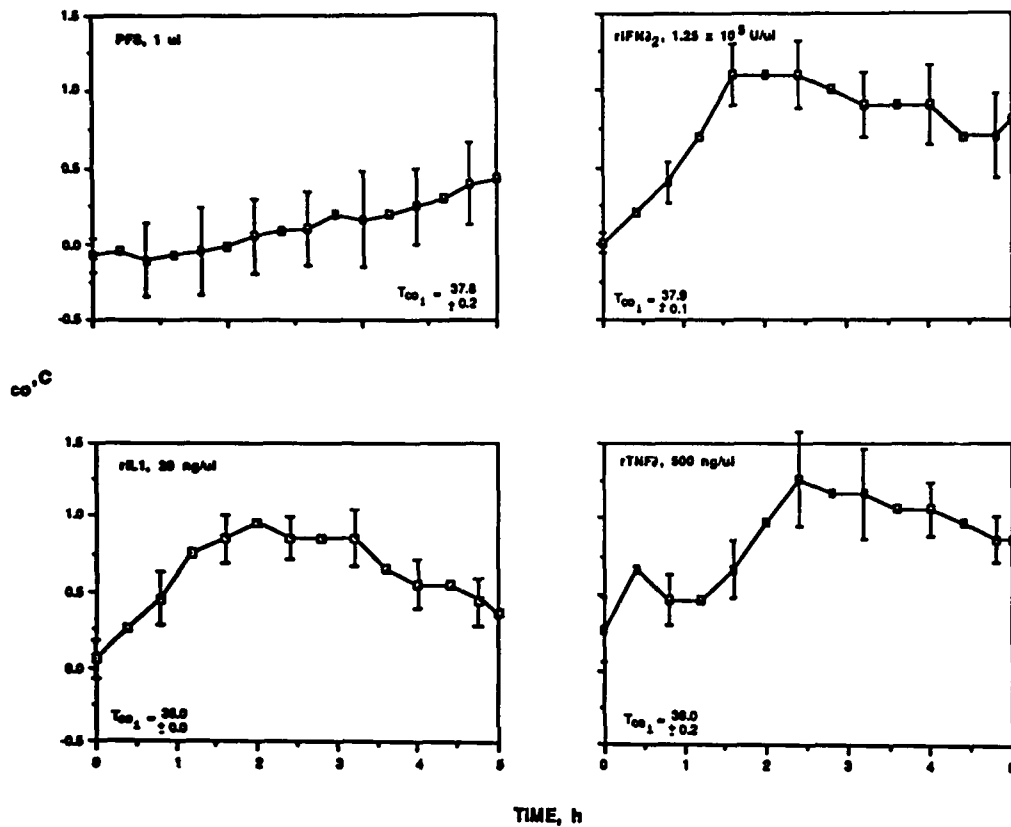
FIGURE 1: Changes in the colonic temperatures (ΔT_c) of guinea pigs produced by the intraPOA injection (1 μl bilaterally at time 0) of ACSF (PFS) (n, 23), rIL1β (n, 11), rIFNα_2 (n, 7), and rTNFα (n, 7). A 90 min stabilization preceded these treatments. Doses are indicated in each graph. T_{ci}, initial T_c (mean ± SEM of 5 consecutive measurements at 6 min intervals during the last 30 min of the stabilization period).

While rIL1α and β induced similar pyrogenic responses on icv ?   .ation into seven rabbits, their somnogenic effects were different (Fig. 2). Fever (not illustr   oited a slow onset (ca. 1 h) and protracted (6 h) course. rIL1β (20-40 ng) characteristically enhanced ŝWS during the first hour after injection, while concurrently depressing REM sleep. These effects waned during the fourth postinjection hour and were not different from control (ACSF) after the fifth hour. By contrast, the onset latency of excess SWS after rIL1α (10-1000 U) was ca. 2 h, and enhanced sleep continued through the 6 h observation period. These patterns are different from those evoked by either rIFNα [8] or rTNFα [9].

Of 48 POA single units recorded, 33 were warm sensitive (W), seven were cold sensitive (C), and eight were thermally insensitive (I). rIL1β, rIFNα_2, and rTNFα, respectively, decreased the FR of 9/11, 10/17 nd 2/5 W, augmented the FR of 1/2, 3/4, and 1/1 C, and variously affected I neurons; i.e., these cytokines generally depressed W and excited C units. However, analysis of the activities of individual neurons that were held long enough to be treated with two or more cytokines (Table I) revealed considerable differences in their responses to these substances. Only 4 of 14 W neurons that were exposed to two cytokines responded in the expected manner to these factors, i.e., their FR were reduced; the remaining 12 W neurons were affected seemingly unpredictably. No W units responded identically to three

cytokines. C and I neurons also were affected differentially, although the sample size was too small to analyze. No relationships were evident in this study between the doses of the cytokines given and the directions, latencies and durations of the responses elicited.
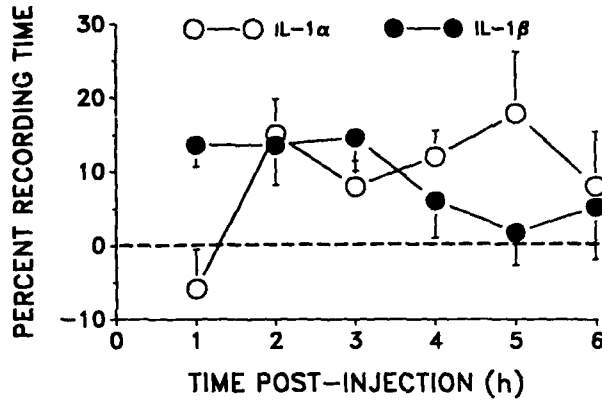
FIGURE 2: Effects on SWS of icv injections (25 μl) of rIL1α (1000 U; n, 7) and rIL1β (20 ng; n, 8) in rabbits. Results are expressed as the differences (means $\pm$ SEM) between these effects and those of ACSF (n, 5) over the same time course.

TABLE I: Changes in firing rates (FR) of individual thermosensitive POA single units exposed (numbers) to two or three cytokines (Cyto) *in vitro*

FR	Warm - sensitive										Cold - sensitive		
Cyto	↓↓	↑↑	↓↑	↓→	→↓	↑→	→→	↓→↓	→↓→	↓↑→	↑↑	→↑	↑↑↑
rIL1β rIFNα_2	2		2	1	1						1	1	
rIL1β rTNFα	2	1		1			1				1		
rIFNα_2 rTNFα				1	1	1							
rIL1β rIFNα_2 rTNFα								1	1	1			1

Order of the arrows from left to right corresponds to order of administration of the cytokines from top to bottom.

DISCUSSION: It is now recognized that many host defense reactions against infectious pathogens are modulated by the CNS and that cytokines play an important intermediary role in the neuromodulation of these responses. Cytokines display some common functional activities in this regard; e.g., they induce fever and enhance SWS. The POA is an important integrative and controlling region for several of their effects. As reported here, both rIFNα_2 and rTNFα caused dose-dependent rises in T_c. However, the courses of these responses were distinct from each other and from those of rIL1β; viz., the fevers after rIFNα_2 generally were protracted, and those after rTNF were bimodal, as compared with those after rIL1β. Moreover, only rIL1β induced hypercupremia from this region. Thus, although they share a common site of action, viz., the POA, the courses and kinds of responses elicited by these cytokines differ. Corresponding differences were noted earlier in the patterns of SWS evoked by icv injections of rIL1β [5], rIFNα_2 [8], and rTNFα [9]. The present results extend those findings further by showing that a distinctive course is also induced by rIL1α.

Previous studies [4,5] showed that the diverse effects mediated by cytokines are separable; e.g., the febrile, acute-phase protein, and somnogenic responses to IL1 can be evoked or suppressed selectively. Moreover, POA thermosensitive neurons and PGE$_2$ are implicated in fever production, but have no part in the induction of the acute-phase protein response [4,10]. This suggests that the cytokines utilize different neuronal substrates and/or neuromodulators to exert their various effects. This possibility is supported by the present findings that, whereas rIFNα_2 and rTNFα generally depressed W and facilitated C neurons, fully two-thirds of the neurons tested responded individually to either of these cytokines in directions different than they did to rIL1β. In this context, it was reported recently [11] that the rIFNα_2-induced depression fo the FR of W units in rat POA slices was blocked by naloxone, while that caused by IL1β was not.

Taken together, these results suggest that rIL1α, rIL1β, rIFNα_2 and rTNFα may stimulate distinct, but partially overlapping sets of neurons, each with characteristic responsiveness and, presumptively, each mediating discrete effector functions. This could explain the coordinated, multiple biological effects elicited by these cytokines, as well as their separability under certain conditions. It is conceivable that different neuronal sets composed of units with various combinations of co-existing sensitivities (e.g., to temperature, osmotic pressure, reproductive steroids, glucose [12-14]) are responsive to one or more of these cytokines and mediate these varied responses. This could explain the observation that not all thermosensitive neurons are also responsive to all the cytokines.

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ANTIPYRETIC MECHANISM OF ACTION OF DEXAMETHASONE

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The acute phase of the immune response to infection involves a variety of host reactions of which fever is the most prominent. In the pathogenesis of fever in response to exogenous pyrogens such as bacterial lipopolysaccharide (LPS) or the interferon inducer polyinosinic: polycytidylic acid (Poly I:C) leucocytes play an important role in releasing cytokines such as interleukin 1 (endogenous pyrogen, IL1/E.P.) which is thought to be the endogenous mediator of fever and inflammation [1]. IL1/E.P. stimulates the production of prostaglandins which are responsible for the symptomatic manifestations of fever and inflammation, i.e., elevated body temperature, pain and oedema [1]. Fever is thought to occur as a result of the actions of PGE_2 on the pre-optic anterior hypothalamic nuclei [see 2 & 3 for review). Thus, it would appear that agents which affect prostanoid production should interfere with the onset of fever. It is well known that non-steroidal anti-inflammatory aspirin like drugs which are potent inhibitors of prostanoid formation are antipyretic when administered systemically [2,3]. We have shown recently that pretreatment of rabbits with dexamethasone (DEX) is antipyretic toward LPS, Poly I:C and IL1/E.P. [4] and that these three agents are capable of producing increases in blood PGE_2 levels which occur simultaneously with the increases in body temperature [5]. Accordingly, in the present study we have investigated the effects of DEX on the level of PGE_2 in the blood and cerebrospinal fluid (csf) and the release of PGE_2 from peripheral blood monocytes *in vitro*.

METHODS: Male Dutch rabbits weighing 2.0-2.3 kg were lightly restrained in conventional stocks during experiments performed at an ambient temperature of 22-24°C. Rectal temperatures were measured using thermistor probes (inserted to a depth of 9 cm) connected to a chart recorder. All drugs were administered via the marginal ear vein and the amount of agent in each dose was adjusted to give an injection volume of 0.4-0.75 ml.

Blood samples (1 ml) were taken from the marginal ear vein at appropriate intervals and collected into 150 μl ketoprofen/EDTA (1 μg/ml and 4.5 mM respectively) and centrifuged. PGE_2 in the resultant plasma was estimated by radioimmunoassay. Recovery of PGE_2 was determined by the addition of [^{3}H]-PGE_2 to the plasma and was estimated to be greater than 90%. None of the agents used in the study was shown to affect the standard curve for PGE_2.

Measurements of csf PGE_2 levels were carried out in rabbits which had stainless-steel Collison-type guide cannulae (21 gauge), implanted stereotaxically into the third cerebral ventricle during pentobarbitone (30 mg/kg) and ketamine (20 mg/kg) anaesthesia. Rabbits were allowed to recover for at least 2 weeks after surgery before being used in any experiments. During experiments the third ventricle was perfused (40 μl/min for 7 min) at various intervals with sterile artificial csf by push-pull perfusion using double lumen "micro" catheter tubing (150 μm internal diameter/lumen) as described by Myers & Gurley-Orkin [6].

Monocytes (adherent cells) were prepared from whole rabbit blood on percoll density gradients [7] followed by adherence to plastic culture dishes over 2 h at 37°C, 5% CO_2, 100% humidity. Cells were then incubated in RPMI 1640 culture medium with the various agents at 37°C, 5% CO_2 100% humidity.

RESULTS: Pretreatment for 1 h with DEX (3 mg/kg iv) attenuated the increases in both body temperature and blood PGE_2 levels in response to LPS (0.2 μg/kg) and IL1/E.P. (Fig. 1). The effect of DEX on the Poly I:C (5 μg/kg) stimulated increases in temperature and blood PGE_2 was similar to the attenuation of the LPS stimulated increases.
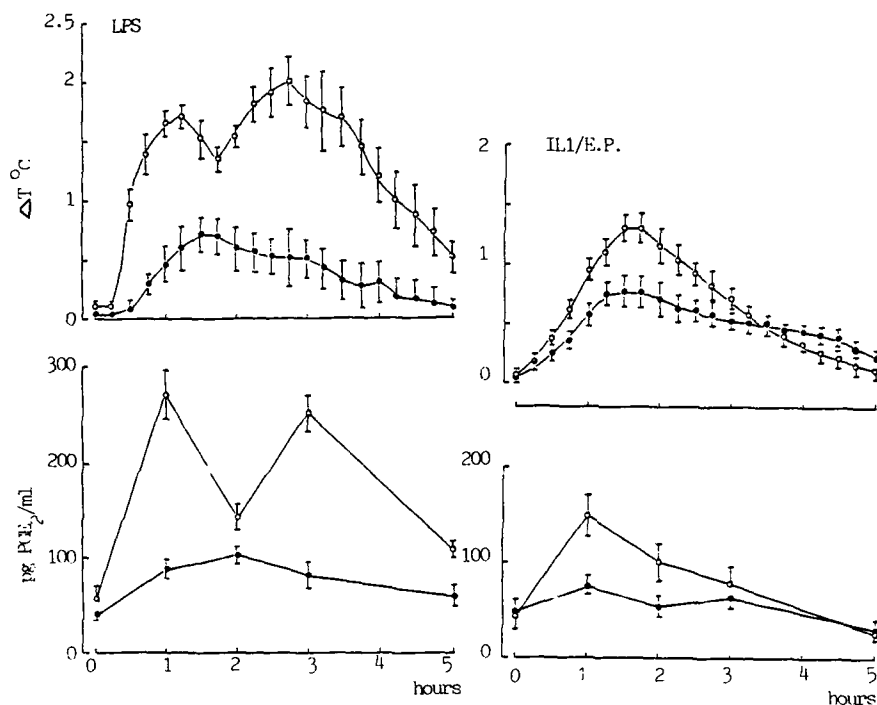
FIGURE 1: Effect of DEX on pyrogen induced fever and elevated blood PGE$_2$ levels. DEX (3 mg/kg iv, filled circles) or saline (open circles) was given 1 h prior to either LPS (0.2 μg/kg iv) or IL1/E.P. (75 units iv, see 4) both of which were given at time zero. Values represent the mean ± S.D. from 4 animals.

The effect of DEX was also assessed on the Poly I:C stimulated increase in PGE$_2$ collected by push-pull perfusion of the third cerebral ventricle (IIIcv) during fever. Samples of perfusate were taken; immediately before administration of DEX (3 mg/kg iv) or sterile saline, 1 h later, being immediately prior to administration of Poly I:C (5 μg/kg iv), and at hourly intervals thereafter. DEX attenuated the Poly I:C stimulated increase in the level of PGE$_2$ collected from the IIIcv (Table I). The results shown are from a single rabbit but are typical of results from 4 different rabbits. The data were not pooled as the PGE$_2$ level at the first peak of the Poly I:C fever (2 h from injection) and the second peak (after 4 h) varied between 204-523 pg/ml perfusate and 300-813 pg/ml respectively. However, increases in the PGE$_2$ level were never observed in the absence of Poly I:C and DEX always attenuated the increase in the presence of Poly I:C. The PGE$_2$ level in perfusates collected either before any treatment or before Poly I:C varied between 48 and 97 pg/ml.

DEX decreased the LPS, Poly I:C and IL1/E.P. stimulated release of PGE$_2$ from monocytes (Fig. 2). The inhibitory action of DEX on IL1/E.P. stimulated PGE$_2$ release was antagonised by the protein synthesis inhibitor anisomycin (Fig. 2).

DISCUSSION: This study demonstrates clearly that the antipyretic actions of DEX occur simultaneously with decreases in the pyrogen stimulated elevation of both blood and csf PGE$_2$ levels. Monocytes were used as a model for pyrogen stimulated PGE$_2$ release as they would contribute to the elevation of blood PGE$_2$ levels in vivo in the presence of pyrogens.

TABLE I   Effect of DEX and Poly I:C on the PGE_2 level in "push-pull" perfusates from the IIIcv

Time sample taken (h)		-1	0	1	2	3	4
Treatment							
1st	2nd						
saline	Poly I:C	60	65	156	204	186	300
DEX	Poly I:C	48	35	96	115	132	102
saline	saline	53	72	62	54	68	82
DEX	saline	60	64	55	67	75	65

DEX (3 mg/kg iv) or saline (1st treatment) was given 1 h prior to either Poly I:C (5 µg/kg iv) or saline (2nd treatment) and samples of IIIcv perfusates were taken at various times (measured from the administration of the 2nd treatment). Values shown are the pg PGE_2/ml of perfusate from a single rabbit and is representative of four separate rabbits (see text).
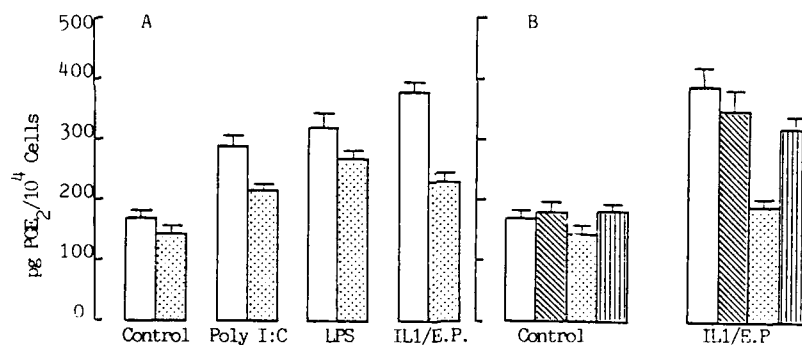
FIGURE 2: Effect of DEX on PGE_2 release from monocytes. A. Monocytes were incubated for 4 h in the presence of either media alone (control), Poly I:C (1 µg/ml), LPS (10 ng/ml) or IL1/E.P. (50 µl) in the absence (unshaded columns) or presence (dotted columns) of DEX (10 µg/ml). B. Monocytes were incubated for 4 h with either media alone (controls) or IL1/E.P. (50 µl) in the absence (unshaded columns) or presence of either DEX (10 µg/ml, dotted columns), anisomycin (1 µg/ml, cross-hatched columns) or DEX + anisomycin (lined-columns). Values are the means ± S.D. of 3 separate incubations and are typical of 3 separate experiments. *P<0.001.

The requirement for a 1 h pretreatment with DEX *in vivo* is consistent with the view that glucocorticoids exert their actions via the induction of protein synthesis. It is thought that the anti-inflammatory actions of glucocorticoids are mediated through the induction of lipocortin, an anti-phospholipase A_2 (PLA_2) protein [8]. Inhibition of PLA_2 prevents the generation of arachidonic acid, the precursor of eicosanoid formation, and consequently a decrease in the biosynthesis of prostanoids. An increase in the basal *in vivo* production of lipocortin has been reported in rats, which occurs within 30 min of administration of DEX (1 mg/kg) [9]. This may be a possible mechanism by which DEX exerts its

antipyretic actions under the present conditions as in the monocyte model of PGE_2 release in the presence of LPS, Poly I:C and IL1/E.P. the effects of DEX were similar to those observed *in vivo*, in blood and csf. In addition, the protein synthesis inhibitor, anisomycin antagonised the inhibitory effects of DEX on the PGE_2 released from monocytes in the presence of IL1/E.P. This indicates that the reduction of PGE_2 release involves a protein synthesis step, possibly the induction of lipocortin which may ultimately be responsible for the antipyretic action of DEX.

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ANTISERUM AGAINST MOUSE IL-1 ALPHA DOES NOT BLOCK STRESS HYPERTHERMIA OR LPS FEVER IN THE RAT

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Psychological stress has been shown to cause a rapid rise in body temperature of rats [1-9], rabbits [10,11], and humans [12,13]. Exposure to a novel environment, a classic stress paradigm for the rat, causes an average increase in body temperature of about 1.4°C [6]. Approximately 70% of this increase can be blocked by intraperitoneal injection of sodium salicylate [6], or indomethacin [14]. Injection of sodium salicylate into the lateral cerebral ventricle (ICV) prior to stress blocked 55% of the temperature rise [14]. The fact that these inhibitors of prostaglandin synthesis block a large portion of the increase in body temperature due to stress has led us to speculate that stress hyperthermia may be a true fever, such as that seen in response to bacterial lipopolysaccharide (LPS). This theory is supported by preliminary data from our laboratory demonstrating that lizards (*Dipsosaurus dorsalis*) tend to show a behavioral fever during the first 2 h of exposure to a novel environment.

In recent years, much work has been done to determine the molecular mechanism of LPS fever. While this pathway has not been fully elucidated, the cytokine interleukin-1, which causes a prostaglandin dependent rise in body temperature, has been thought to play a major role [see reviews 15,16]. However, since IL-1 has not been detected in the plasma during LPS fever, it is unclear if IL-1 enters the circulation in response to this stimulus.

In the present study, we used antiserum against mouse IL-1 alpha to examine the role of circulating IL-1 alpha in stress hyperthermia and LPS fever. We first determined how much antiserum had to be administered to rats to prevent the febrile response to a moderate dose of IL-1 alpha. We then injected the antiserum into rats prior to exposure to a novel environment or to LPS injection to determine whether the antibodies could block the subsequent rise in body temperature.

METHODS: Specific pathogen-free male Sprague-Dawley rats weighing 200 to 475 g were housed in individual plastic cages in a room maintained at 26 ± 1°C, i.e., in the thermoneutral zone for rats, with a photoperiod consisting of 12 h of light and 12 h of dark. Tap water and rodent chow were provided *ad libitum*.

Body temperature was measured using battery operated biotelemetry devices implanted intraperitoneally into each rat four or more days before experimentation began. Each transmitter was calibrated prior to implantation. Output (frequency in Hz) was monitored by a mounted antenna placed under each animal's cage and fed into a peripheral processor connected to a microcomputer. Temperatures were monitored and recorded at 5 min intervals during the stress experiments, and at 15 min intervals during the IL-1 and LPS experiments. Prior to any experimental manipulation, basal temperatures were recorded for at least 10 h.

Some of the rats were implanted with intracerebroventricular cannulae using the following procedure. Rats were anesthetized with ketamine hydrochloride (87 mg/kg im) and xylazine hydrochloride (12.9 mg/kg im) and placed in a stereotaxic apparatus. A stainless steel cannula was placed in the right lateral cerebral ventricle as described by Bailey *et al.* [17]. At least 1 week was allowed for recovery before the rats were used in an experiment. During experimental injections, a 27 gauge needle with a 0.5 ml syringe attached to its distal end was inserted into the cannula and gently pushed through the protective rubber diaphragm. The fluid (see below) was injected (ICV injection) over several seconds. The entire procedure took approximatley 30 sec, during which the rat was held loosely with the head stabilized by one finger holding it just above the eyes. The rats were conditioned to the injection procedure by at least two separate previous injections of artificial cerebral spinal fluid (ACSF) not less than 24 h prior to the experiment. The animals did not struggle or squeal during the injection procedure.

Acute psychological stress in rats can be induced by exposure to an open field [6,18]. The open field used in these experiments consisted of a 152 x 97 x 206 cm high white acrylic spray finish temperature controlled chamber illuminated by two fluorescent lights suspended above. The temperature within the open-field box was the same as that of the rats' home cages, 26°C. The experimental protocol for stress involved transporting each rat from the home room to the stress room, removing the rat from his cage, and placing him in the open-field chamber. After 30 min, the rat was returned to its cage and taken back to the housing area. Care was taken not to disturb animals before or after the stress exposure. All animals were conditioned to handling at least 2 weeks prior to the experiments. To minimize any confounding effects of the circadian rhythm, all stress experiments were performed between 9:30 h and 12:30 h.

The recombinant mouse IL-1 alpha used in this study was a gift of Dr. Ivan Otterness of Pfizer Corporation. Antibodies against IL-1 were raised by injecting the protein into a goat. Blood was drawn from the goat, and the serum was isolated and kept frozen at -20°C. Dr. Steven Kunkel provid with goat antiserum against this IL-1. Control animals received goat serum. Care was taken to ke   sera sterile.

Lyophilized LPS 0111:B4 phenol extract, number L-2430 was used. The LPS was reconstituted in 0.9% saline to a concentration of 10 μg/ml.

Before we could determine if the antiserum could block the rise in body temperature due to stress or LPS, we had to ascertain that it could block a fever due to IL-1 alpha. Two experiments were performed.

In the first study, IL-1 alpha was incubated in vitro with either antiserum against IL-1 alpha or control serum, then injected icv. Antiserum (396 μl) that had been diluted 1/100 with ACSF was added to 4 μl of IL-1 (500 ng/ml) and incubated for 35 min at 37°C. As a c   ol, the same dose of IL-1 was incubated with diluted goat serum (control serum). This resulted in a concentration of IL-1 of 5 ng/ml ACSF.
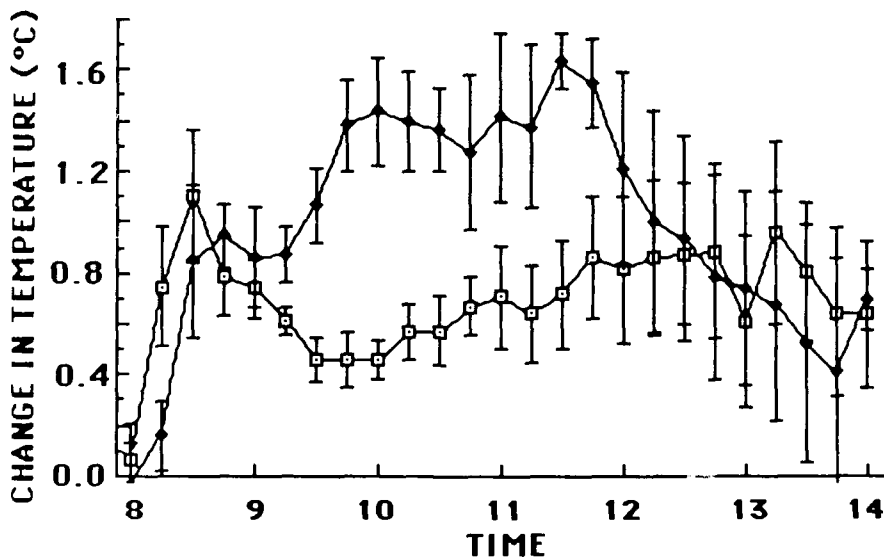
FIGURE 1: The change in temperature following the 8:00 h injection of mouse IL-1 alpha that had been preincubated with antiserum to mouse IL-1 alpha (open symbols) (N, 6) or control serum (closed symbols (N, 5). The change in temperature in the two groups differed significantly (p<0.05) between 9:30 h and 11:30 h.

Six rats received 20 μl (0.1 ng of IL-1) of the IL-1-antiserum mixture. Another group of five received 20 μl of the IL-1-control serum mixture. All injections were immediately followed by a 30 μl flush with ACSF.

In the second study, ip injection of antiserum against IL-1 alpha was followed by im injection of IL-1 alpha two hours later. Seven rats received injections of antiserum (2.4 ml/kg ip) and 8 rats received the same dose of control serum at 8:00 h. Two hours later, all animals received a dose of IL-1 (600 ng/kg im). Preliminary studies (data not shown) had demonstrated that neither antiserum nor control serum injected alone had a significant effect on body temperature.

The next experiment was performed to determine if the antiserum could block the rise in temperature due to open-field stress. Six rats received injections of antiserum against IL-1 alpha (2.4 ml/kg ip). Five other rats received the same dose of control serum. 3.5 h later, the animals in both groups were individually exposed to open-field stress.

The final experiment tested whether the antiserum against IL-1 alpha could block the febrile response to LPS. Ten rats received antiserum against IL-1 alpha (2.4 ml/kg) and 10 others received the same dose of control serum at 8:00 h. At 10:00 h, rats from both groups received LPS (10 μg/kg).

Values reported are mean ± 1 S.E. Comparisons between each experimental and control group were analyzed for statistical significance using the Student's t-test.

RESULTS: The response of the rats to the icv injection of IL-1 alpha that had been incubated with either antiserum against IL-1 alpha or control serum are shown in Fig. 1. Prior to injection, the mean body temperature of the rats which received the antiserum-IL-1 mixture was 37.2 ± 0.2°C, and that of the rats that received the control serum-IL-1 mixture was 37.6 ± 0.3°C (p>0.20) (Fig. 1).
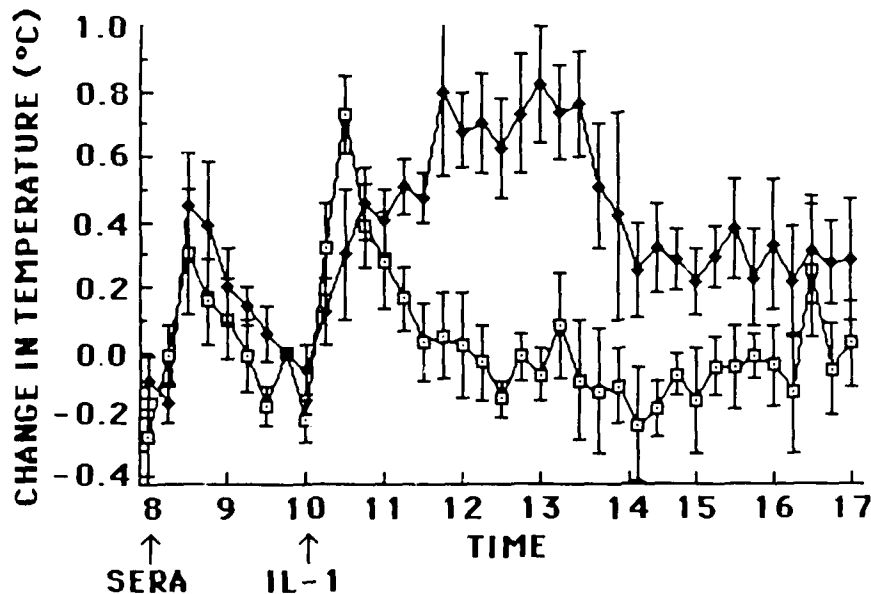
FIGURE 2: The change in temperature in response to the injection of mouse IL-1 alpha at 10:00 h following injection of antiserum to mouse IL-1 alpha (open symbols) (N, 7) or control serum (closed symbols) (N, 8) at 8:00 h. The change in temperature in the two groups was significant (p<0.001) between 11:00 and 17:00 h.

Rats in both groups showed initial rises in body temperature following the injections. Preliminary studies (data not shown) demonstrated that the injection of either antiserum or control serum caused an increase in body temperature of about 0.4°C that lasted several hours. This increase is commonly seen in response to the injection of any type of serum into the brain, and may be due to the introduction of foreign proteins.

The rats that received control serum mixed with IL-1 subsequently developed fever that was significantly higher than that of the animals that received antiserum mixed with IL-1 (p<0.05).

The temperature changes in response to the ip injection of either antiserum against IL-1 alpha or control serum, followed by the im injection of IL-1 alpha are shown in Fig. 2. The mean temperature of rats prior to the injection of IL-1 was 37.5 ± 0.1°C and 37.6 ± 0.2°C (p>0.85) for the animals that had received antiserum or control serum respectively. Rats in both groups showed typical stress responses to the injections of both the sera and the IL-1. However, rats that received antiserum prior to the injection of IL-1 showed no febrile response whereas the rats that received control serum showed a normal fever (Fig. 2).

The change in temperature due to the stress period, calculated by subtracting the pre-stress temperature (temperature immediately prior to removing the rat from his home cage) from the post-stress temperature (temperature immediately after returning the rat from the open-field to his cage) is shown in Fig. 3. Prior to the open field stress, the mean temperature of the animals in both groups was 37.1°C. Following the 30 min stress period, the mean rise in body temperature of the rats injected with antiserum was 1.43 ± 0.48°C. This did not differ significantly from the mean rise of 1.40 ± 0.16°C seen in the animals that received control serum prior to stress.
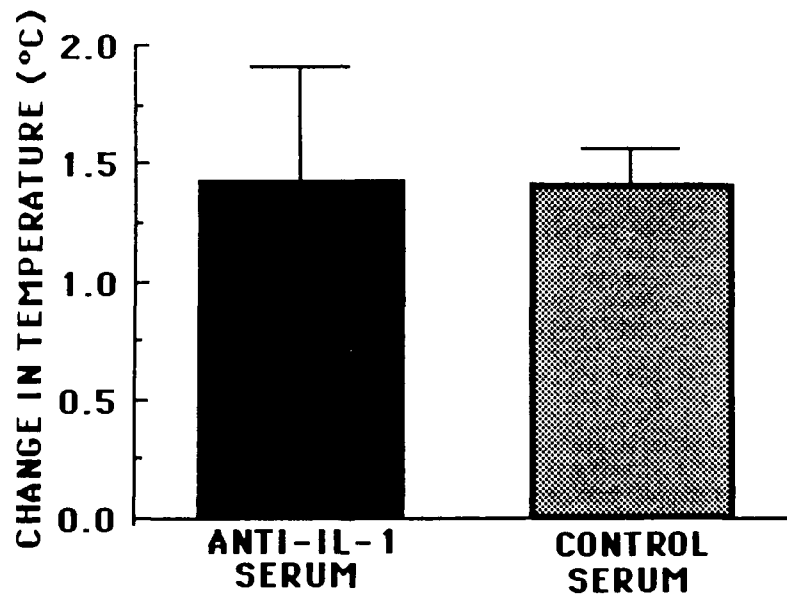

FIGURE 3: The mean change in body temperature in response to open field stress in rats that were injected with antiserum against mouse IL-1 alpha (N, 6) or control serum (N, 5) 3.5 h prior to the beginning of the 30 min stress period. These changes in temperature did not differ significantly (p>0.97).
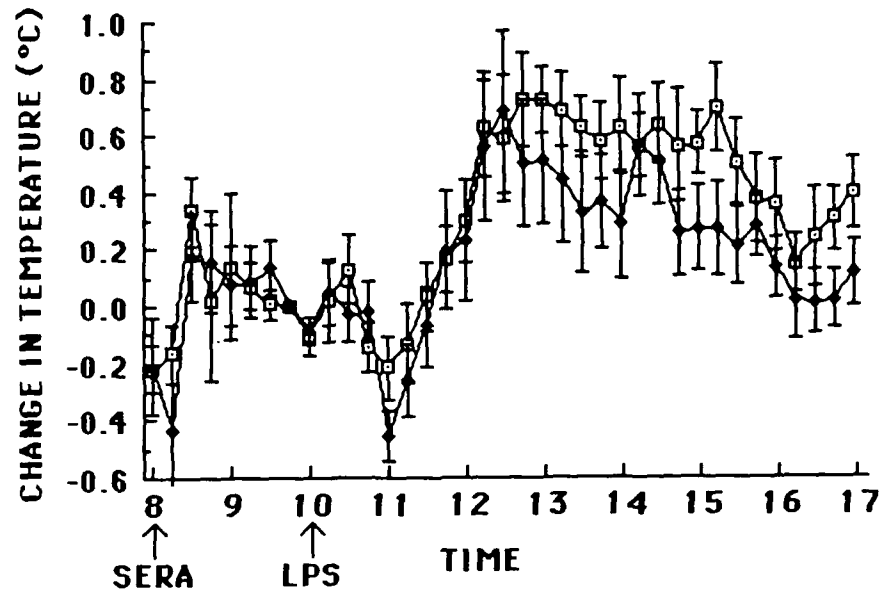
FIGURE 4: The mean change in body temperature in response to the injection of LPS at 10:00 h in rats that were injected at 8:00 h with antiserum against mouse IL-1 alpha (open symbols) (N, 10) or control serum (closed symbols) (N, 10). The changes in temperature did not differ significantly (p>0.21 between 13:00 h and 15:30 h).

The febrile response to the injection of LPS in rats which had been injected with either anti-serum against IL-1 alpha or control serum are shown in Fig. 4. Prior to the injection of LPS, the mean body temperature of the rats that had received antiserum was 37.6 ± 0.2°C. The body temperature of those that had received control serum was 37.5 ± 0.2°C (p>0.85). Again, rats in both groups showed normal stress responses to the injections of sera and of LPS. The rats that were injected with antiserum prior to the injection of LPS showed a tendency to develop higher fevers than rats injected with control serum. However, this difference was not significant (p>0.21) (Fig. 4).

DISCUSSION: The data from this study do not support the hypothesis that circulating IL-1 alpha is responsible for the increase in body temperature due to stress or LPS.

From the results of the second study we concluded that antiserum was able to enter the circulation and bind to administered IL-1 before the IL-1 could reach its target tissue and cause a fever. Our logic, therefore, was that this dose of antiserum should be able to block an equivalent fever (approximatley 1°C) due to stress or LPS if the fever were due to circulating IL-1 alpha. However, a potential problem with this logic is that we are using antibodies against mouse IL-1 alpha to try to block rat IL-1 alpha. Reports in the literature [19,20] have shown that antibodies to mouse IL-1 alpha cross react with rat IL-1 alpha. Moreover, the particular antiserum used in our experiments has been shown to attenuate the proliferation of thymocytes due to monocyte supernatant from the rat (Dr. Steve Kunkel, personal communication). Thus, the antibodies to mouse IL-1 alpha seem to bind to rat IL-1 alpha.

Antiserum to mouse IL-1 alpha did not block any of the increase in body temperature due to open field stress (or injection stress in any of the experiments). Hence, these data do not support a role for IL-1 alpha in mediating this response.

The antiserum against mouse IL-1 alpha did not block any of the increase in body temperature due to LPS (in fact, there was a tendency for the animals injected with antiserum to develop higher fevers in response to endotoxin as compared to the control serum injected animals).

While the data from these experiments do not support the hypothesis that circulating IL-1 alpha is a major mediator of stress hyperthermia or LPS fever, they do not rule out the possibility that brain IL-1 alpha is the mediator [21]. Since the antibodies may not cross the blood-brain barrier, they would not block the response if the stress or LPS were to cause the release of IL-1 alpha directly into the central nervous system rather than into the systemic circulation.

Another possibility is that a different prostaglandin-mediated cytokine may be released into the circulation or brain in response to stress or LPS, and may be responsible for the temperature increase associated with these stimuli. One possibility is that IL-1 beta, which is released in response to the same stimuli as is IL-1 alpha, is the mediator [22]. Another likely candidate is tumor necrosis factor (TNF) [23] which is released from macrophages in response to LPS and other infectious states, and the pyrogenic action of TNF can be blocked by prostaglandin inhibitors. It is also possible that IL-6 (interferon beta-2), another endogenous pyrogen [24], could be involved in these responses.

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SPLANCHNIC OXYGEN UPTAKE DURING FEVER IN HUMAN SUBJECTS

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Brown fat as a thermogenic source of importance in adult human subjects is probably negligible
[1]. The splanchnic region, and especially the liver, has been suggested as an important source of
thermogenesis. In order to test this hypothesis oxygen uptake in this region, as well as in one leg, was
measured in patients with fever due to infection.

PATIENTS AND METHODS: Seven patients admitted to the department of infectious diseases
were studied. They were previously healthy, not taking any drugs, with the exception of antibiotics. Five
of the patients had pneumonia and two had pyelonephritis. Patients who were febrile with a temperature
above 39°C on admission and more than 38.5°C on the following investigation day were studied. Four
patients had a slight increase (less than twice the upper normal limit) in the serum of one or more of the
hepatic enzymes alanine aminotransferase, asparagine aminotransferase and alcaline phosphotase. No
patient developed hyperbilurubinemia. The investigation was approved by the Ethics Committee of the
Medical Faculty, Uppsala University.

All patients were premedicated with diazepam (10-15 mg po) and atropine (0.5 mg sc). After ap-
propriate infiltration anaesthesia a 1.2 mm arterial catheter was inserted into the femoral artery and a
1.25 mm venous catheter into the femoral vein of one leg. The catheters were placed with their tips at the
level of the inguinal ligament. Additionally, a 1.7 mm heparin-coated catheter was inserted by per-
cutaneous puncture of a cubital vein and, under fluoroscopic control, its tip was advanced to a right
hepatic vein, 2-3 cm from the wedge position. A 1.0 mm catheter was inserted into a radial artery. After
this procedure the patients were allowed to rest for at least 30 min. Those who required intravenous fluid
to maintain a normal state of hydration received appropriate amounts of saline during the investigation.
In the other subjects catheter patency was maintained by a slow infusion of saline. Leg blood flow was
determined by means of a constant infusion of indocyanine green into the femoral artery according to a
modification of the method of Jorfeldt and Wahren [2]. Since indocyanine green is eliminated by the liver
the splanchnic blood flow could be determined according to the modification by Caesar et al. [3] of the
original technique described by Bradley et al. [4]. Blood samples from the radial artery, femoral vein and
hepatic vein for indocyanine green concentration were drawn simultaneously in triplicate on two occasions
20 min apart. On these two occasions blood samples were similarly drawn for analysis of oxygen satura-
tion, hemoglobin and pO_2. These samples were analysed in duplicate. Simultaneously blood samples were
drawn for analysis of plasma 3-methylhistidine. After this procedure the metabolic rate (systemic oxygen
uptake) was measured by an open flow-through system [5]. Urinary nitrogen losses were followed for
4 days from the day of investigation. The patient's basal metabolic rate was calculated according to the
Harris Benedict formula and an increase of 13.4% per 1°C in oxygen uptake was anticipated when correct-
ing the oxygen uptake for the increase in body temperature.

Values are presented as median values and the 25th and the 75th percentiles. Linear regression was
used for the correlation between temperature corrected oxygen uptake and measured oxygen uptake.

RESULTS: Blood flows were unchanged throughout the procedure. Median splanchnic blood flow
was 1947 ml/min (1490-2076). Median leg blood flow was 339 ml/min (287-437).

Median systemic oxygen uptake was 12.86 mmol/min (11.63-15.44). Median splanchnic oxygen
uptake was 3.44 mmol/min (2.86-4.19). Median oxygen uptake in one leg was 0.77 mmol/min (0.70-0.92).

The calculated basal oxygen uptake, core temperature, measured oxygen uptake, splanchnic oxygen
uptake and oxygen uptake in one leg are shown in Table I.

TABLE I   Calculated basal oxygen uptake (H-B.$\dot{V}O_2$), core temperature, systemic oxygen uptake ($\dot{V}O_2$), splanchnic oxygen uptake and oxygen uptake in one leg

No.	H-B.$\dot{V}O_2$	Temp.	Syst. $\dot{V}O_2$	Spl. $\dot{V}O_2$	$\dot{V}O_2$ one leg
1	8.83	39.0	9.73	2.92	0.70
2	8.57	38.8	11.29	2.84	0.
3	11.07	38.5	16.12	3.66	0.69
4	8.30	39.1	12.63	3.44	0.70
5	11.29	38.8	12.86	4.36	0.92
6	12.28	40.6	17.41	6.78	1.58
7	10.80	38.5	13.39	2.69	0.83

DISCUSSION: The oxygen uptake over one leg is usually considered to represent 25% of peripheral oxygen uptake. In Fig. 1 it is seen that splanchnic oxygen uptake and the peripheral oxygen uptake account for 6.5 mmol/min. The heart (10%), kidneys (10%), brain (20%) and other organs are assumed to account for the last 6.3 mmol/min. Ideally the splanchnic oxygen uptake, leg oxygen uptake and systemic oxygen uptake should have been obtained from the same subjects after reconvalescence. However, splanchnic oxygen uptake has been measured with the same methodology in healthy subjects in our department. The splanchnic oxygen uptake was consistently found to be in the order of 25% of systemic oxygen uptake [6,7]. The patients in this study had a 31.4% increase in oxygen uptake and an average temperature increase of 2°C. The splanchnic $\dot{V}O_2$ was 28% of total $\dot{V}O_2$. The relative contribution of this region to the total heat production therefore seems to be unchanged.
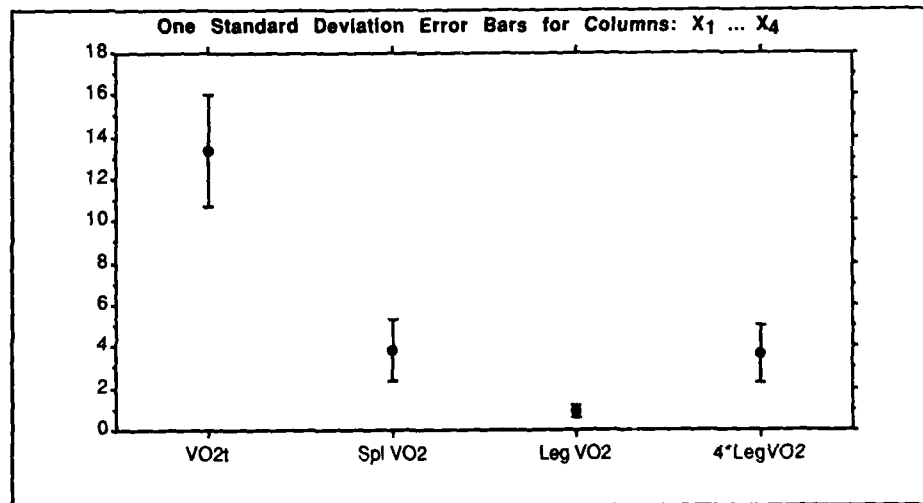
FIGURE 1: Systemic oxygen uptake ($\dot{V}O_2$t), splanchnic $\dot{V}O_2$ (spl. $\dot{V}O_2$), oxygen uptake in one leg (leg $\dot{V}O_2$) and in 4 x one leg (4 x leg $\dot{V}O_2$).

Measured systemic oxygen uptake in two patients was found to be higher than the expected temperature corrected oxygen uptake. It is interesting that these two patients were the most catabolic patients as estimated by the release of 3-methylhistidine and urinary nitrogen losses. The r-squared value of the linear regression between temperature corrected oxygen uptake and the measured oxygen uptake is 0.66, meaning that only approximately 66% of the increase in oxygen uptake is explained by the increase in temperature.

In conclusion we found no evidence of a relative increase in heat production from the splanchnic region in febrile patients.

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ARGININE VASOPRESSIN (ADH) ALLEVIATES POOR HEAT TOLERANCE IN A CHILD WITH HYPODIPSIC HYPERNATREMIA WITH PARTIAL ADH DEFICIENCY

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Hypodipsic hypernatremia is an uncommon syndrome that has been observed in children and adults with various congenital or acquired diseases of the brain that affect mainly the hypothalamus. The syndrome is characterized by chronic or recurrent episodes of severe hypernatremia associated with dehydration and a lack of thirst. In all previous carefully evaluated cases, hypodipsia has been associated with some defect in secretion of the antidiuretic hormone, arginine vasopressin (ADH), which suggests that there is hypoplasia or destruction of the hypothalamic osmoreceptors that regulate thirst and vasopressin secretion [1,2].

We here describe a child with central nervous system abnormality who had hypernatremia and lack of thirst and in addition suffered from multiple episodes of elevated body temperature in the summer months after short exposure to moderate ambient temperature. Administration of ADH or desmopressin acetate (DDAVP) even in the presence of dehydration and hyperosmolality alleviated the poor heat tolerance of the patient and improved her water regulation to some degree as well. The discovery of an isolated defect in thermoregulation and its correction with antidiuretic hormone confirms some predictions about the antipyretic role of ADH in the control of fever.

CASE REPORT: A 3 year old girl presented with marked developmental delay, hypernatremia and multiple episodes of high body temperature occurring shortly after exposure to ambient temperature such that usually prevail during the summer months in Israel (approximately 30°C). She was born after an uneventful pregnancy and delivery to unrelated healthy parents of Jewish Ashkenazi origin. At the age of 4 months following correction of bilateral cleft lip and palate she developed high fever and bilateral otitis media. Hypernatremia, hyperchloremia with mild to moderate signs of dehydration were found and persisted during periods of apparent good general health. She did not show any signs of thirst even at very high serum osmolality levels and refused to drink almost any liquids. A water deprivation test performed at the age of 5 months, during which she lost 3% of her weight, showed increases in urine osmolality from 220 to 351 mOsmol/kg H_2O, serum sodium increased from 150 to 159 mmol/l. Plasma ADH levels were very low (1.0-2.1 pg/ml) during the whole test. Following administration of aqueous pitressin (0.5 microgram im), urine osmolality increased two-fold, thus eliminating the possibility of renal tubular defect. Serum ADH levels during a wide range of plasma osmolality values (275-330 mOsm/kg H_2O) were much lower than expected.

At the age of 8 months she presented with infantile myoclonic seizures and an EEG hypsarrhythmia pattern was found. Seizures were successfully controlled with adrenocorticotropic hormone. CT and MRI disclosed mild dilatation of the lateral ventricles with absence of the anterior parts of the corpus callosum. Karyotype examination was normal, 46XX. Free T4, TSH, growth hormone, cortisol, LH and FSH were normal for her age. Serum prolactin levels were elevated. Other routine biochemical studies were within normal limits.

Starting at the age of 18 months, she developed multiple episodes of high temperature of 39°-39.5°C, following short exposures to usual summer temperatures that prevail in the area. The fever used to return to normal values when the patient was kept in an air conditioned room (20°C) for several hours. Almost no such episodes occurred during the winter months. Laboratory work-up did not disclose any infectious or inflammatory cause for these episodes. Repeated water deprivation tests during which the

patient lost 5% of her weight followed by DDAVP administration, disclosed clearly partial deficiency of ADH secretion. Since it was difficult to maintain normal hydration through forced drinking, DDAVP (1 μg twice daily) was started. While on this treatment it was noted that there was marked improvement in her tolerance to environmental heat exposure. To test this effect in more detail we followed changes in her rectal temperature during a 1 h stay in a warm and humid environment (34-35°C, 80% relative humidity). The test was repeated at four successive weeks at the same time of the day (15:00 h) at different hydration states, serum sodium levels and drug administration. Rectal temperature at the beginning of the test was 37.0-37.4°C.

The change in the rectal temperature during the 1 h test periods is depicted in Fig. 1. Following 48 h of intravenous fluid administration normal hydration was achieved and serum sodium value was 143 mmol/l. Rectal temperature rose by 0.8°C (A). During chronic dehydration state, with serum sodium values of 151 mmol/l, the rectal temperature rose by 0.7°C (B). Intravenous administration of aqueous ADH immediately before the test while the patient was moderately dehydrated with serum sodium of 146 mmol/l caused a 0.4°C increase after 30 min with return to initial temperature at 60 min (D). Chronic administration of DDAVP twice daily resulted in some correction of the dehydration and hypernatremia (sodium values were 144 mmol/l). Rectal temperature during the test increased by 0.4°C 15 min following exposure, and towards the end of the test, gradually decreased to 0.2°C above the initial temperature (C). Very few episodes of fever occurred after initiation of DDAVP therapy.
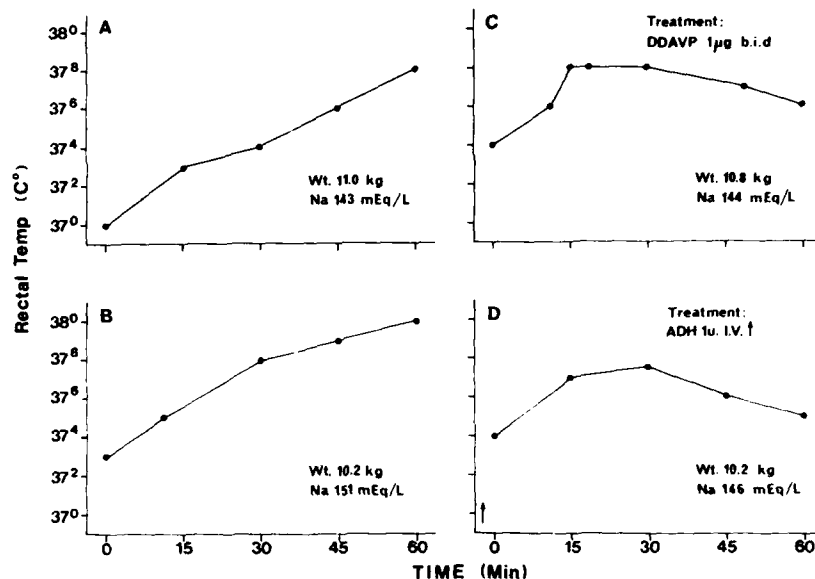
FIGURE 1: Increase in rectal temperature during 1 h exposure to ambient temperatures of 34-35°C, 80% relative humidity. A - Normal hydration and normonatremia - no treatment; B - Moderate dehydration, hypernatremia - no treatment; C - Normal hydration, normonatremia - chronic intranasal DDAVP treatment; D) Moderate dehydration, mild hypernatremia - Acute i.v. ADH administration.

DISCUSSION: The abnormal heat regulation in our patient, manifested by extremely high fever on short exposure to high ambient temperatures, was an extremely unusual phenomenon in this unusual brain damaged patient who had the characteristics of hypodipsic hypernatremia. Although hypothalamic lesions with abnormal hormonal secretion was described in patients with cleft lip and palate [3], the association of partial deficiency of ADH, hypodipsia and poor temperature regulation has not been described previously. It was surprising that ADH administration either as vasopressin or in the form of the more

stable analogue DDAVP should have alleviated the poor heat tolerance, while the level of hydration and serum sodium by themselves did not have any effect on heat tolerance. Previous animal experimental studies showed that endogenous ADH secretion suppress fever due to intravenous or intracerebroventricular pyrogen administration, with no effect on normal body temperature [4]. The present study indicates that vasopressin affects normal thermoregulation, even in the absence of pyrogen fever.

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CORD COOLING AND FEVER CAUSED BY LPS AND PGE$_2$

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Central and peripheral thermal loads induce antagonistic changes in cutaneous and visceral sympathetic outflow [1]. During hypothalamic, spinal and peripheral cooling the skin sympathetic nerve activity increased, while the activity of splanchnic nerves decreased.

If fever is caused by an upward shift of the set-point for temperature regulation, it is expected that the pattern of sympathetic differentiation during fever genesis should be identical with that to cooling.

In the present investigation, we observed splanchnic and renal sympathetic nerve activity as the "visceral" sympathetic tone and ear skin temperature as the index of "skin" sympathetic activity in anesthetized rabbits during fever caused by lipopolysaccharides (LPS) and prostaglandin E$_2$ (PGE$_2$) in order to clarify the role of PGE$_2$ in LPS fever.

METHODS: The experiments were performed in 31 male rabbits with body weights of 1.8-3.2 kg. In a preliminary operation at least 5 days prior to an experiment, a polyethylene cannula was implanted into the left lateral ventricle for the injections of PGE$_2$ as described elsewhere [4] and a U-shaped polyethylene tubing, which served as the thermode, was inserted into the peridural space [2] under sodium pentobarbital anesthesia.

The experiments were carried out under urethane anesthesia (1.3-1.5 g/kg ip). Rectal temperature (T-rect) and ear skin temperature (T-ear) were measured with thermistor probes. For arterial blood pressure and heart rate (HR) recording a catheter was inserted into the left femoral artery.

In 16 animals out of 31, the renal nerve bundle running along the left renal artery was dissected free and placed on a bipolar silver wire electrode. After crushing the distal end of the nerve to eliminate afferent nerve activity, we molded the electrode and the nerve together with silicone gel for the electrical insulation. In the other 15 animals the left major splanchnic nerve was prepared below the diaphragm and set on the electrode as for the renal nerve recording. The renal sympathetic nerve activity (RSNA) and splanchnic nerve activity (N-spl) were amplified and fed into an integrating circuit. The original discharges and the integrated nerve activities of 5 sec intervals were recorded on an oscillograph. Then the integrated nerve activity was evaluated in percent: the average value over 5 min before stimulation or pyrogen injection was taken as 100%.

The ambient air temperature was kept at 24-26°C. The animals were placed on a heating pad, which was perfused with water of 39-40°C to maintain T-rect at 38.0-39.0°C. After the stabilizing period for 1 h, spinal cord cooling was carried out. Water of 15°C was perfused through the thermode for 3 min, and the average peridural temperature was decreased to 20.0°C at the end of cooling. One hour after spinal cooling, LPS (100 ng/kg) was injected intravenously. In the other rabbits PGE$_2$ (8 μg/kg) was injected into the cerebral ventricle.

RESULTS: The effects of spinal cooling on splanchnic and skin sympathetic nerve activity are seen in Fig. 1 (A). The spinal cooling induced an activation of ear sympathetic efferents as shown by a fall in T-ear, while the activity of N-spl was rapidly decreased.
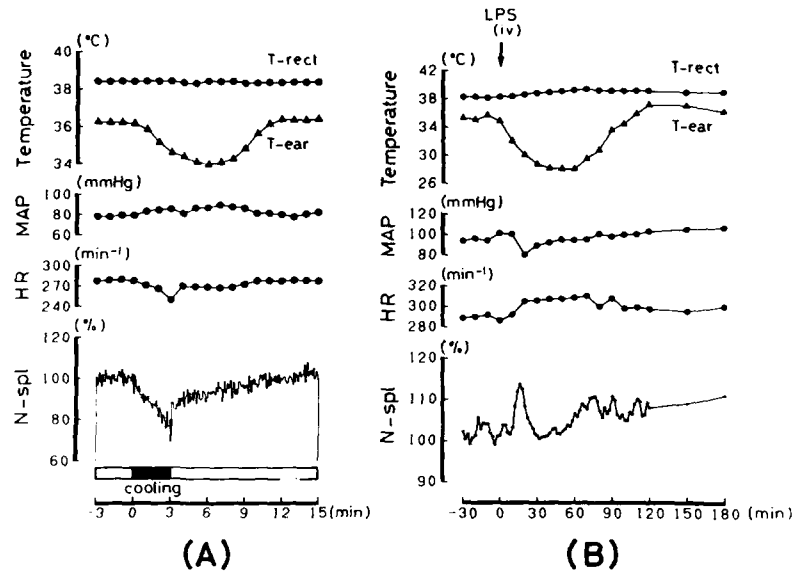
FIGURE 1: Time courses of temperatures, splanchnic nerve activity (N-spl), mean arterial pressure (MAP), and heart rate (HR) during spinal cord cooling (A) and fever induced by LPS (iv) injection (B) in one experiment.

In 7 rabbits LPS was injected intravenously. Fig. 1 (B) shows the effects of LPS in the same rabbit as (A). T-rect rose and reached the maximum, 0.9°C higher than the control temperature 1 h after LPS administration. T-ear started to decrease within 15 min, accompanied by activation of N-spl. MAP shows a slight and transient decrease, however, this was not statistically significant. HR was increased and reached the maximum at 1-1.5 h after LPS injection. T-ear recovered to the control level within 2 h with the subsidence of fever. N-spl showed a tendency to increase even after T-ear recovered.

In 8 rabbits PGE_2 was injected into the lateral ventricle. Fig. 2 shows a typical example. T-rect began to rise within 5 min after the injection with ear skin vasoconstriction which caused T-ear to decrease. T-rect reached the maximum (1.5°C above the control level) 1.5-2 h after injection. The activity of N-spl increased within 1 min after the injection. HR also increased. MAP showed a tendency to rise slightly but not significantly.

In 8 rabbits RSNA was recorded during fever caused by PGE_2 (icv). Fig. 3 shows an example. RSNA reached the minimum value within 5 min after injection and then gradually increased and returned to the control level when skin vasoconstriction ceased. MAP showed a tendency to slightly increase. We performed sino-aortic denervation in 8 rabbits in order to confirm whether this decrease in RSNA is a result of baroreflex due to MAP increase with PGE_2 injection. In denervated animals, increases in MAP and HR after PGE_2 injection were pronounced as was expected, however, RSNA did not change uniformly: an increase in some animals and decrease in others. Thus, RSNA decreases during PGE_2 fever mainly due to baroreflex. The effect of icv injection of PGE_2 on RSNA was not determined in the present experiments. In 4 rabbits PGE_2 was injected after cervical vagotomy. The change in each parameter was qualitatively similar to the intact group but more pronounced. The increase of HR in vagotomized animals suggests that PGE_2 (icv) activates cardiac sympathetic outflow.

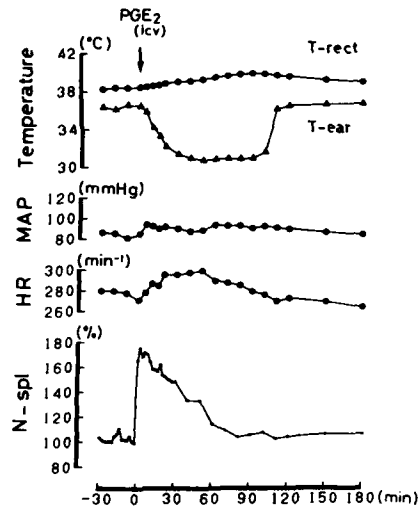**FIGURE 2:** Time courses of temperatures, splanchnic nerve activity (N-spl), mean arterial pressure (MAP), and heart rate (HR) during fever induced by PGE_2 (icv) injection in one experiment.
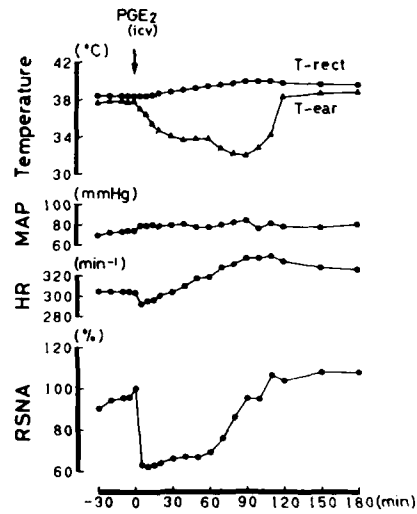

FIGURE 3: Time courses of temperatures, renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and heart rate (HR) during fever induced by PGE_2 (icv) injection in one experiment.

DISCUSSION: The direction of changes in regional sympathetic activity during spinal cooling and fever induced by LPS and PGE$_2$ are summarized in Table I.

TABLE I   Comparison of changes in regional sympathetic activity during spinal cord cooling and fever induced by PGE$_2$ and LPS administration

			Ear	Renal	Splanchnic	HR
Spinal cooling			+		−	−
PGE$_2$	(icv)	0-60 min	+	−[1]	+	+
		>100 min	0	0	0	~
LPS	(iv)	0-60 min	+	(−)[2]	+	+(+−)[3]
		>100 min	0	(+)[2]	+	~

+ : activation, − : inhibition, 0 : control level, ~ : tend to return
[1] : Baroreceptor influence is dominant.
[2] : W. Riedel et al. 1982 [4]
[3] : modulated by ambient temperature, W. Riedel et al. 1986 [5]

The reciprocal changes in cutaneous and visceral sympathetic activity which are typical for the cooling were observed in ear skin and RSNA during PGE$_2$ fever. But the pattern of changes in splanchnic nerve activity and HR caused by PGE$_2$ administration was different from that of cooling.

There was a similarity in the pattern of regional sympathetic activities between PGE$_2$ administration and early phase of LPS fever. This supports the concept that PGE$_2$ mediates the generation of LPS fever. But the activation of RSNA during the late phase of LPS fever [4] is not observed in any course of PGE$_2$ fever. This fact suggests that PGE$_2$ is probably involved in producing the early phase of LPS fever, but the later phase of LPS fever is not solely mediated by PGE$_2$.

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RELATIONSHIP BETWEEN BLOOD GLUCOSE AND HOT FLUSHES IN WOMEN AND AN ANIMAL MODEL

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Seventy-five to 85 percent of all women experience hot flushes as a result of the decline in go-
nadal steroids at the menopause [1]. Hot flushes appear to result from the inappropriate activation of heat
dissipatory mechanisms. Associated with this is the activation of the sympathetic nervous system resulting
in a peripheral vasodilation [2], an increase in heart rate [3], release of adrenal cortical [4] and medullary
hormones [5], β-endorphin [6], and luteinizing hormone [4,7].

The mechanism by which the decline in gonadal steroids causes the activation of sympathetic heat
dissipation is currently not known. Perplexing also is the observation that 15 to 25% of post-menopausal
women do not experience hot flushes and, of symptomatic patients, the number and intensity of hot
flushes varies greatly between individuals [1]. The difference between symptomatic and asymptomatic
women cannot be explained by the extent of decline in estradiol or other estrogen at the menopause [8],
although an association between symptoms and the decline in "free" estradiol has been observed [9].

In diabetic patients who receive aggressive insulin therapy, hypoglycemic episodes are associated
with many of the same symptoms experienced by post-menopausal patients. Among the counter-
regulatory mechanisms used to increase blood glucose in these patients is acute activation of the
sympathetic nervous system. This hypoglycemia causes increased release of adrenal medullary [10] and
cortical hormones [11], tachycardia [11-13], and β-endorphin release [14]. These patients also experience
hot flushes and chills during the hypoglycemia attack [11-13], but this aspect of the hypoglycemic episode
is not well studied.

We proposed then that the decline in estradiol at the menopause may result in an inappropriate
change in the availability of glucose to brain neurons and the hot flush observed in post-menopausal
patients may reflect the compensatory activation of the sympathetic mechanism involved in blood glucose
regulation. As an initial evaluation of this hypothesis we monitored blood glucose concentrations and the
occurrence of hot flushes in post-menopausal women. Further, we induced hypoglycemia in rats and
evaluated the occurrence of hot flushes. The present report documents the results of these studies.

METHODS: Three postmenopausal women were admitted to our clinical research center for an
evaluation of the occurrence of hot flushes and the association of hot flushes with blood glucose con-
centrations. Women were comfortably seated in bed, given a "light meal" consisting of orange juice and
toast at 7:00 h and were fitted with a heparin lock for repeated blood sampling. A thermistor lead was at-
tached to the dorsal surface of the right ring finger and a thermistor probe was inserted into the vagina.
Skin, vaginal and ambient temperatures were recorded at 2 min intervals and blood samples were obtained
every 15 min from 5 to 7 h. This procedure was repeated on the next day to achieve 6 temperature and
blood glucose profiles.

Hot flushes were defined as skin temperature increases of >1°C. The following parameters of hot
flushed were quantitated: the time (min) from the meal to the first hot flush, blood glucose concentration
at the time of the first hot flush (mg/dl) and the number of flushes at blood glucose concentration ranges
of >125 mg/dl; 100 to 124 mg/dl; 75 to 99 mg/dl and 50 to 74 mg/dl.

Adult male Sprague Dawley rats were obtained from Charles River Breeding Laboratories.
Animals weighed 225-250 g upon arrival and were maintained in a light (lights on 05:00 h to 19:00 h) and
temperature (25±1°C) controlled room with food and water provided *ad libitum*. All animals were
maintained under these conditions for at least 1 week prior to the initiation of the following studies.

An initial experiment was performed to determine the effects of acute insulin exposure on blood glucose concentrations. Rats were administered protamine zinc insulin at doses of 5, 10 or 20 IU/kg or a saline vehicle (1.0 ml/kg) by a subcutaneous injection. Two hours later a single blood sample was obtained by cardiac puncture while animals were under light ether anesthesia. A second group of rats was divided into 2 subgroups which received either protamine zinc insulin (5 IU/kg) or saline (1 ml/kg) and 2 h later a single blood sample was obtained. Additionally, separate groups of rats were treated with protamine zinc insulin (5 IU/kg) or the saline vehicle every 12 h (08:00 h and 20:00 h) for 24 h (3 injections) or 96 h (7 injections) and a single blood sample was obtained at 2 h after the last injection. Blood samples were centrifuged and sera was stored frozen (-20°C) for later assay of glucose concentrations.

To assess the effects of chronic hypoglycemia on tail skin temperature (TST), a second study was conducted in which 11 rats received twice daily (08:00 h and 20:00 h) injections of protamine zinc insulin (5 IU/kg) and 7 rats received a twice daily injection of a saline vehicle. Two hours after the 19th injection (08:00 h or the 10th day of the study), rats were lightly restrained in wire mesh tunnel cages and a copper-constantan thermocouple was taped to the dorsal region of the tail at approximately 2 cm from its base. Skin temperatures were recorded at 2 min intervals for 2 h without further disturbance of the rats.

A third experiment was conducted to assess the temporal association of chronic insulin exposure with the development of TST pulses. Rats were treated with a single injection of saline or protamine zinc insulin (5 IU/kg) and were evaluated for tail skin temperature response at 2 h later or were treated daily with the saline vehicle or with insulin (5 IU/kg) for 1 day (3 injections), 4 days (7 injections) or 8 days (15 injections). At 2 h after the last insulin or saline injection, rats were restrained and TST was evaluated as described above.

The effects of insulin dose on blood glucose concentrations was determined by one-way analysis of variance and Student-Newman-Keuls tests. Blood glucose concentrations at various times after saline or insulin injection was determined by Student's t tests. The effects of multiple insulin injections on TST pulse amplitudes was determined by one-way analysis of variance and Student-Newman-Keuls tests.

RESULTS: A typical plasma glucose profile for a postmenopausal patient is depicted in Fig. 1. In this patient plasma glucose was elevated to levels of 200 mg/dl or greater at the initial sampling time (1 to 2 h post-meal) then declined for the next 1 to 1.5 h. Blood glucose concentrations then normalized in a ranged 75 to 100 mg/dl. No hot flushes were noted in this patient prior to 2 h (3 h post-meal), then occurred frequently over the next 3 h period.
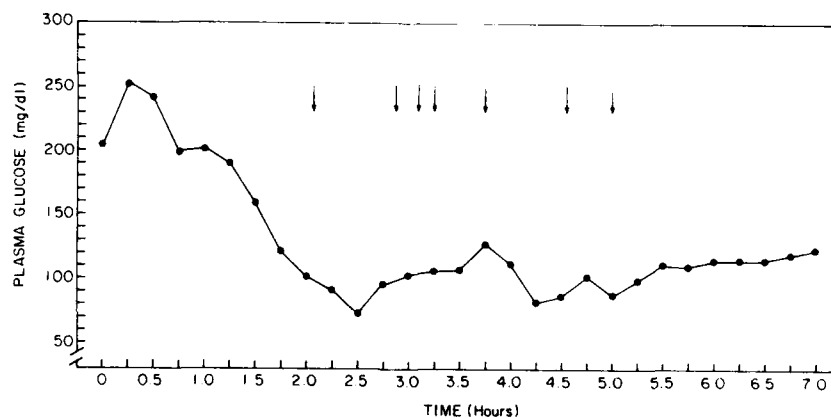
FIGURE 1: Representative plasma glucose profile of a post-menopausal woman from 1 to 8 h after consuming a light meal. The patient received orange juice and toast at 07:00 h and blood sampling began (time 0) at 08:00 h. Arrows indicate the time of objectively identified hot flushes.

Data from the 6 runs indicated that the time from the morning meal to the first hot flush was 140±23 min (mean ± SEM) (Table I). The first hot flush was observed when blood glucose concentrations were 81.6±7.4 mg/dl (mean ± SEM). Interestingly, for 17 of 24 (71%) hot flushes observed, blood glucose levels were elevated in the sample immediately following the onset of the flush. For all flushes observed, only one occurred when blood glucose was greater than 125 mg/dl; 5 occurred when glucose was 100 to 124 mg/dl; 12 occurred when glucose was 75 to 99 mg/dl and 5 occurred when glucose was 50 to 74 mg/dl (Table I).

TABLE I   Relationship between blood glucose and flushing episodes in postmenopausal women

Time from meal to 1st hot flush (min)	140±230 (6)[1]
Blood glucose concentrations at 1st hot flush (mg/dl)	81.6±7.4 (6)[1]
Number of flushes when blood glucose concentrations were:	
Greater than 125 mg/dl	1
100 to 124 mg/dl	5
75 to 99 mg/dl	12
50 to 74 mg/dl	6

[1]mean ± SEM (n).

In an attempt to mimic the postprandial decline in blood glucose observed at 2 to 3 h in women, we administered insulin to rats at various doses (Table II). Insulin caused a significant and dose-dependent reduction in blood glucose from 120 to 56 mg/dl over the 0 to 10 IU/kg dose range. The 20 IU/kg dose caused no further reduction in blood glucose. When the 5 IU/kg dose of insulin was administered every 12 h and a blood sample was obtained at 2 h post injection, blood glucose was reduced by a similar magnitude at each of three sampling times through 4 days (Table II). Thus, this dosing regimen (5 IU protamine zinc insulin/kg every 12 h) was used to evaluate the effects of chronic reductions in blood glucose on skin temperature regulation. In an initial study rats were administered protamine zinc insulin (5 IU/kg sc) or saline every 12 h for 10 days. Representative TST profiles of 2 of 11 insulin treated rats and 2 of 7 saline treated rats are depicted in Fig. 2. TST in saline trated rats were stable over the 2 h time course of this evaluation. In contrast, insulin treated animals show TST instabilitiy and exhibited major TST surges as well as minor TST fluctuations. The magnitude of this TST instability was quantitated by determining the area under the TST response curve (AUC) and the maximal increase in TST (MAX TST) or the saline and the insulin treated rats. The AUC for the TST response was -7.9±14.6°C-h (n, 7) for the saline group and 115±41°C-h (n, 11) for the insulin treated groups (p<0.05). While the MAX TST for the saline group was 0.7±0.3°C, the MAX TST for the insulin treated group was 3.5±0.5°C (p<0.05). In animals exposed for varying lengths of time to insulin or saline, the TST pulses observed were either low amplitude pulses (0.5 to 1.0°C) or high amplitude pulses (>2.0°C) (Table III). No saline treated rats showed any high amplitude TST pulses at any sampling time. A few low amplitude pulses were observed whose amplitude was similar to the MAX TST for saline treated rats in the previous study. In contrast, after a single insulin injection (Day 0) or after 3 injections (Day 1) rats exhibited a 4 to 5 fold increase in the number of low amplitude TST pulses and a significant increase in their magnitude. Additionally, high amplitude pulses of 3.4°C were observed in both of these groups at a frequency of about 1 rat/2 h. After 4 and 8 days of insulin treatment, the height of low amplitude pulses remained constant and their number decreased. This was associated with an increase in the number of high amplitude pulses to a frequency of about 2/rat/2 h. The amplitude of these high amplitude pulses remained constant, however.

TABLE II   Effects of protamine zinc insulin on plasma glucose concentrations in male rats

	Dose of Insulin			
	0 IU/kg	5 IU/kg	10 IU/kg	20 IU/kg
Plasma glucose (mg/dl)	120±15	76±11[1]	56±4[1]	67±8[1]

	Time after Initial Insulin Exposure		
	2 h	26 h	98 h
	Plasma glucose (mg/dl)		
Saline	143±9	127±3	125±3
Insulin (Protamine Zinc; 5 IU/kg/12 h)	87±6[1]	83±4[1]	74±7[1]

[1]p<0.05 vs respective saline treated control; analysis of variance and Student-Newman-Keuls Tests.
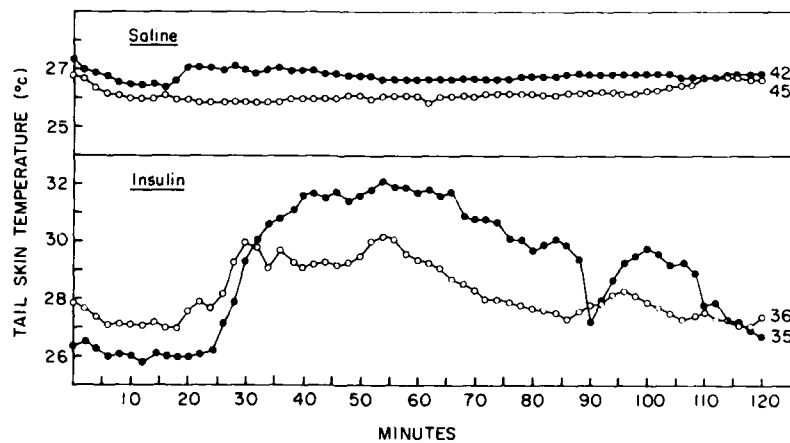

FIGURE 2: Tail skin temperature profiles of rats treated for 10 days with saline (upper panel) or protamine zinc insulin (5 IU/kg/12 h; lower panel). Tail skin temperature measurements began 2 h after the last insulin (saline) injection. The number to the right of the temperature profile indicate the number of the individual animal.

TABLE III Effects of continuous insulin exposure on the amplitude and frequency of skin temperature pulses in male rats

	Low Amplitude Pulses (0.5 to 1.9°C)	High Amplitude Pulses (>2.0°C)	All Pulse
		PULSE AMPLITUDE (°C)	
Day 0			
Saline	0.75±0.09 (6)	0(0)	0.75±0.09 (6)
Insulin	1.17±0.07 (25)[1]	3.36±0.44 0(5)	1.49±0.18 (30)
Day 1			
Saline	0.52±0.04 (5)	0(0)	0.52±0.04 (5)
Insulin	1.00±0.07 (24)	3.38±0.34 0(6)	1.48±0.19 (30)
Day 4			
Saline	0.91±0.13 (9)	0(0)	0.91±0.13 (9)
Insulin	1.20±0.10 (18)[1]	3.46±0.29 (13)	2.14±0.25 (31)[2]
Day 8			
Saline	0.63±0.03 (6)	0(0)	0.63±0.03 (6)
Insulin	1.15±0.12 (15)[3]	3.75±0.34 (12)	2.31±0.30 (27)[3,4]

[1]$p<0.05$ vs. Saline; [2]$p<0.005$ vs. Saline; [3]$p<0.025$ vs. Saline; [4]$p<0.05$ vs. Day 0 and Day 1, Insulin-Treated rats. Analysis of variance and Student-Newman-Keuls Tests.
The number in parenthesis indicates the number of pulses observed.
n, 7 to 8 rats/group.

DISCUSSION: · We have observed that during the postprandial period in post-menopausal women, elevation in blood glucose concentrations is associated with a hot flush-free period; whereas the subsequent decline in blood glucose is associated with the appearance of this symptom of the menopause. Thus, only one flushing episode was observed when blood glucose levels were greater than 125 mg/dl. This absence of flushes during the postprandial hyperglycemia period suggests that elevations in blood glucose may prevent flushes by preventing counter-regulatory activtion of sympathetic output. It has been observed that body weight of post-menopausal women is inversely related to the number of hot flushes [9]. It can be argued that since obesity causes insulin resistance and an elevation in blood glucose [15,16], obese women may supply more glucose to brain sympathetic centers. Support for this proposal comes from our recent observation that the administration of glucose can block the hot flush induced in morphine dependent rat by the administration of naloxone (Simpkins et al., unpublished observation). Thus, hyperglycemia induced by food consumption in women or by parenteral administration of glucose in an animal model can prevent hot flushes.

At 2 to 3 h after a light meal in our patients, the blood glucose decline, which resulted in part from insulin release, was associated with the appearance of hot flushes. This observation suggests that the activation of counter-regulatory sympathetic mechanisms may be involved in the etiology of the hot flush. It is of interest that the incidence of occurrence of hot flushes is higher during sleep than at other times of the day [4]. Unfortunately, the association of hot flushes with the time of meals or with the amount of glucose consumed has not been studied.

Based upon these preliminary clinical observations, it was reasonable to predict that the insulin induced hypoglycemia in rats would cause flushing episodes. Hypoglycemia caused flushing episodes in rats and the amplitude of the flushes observed increased with the length of the induced hypoglycemia. It ap-

pears that with chronic exposure to insulin, a periodic activation of sympathetic output results in acute peripheral vasodilation and transient surface temperature increases in the rat. As such, the described animal model may be useful in further evaluating the mechanisms of the menopausal hot flush.

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FURTHER EVIDENCE FOR AN ANIMAL MODEL FOR THE HOT FLUSH

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The most distressing manifestation of the menopause is the hot flush. This episodic disturbance of thermoregulation is characterized by a *sensation* of heat followed by vasomotor changes which results in an elevation in skin temperature. The rise in skin temperature, which is preceded by a transient tachycardia, is generally considered to be the primary objective index of the vasomotor flush [1-3]. Core temperature has also been shown to decline rapidly during and following the flush episode [1,4,5]. It has been suggested that the physiological changes that characterize a hot flush are consistent with a downward resetting of the thermoregulatory set point and suggests an integrated thermoregulatory event [4,5]. The main accompanying neuroendocrine event of the hot flush is a pulsatile luteinizing hormone (LH) secretion which accompanies the flushing episode [5-8]. LH pulses alone do not induce hot flushes and a more probable hypothesis is that this hormonal expression results from altered neurotransmitter functions within the hypothalamus which trigger the vasomotor symptoms [5,8].

We have suggested previously [9-11] that the morphine dependent rat can serve as a model to study the neuroendocrine mechanisms associated with the menopausal hot flush. Administration of opioid antagonists to morphine dependent female rats produces a tachycardia, followed by a surge in LH and a prompt rise in tail skin temperature (TST) with a subsequent fall in core temperature [9,11]. These physiological changes are similar in magnitude, duration and temporal association to those observed in women undergoing menopausal hot flushing. Additionally, we have demonstrated that these changes are mediated by a central mechanism [12,13]. We recently demonstrated that castration did not alter the TST response to naloxone in morphine dependent male rats [10]; however, it appears that the TST response to naloxone (1 mg/kg) in the morphine dependent rat ovariectomized for 1 week [9] was greater than the TST response produced in the intact female rat [10]. One goal of the current study was to evaluate the TST response in age matched intact and ovariectomized, morphine dependent rats to a threshold challenge of an opiate antagonist in order to determine if ovariectomy alters the sensitivity of the temperature response. Additionally, the role of estrogen replacement on TST response was evaluated in this animal model.

METHODS: Female Charles River CD rats, initially weighing 200-225 g were housed in groups of 2 in hanging stainless steel cages in a room maintained at 25±1°C and illuminated from 05:00 to 19:00 h daily. Food and tap water were provided *ad libitum.*

Morphine dependence was produced as described previously [9-13] by subcutaneous implantation of one pellet containing morphine (75 mg) on day 1 and 2 additional morphine pellets on day 3. All temperature experiments were performed 2 days after the last morphine pellets were implanted in a room maintained at 25±1°C.

In the first experiment, 24 female rats were divided into two equal groups: 12 were ovariectomized (OVEX) while under light ether anesthesia while the remaining 12 underwent sham treatment. All rats were rendered morphine dependent as described above. Six OVEX and 6 control rats at 1 and 2 weeks after ovariectomy were lightly restrained in wire mesh tunnel cages with a wooden floor. Tail skin temperature (TST) was measured with a copper-constantan thermocouple taped to the dorsal region of the tail so that the thermocouple contacted the skin near the base of the tail. Colonic temperature (TC) was measured with a similar, silastic coated thermocouple inserted 6 cm beyond the anus and taped to the base of the tail. Temperatures were measured simultaneously with a data acquisition and control system interfaced to a microcomputer. The rats were allowed to adjust for 60 min after they were placed into the restraining cages prior to administration of naloxone. At the end of the equilibration period the 6 control and 6 OVEX animals were administered naloxone (0.25 mg/kg sc). Tail skin and colonic temperatures were recorded for 60 min following administration of naloxone at 5 min intervals. Previous studies have

shown that neither subcutaneous saline administration to morphine dependent rats nor naloxone administration to placebo treated rats produce any changes in tail skin or colonic temperatures [9-13].

In a second experiment, 12 female rats were ovariectomized as previously described. Six received a pellet of estrogen (0.05 mg) (17β-estradiol) while the remaining 6 rats were administered a subcutaneous pellet consisting of carrier material. The carrier material in the pellets was cholesterol-methyl cellulose-α-lactose. These pellets are reported to release hormone at a constant rate for a period of 3 weeks. All animals received the first morphine pellet on the 16th day of estrogen/placebo treatment, followed 2 days later by an additional two pellets of morphine, sc. Tail skin and colonic temperature were recorded at 5 min intervals, 30 min before and 60 min following administration of naloxone (0.25 mg/kg sc) on the 21st day of the study as described above.

Following the temperature studies, animals were sacrificed by decapitation and trunk blood was collected. The blood was centrifuged, plasma collected and stored at -20°C until assayed. Plasma estrogen was assayed in duplicate utilizing solid phase ^{125}I radioimmunoassay. The estrogen assay has an intra-assay variation less than 7%. Additionally, the anterior pituitary and uterus were removed from each animal, cleaned of adherent tissue, and weighed.

All data were expressed as mean ± 1 SEM and differences between groups were analyzed by students T-test with significance set at the 95% confidence limits.

TABLE I   Effect of ovariectomy and estrogen treatment on tail skin temperature (TST) and colonic temperature (TC) in morphine-dependent female rats

Experimental Groups	Body wt (g)	Baseline TST (°C)	Mean Maximal TST (°C)	Area Under the 60 min TST Curve	Baseline TC (°C)	Mean Maximal
Experiment 1						
1 week ovariectomized	240	25.7	3.5	70.5	39.7	-1.4
(n, 6)	±4[1]	±0.1	±0.8	±10.5[2]	±0.3	±0.2
intact controls	231	26.0	1.6	29.2	39.6	-1.1
(n, 6)	±6	±0.3	±0.8	±11.7	±0.3	±0.3
2 week ovariectomized	249	26.3	4.6	122.6	39.6	-2.5
(n, 6)	±6	±0.3	±0.6[2]	±8.0[2]	±0.2	±0.7
intact controls	255	26.1	2.1	59.8	40.2	-1.8
(n, 6)	±5	±0.3	±0.9	±9.1	±0.2	±0.2
Experiment 2						
ovariectomized control	265	26.0	5.5	161.0	39.9	-2.8
(n, 6)	±5	±0.2	±0.3	±9.7	±0.3	±0.3
Estrogen-treated	232	26.1	1.6[3]	54.1[3]	39.4	-2.5
(n, 6)	±13[2]	±0.1	±1.0	±18.4	±0.6	±0.3

[1]mean ± standard error of mean; significantly different from respective control.
[2]$p < 0.05$; [3]; < 0.01

RESULTS: Table I summarizes the results from all 3 studies. There was no effect of ovariectomy or estrogen treatment on basal TST or TC between groups in any of the studies. Systemic administration of naloxone resulted in a significant elevation in TST with a corresponding fall in TC. Animals ovariectomized for both 1 and 2 weeks appeared to develop an enhanced TST response to naloxone administrtion when compared to their age-matched, control animals. Not only was the maximal rise in TST elevated in both 1 and 2 week OVEX rats but the integrated area under the TST curve also displayed a similar twofold increase over intact, control female rats; although complete significance was not observed until animals were ovariectomized for at least 2 weeks (Table I, Fig. 1). Ovariectomy did not alter the response of TC to administration of naloxone. Within each study the fall in TC was similar between intact control and OVEX rats; however there appeared to be a trend for a greater fall in TC in the 2 week study compared to the 1 week study.
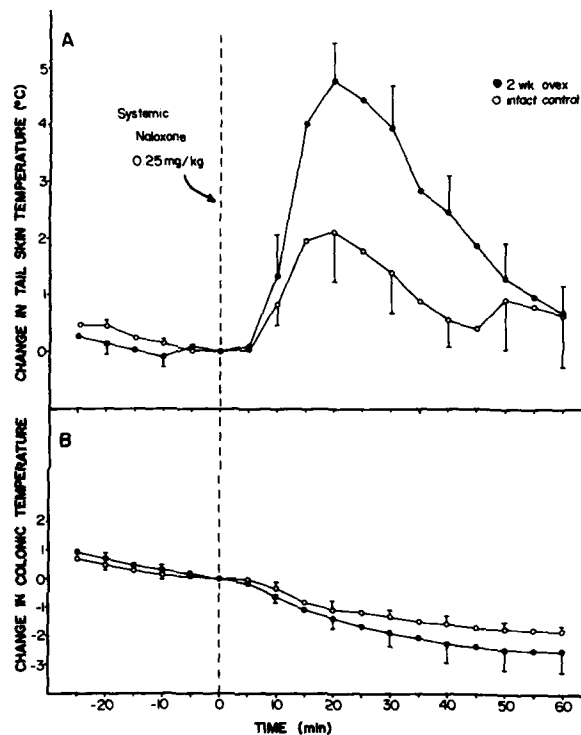
FIGURE 1: Effect of systemic administration of naloxone (0.25 mg/kg sc) on changes in mean tail skin temperature (A) and change in mean colonic temperature (B) in intact (open circle) and 2 week ovariectomized, (closed circles) morphine-dependent female rats. Each point represents the mean of 6 animals and the vertical bar represents one standard error of the mean.

In the second experiment, estrogen treatment significantly reduced the rise in TST associated with naloxone administration in OVEX rats (Fig. 2, Table I). A 3 fold reduction in both the maximal rise in TST and the integrated area under the 60 min TST curve was observed in the estrogen treated rat. The TST values observed in these animals were similar to those observed in the 2 intact, control groups of the first study. Another observation, not statistically verified, was the apparent elevated TST response observed in the OVEX groups with time. Estrogen treatment, however, did not significantly alter the TC response to naloxone as demonstrated by the similar reduction in TC between the 2 groups (Fig. 2, Table I).
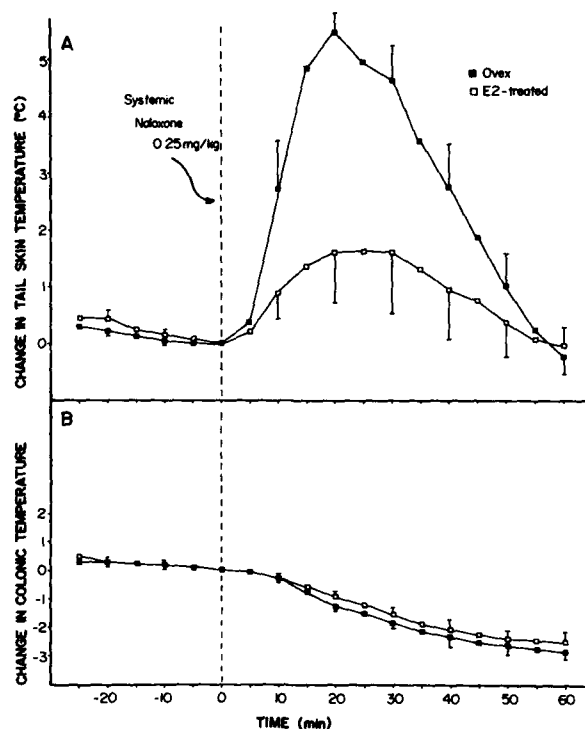
FIGURE 2: Effect of systemic administration of naloxone (0.25 mg/kg sc) on changes in mean tail skin temperature (A) and changes in mean colonic temperature (B) in estrogen-treated (open squares) and ovariectomized (closed squares) morphine-dependent female rats. Each point represents the mean of 6 animals and the vertical bar represents one standard error of the mean.

Serum estrogen levels were significantly elevated in the estrogen treated (65.9±7.9 pg/ml) when compared with the OVEX, controls (14.7±2.1 pg/ml). Although estrogen values were not much above those observed during estrus in rats this prolonged elevation in estrogen levels in OVEX rats resulted in a significant elevation (p<0.001) in both uterine (995±105 mg) and pituitary (30.0±2.1 mg) weights when compared to OVEX controls (244±62 g and 8.7±1.0 g, respectively). In addition, body weight was also significantly depressed in the estrogen treated group (Table I).

DISCUSSION: Although the role of estrogens in the etiology of the hot flush needs to be clarified, there is a strong relationship between low plasma levels of estrogens and the incidence of hot flushes in women. Judd [14] has reported that the level of free circulating estrone and estradiol are lower in women with hot flushes than in women who do not exhibit hot flushes. The results reported here indicate that estrogen removal enhances and estrogen replacement attenuates a rise in skin temperature in the morphine dependent rat suggesting the animal model may be one which can be used to study the mechanisms of the hot flush syndrome.

There are several lines of evidence which suggest an interaction of sex steroids and endogenous opioid peptide (EOP) activity. Support of a functional interaction between estrogens and opioids is the absence of detectable levels of β-endorphin in the hypophyseal portal blood of OVEX monkeys [15] and rats [16] and in postmenopausal women [17]. Furthermore, β-endorphin levels are restored to normal following treatment with sex steroids [16]. These reports would suggest that the activity of EOP's are either absent or diminished when estrogen levels are lowered. Additionally, endogenous opioids have been shown to modify body temperature regulation [18]. Thus, it has been suggested [8-13] that a decline in

the level of sex steroids may decrease EOP activity in the menopause and that this reduced opioid activity may be an integral part of the central mechanism that participates in the genesis of the hot flush response.

The development of the morphine dependent rat as a model to study the mechanism of the menopausal hot flush was one in which EOP is artificially elevated then precipitously reduced with the administration of an opioid antagonist. This withdrawal like response resulted in an increase in heart rate, LH secretion, skin temperature and a fall in core temperature [9-11] that was similar to that observed in women undergoing a menopausal hot flush episode. We have subsequently demonstrated both central adrenergic [19] and dopaminergic [20] changes which are consistent with those reported in postmenopausal women [21,22]. Estradiol and not progesterone reduced the TST response to naloxone in our animal model [23]. However, in the latter study, pharmacological doses of estradiol were evaluated. Previously we were unable to document an effect of ovariectomy. Possible reasons for this were the duration of ovariectomy and the high dose of naloxone (1 mg/kg) that was utilized in these earlier studies. In the current study we used a threshold dose of naloxone to determine if there was any sensitivity difference between intact and ovariectomized rats. The results demonstrate that ovariectomy greater than 1 week duration does result in a more pronounced rise in TST suggesting a role of reduced estrogens in the development of a flushing episode. The current study also utilized a more physiological replacement level of estrogen (2.5x lower than we previously used [23]) to demonstrate that estrogen replacement can attenuate the rise in TST. The serum concentration of estrogen observed in the present study is in a similar range observed by Nequin et al. [24] in the rat during proestrus, although the rat normally does not have this level maintained constantly. This prolonged duration of estrogen treatment could account for the effects on pituitary and uterine weights in the ovariectomized rats.

Nonetheless, these results would support the use of the morphine dependent rat to study the mechanisms of the menopausal hot flush. Utilization of this animal model may allow for determination of the site of action of estrogenic agents in modifying the flush response. Additionally, this animal model can be used to evaluate central neuronal changes which may mediate the flush response.

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HUMAN TYMPANIC MEMBRANE TEMPERATURE DURING WEIGHT LIFTING EXERCISE

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There have been few observations of changes in core temperature (tympanic, T_{ty}) during exercise [1-3,5,8], and no reports of responses of T_{ty} to knee joint extreme flexion and extension (KJEFE) or weight lifting exercises. Thus, the thermal responses, particularly changes in T_{ty}, during KJEFE and the weight-lifting training, were examined in this study.

METHODS: Six normal male university students in physical education participated. T_{ty} was measured with a spring-type probe with $1/100°C$ resolution and $5/100°C$ accuracy [10]. Rectal temperature (T_{re}) and skin temperature (T_s) were measured using thermistor probes. During stage 2 and 3 of the experiment O_2 intake ($\dot{V}O_2$) and heart rate (HR) were measured.

Experimental procedures consisted of the following: Stage 1: The subjects performed KJEFE exercise 12 times for 1 min; Stage 2: The subjects performed KJEFE exercise 30 times/min for 5 min; Stage 3: the subjects performed bicycle ergometer exercise at a rate of 150 watt for 5 min; Stage 4: One of subjects weight lifted for 60 min.

RESULTS: Fig. 1 shows the results of stage 1 experiments on 6 subjects. KJEFE caused a decrease in T_{ty} by 0.06–0.1°C with 7–10 sec latency from the start of exercise for 1 min, a decrease in T_s of forehead and thigh regions by 0.05–0.45°C, 0.2–0.5°C respectively, with a 4–7 sec latency after the start of exercise for 1 min. T_{re} remained almost unchanged.

Fig. 2 shows the results of a stage 2 experiment on subject S.T. KJEFE caused a decrease in T_{ty} of 0.05°C with 7 sec latency and a fall of 0.13°C after 5 min of exercise. A rise in T_{re} (0.15°C) occurred at the end of exercise. KJEFE caused a decrease in T_s of the forehead and thigh regions 0.2, 0.5°C respectively with 4 sec latency. $\dot{V}O_2$ measured after exercise for 5 min was 1.90 l/min. HR increased after exercise for 5 min to a peak level of 156 beats/min.

Fig. 3 shows the results of a stage 3 experiment on subject S.T. T_{ty} remained unchanged for 1 min after the start of ergometer exercise and then gradually decreased to reach a minimum level after 3 min. T_{re} increased during exercise and during a 5 min recovery period reached its maximum level. $\dot{V}O_2$ at the end of exercise was 2.04 l/min. HR 5 min after the start of the exercise rose to 150 beats/min.

Fig. 4 shows change in T_{ty} during KJEFE and 150 watt bicycle ergometer exercise. The decrease in T_{ty} during KJEFE was greater than during bicycle exercise by 0.09°C and the increase after KJEFE was greater than after bicycle exercise by 0.1°C.

Fig. 5 shows an increase in T_{re} during KJEFE and 150 watt bicycle ergometer exercise. The increase in T_{re} during KJEFE was greater than during bicycle exercise by 0.07°C. Also, T_{re} during 10 min recovery after KJEFE was greater than during recovery from bicycle exercise by 0.24°C.

Fig. 6-a and Fig. 6-b show body temperature changes during weight lifting training for 60 min. Subject rested in a standing position. Exercises were: weight (20 kg) lifting 3 times; military press (30 kg 3 times, 40 kg 5 times); snatch (60 kg 3 times, 80 kg 1 time); performed in an 18 min period. Difference between T_{re} and T_{ty} after the trials increased by 1.35°C (Fig. 6-a). Following trials of standing press 60 kg and set press 60 kg respectively 1 time, standing press 40 kg 5 times, press 90 kg, jerk 90 kg, power jerk 90 kg, jerk 110 kg, jerk 90 kg performed in 30 min. T_{ty} decreased by 0.4°C and T_{re} increased by 0.25°C. The difference between T_{re} and T_{ty} was 1.38°C (Fig. 6-b).
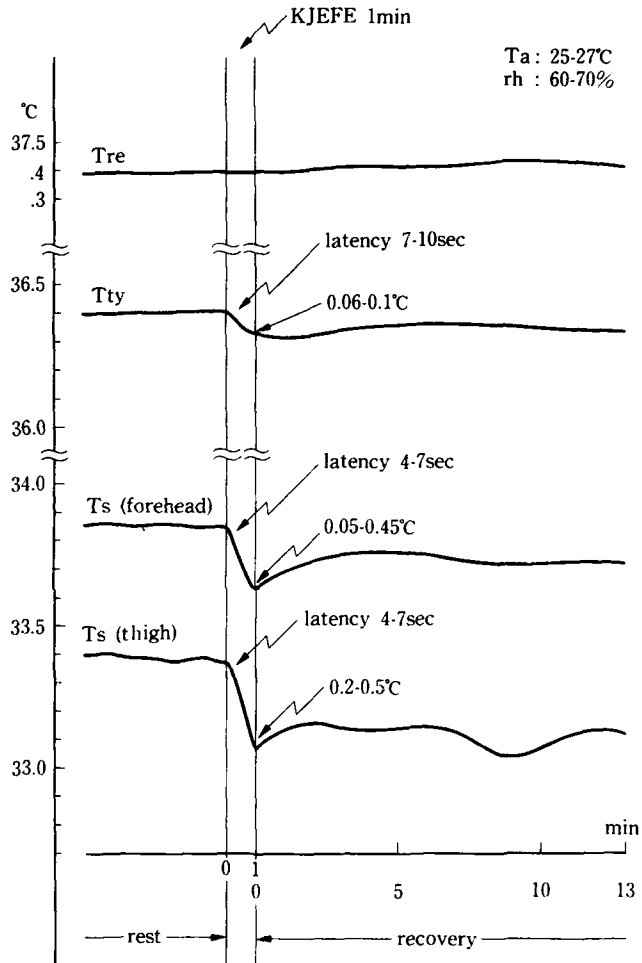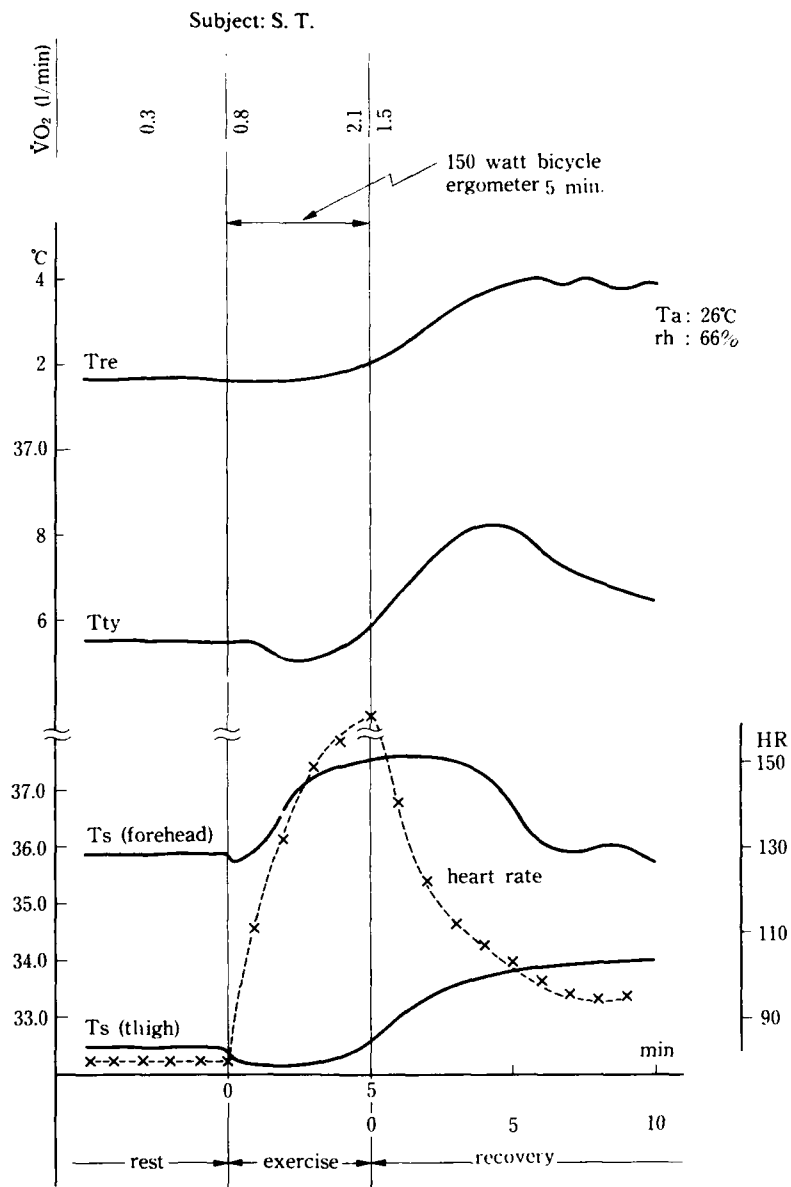

FIGURE 1: Changes in temperature during knee joint extreme flexion and extension (KJEFE) 12 times/min.
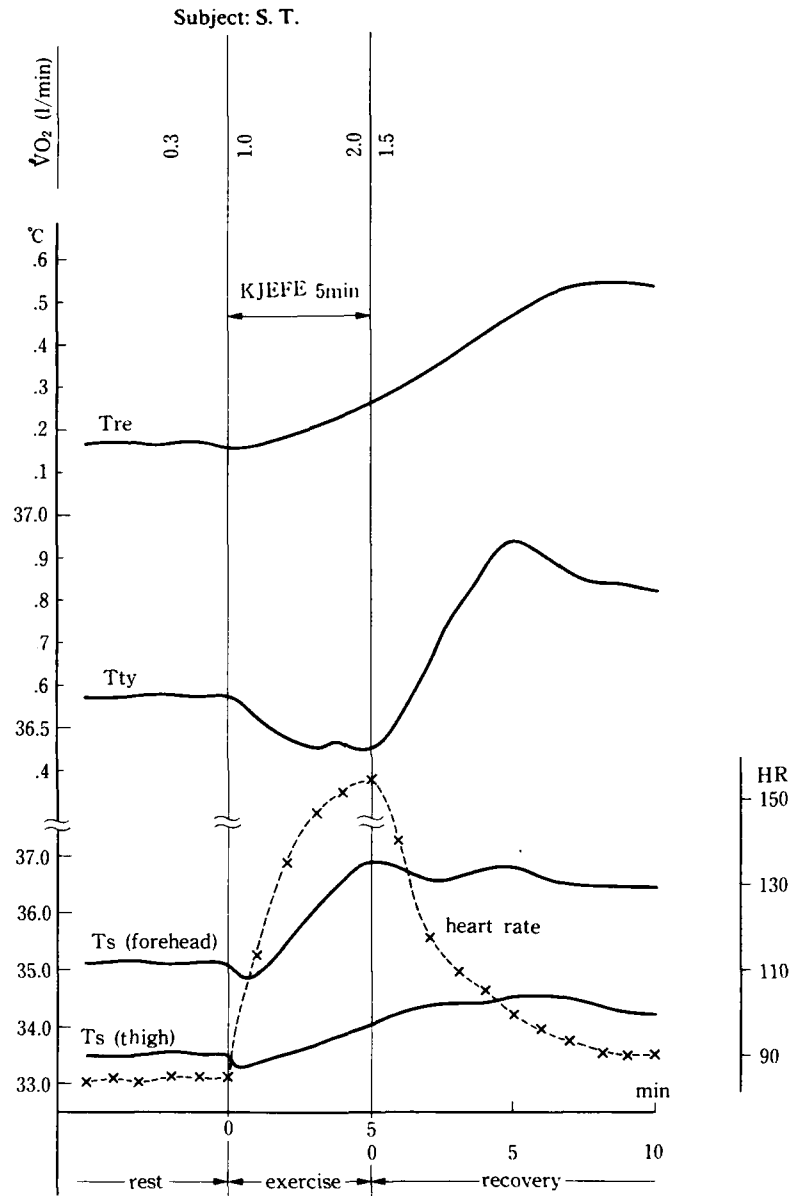
FIGURE 2: Changes in temperature, oxygen intake ($\dot{V}O_2$, l/min) and heart rate (HR) during knee joint extreme flexion and extension (KJEFE) 30 times/min for 5 min.

FIGURE 3: Changes in temperatures, oxygen intake ($\dot{V}O_2$, l/min) and heart rate (HR) during bicycle ergometer (150 watt) exercise for 5 min.
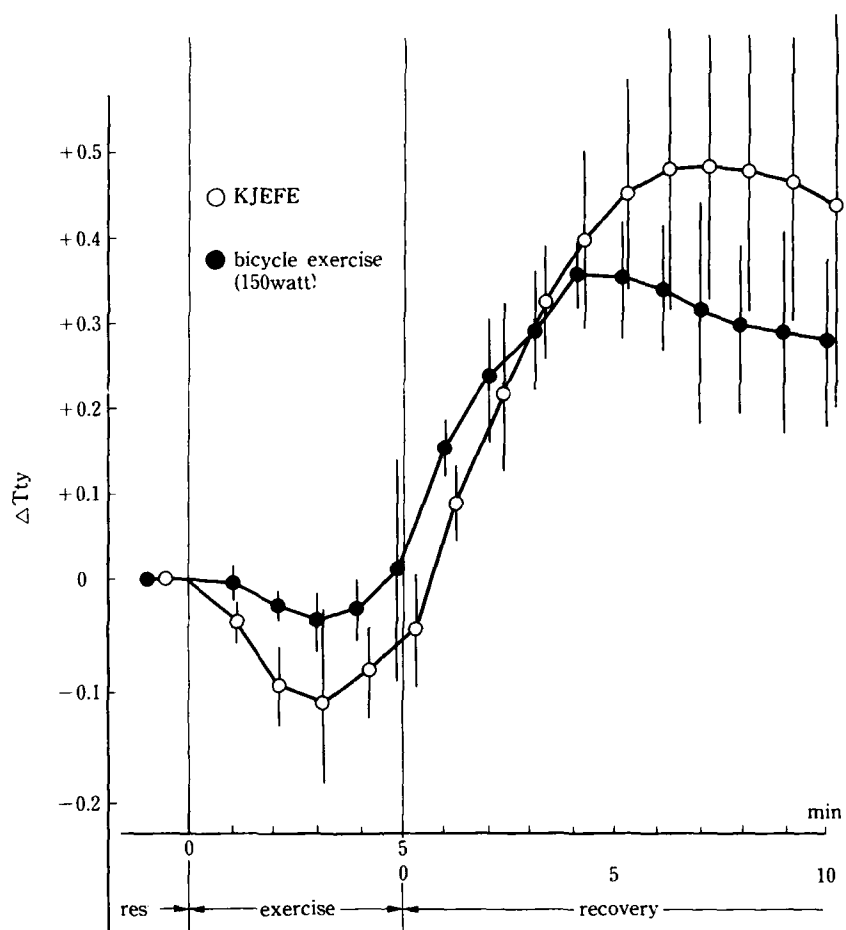
FIGURE 4: *Increases in tympanic membrane temperature* (T_{ty}) *during extreme knee joint flexion and extension (KJEFE), and bicycle ergometer exercise (150 watt) in 3 subjects.*
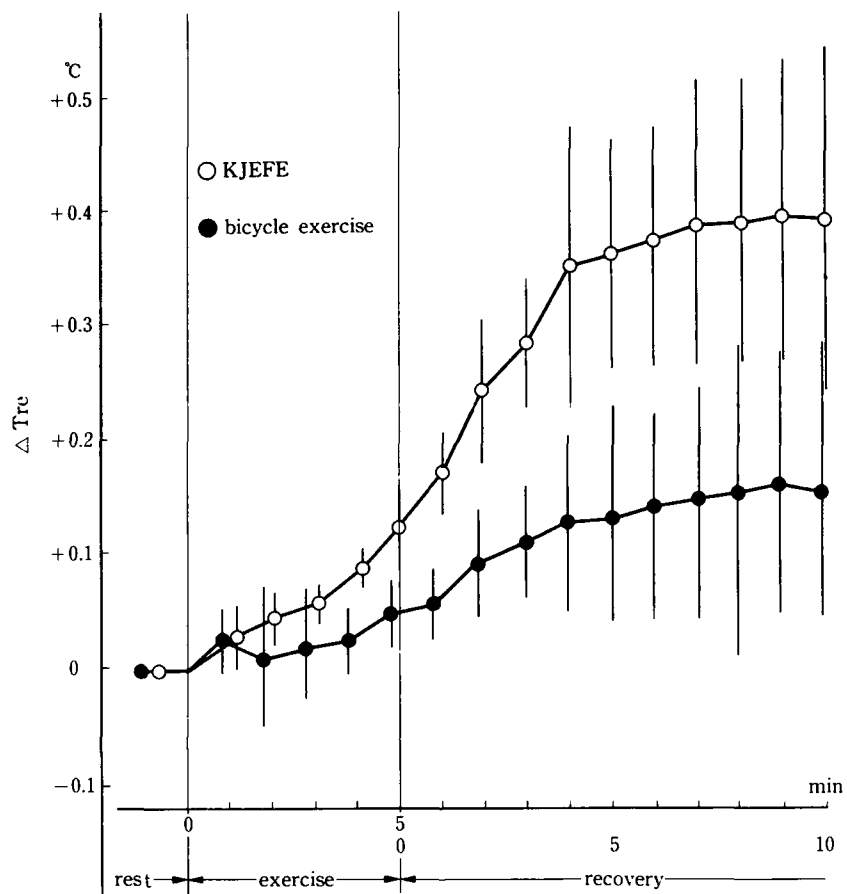
FIGURE 5: Increases in rectal temperature (T_{re}) during extreme knee joint flexion and extension (KJEFE), and bicycle ergometer exercise (150 watt) in 3 subjects.
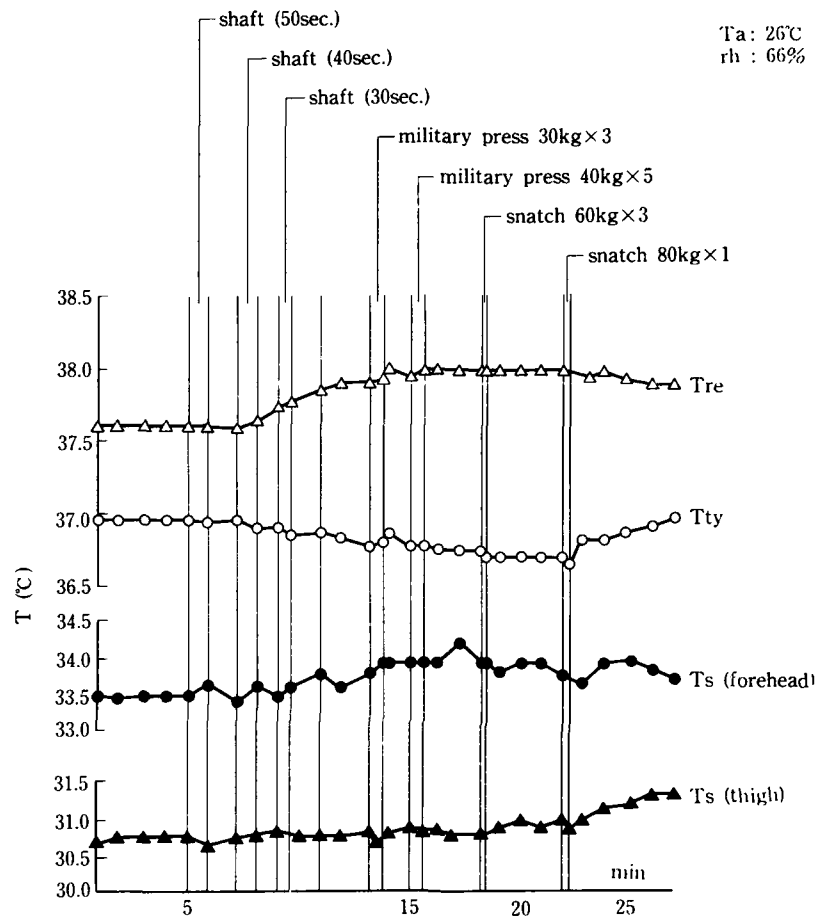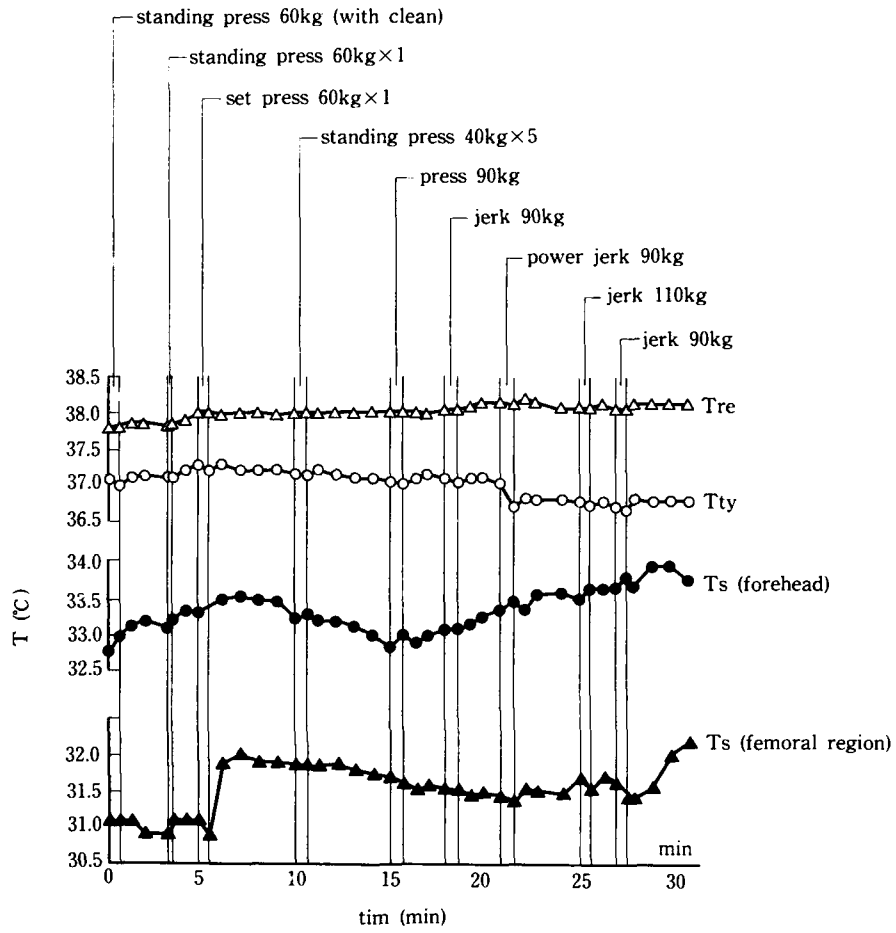
FIGURE 6-a: Relationship between tympanic membrane temperature (T_{ty}), rectal temperature (T_{re}) and skin temperature (T_s) 30 min after the start of weight lifting training.

FIGURE 6-b: Relationship between tympanic membrane temperature (T_{ty}), rectal temperature (T_{re}) and skin temperature (T_s) from 30 min to 60 min after the start of weight lifting training.

DISCUSSION: After the start of the exercises, T_{ty} slightly decreased with a latency of more than 30 sec and thereafter increased during exercise. The initial fall in T_{ty} is probably due to vasoconstriction in the skin from sympathetic excitation. Cold blood in the skin flows into the body core cooling the arterial blood by counter current heat exchange. This cools the blood in the internal carotid artery and results in the initial fall in T_{ty} with latency of more than 30 sec [3]. It was found that T_{ty} during KJEFE, or weight lifting from a squatting to a standing position, dropped by 0.06-0.1°C and 0.05°C, respectively, with considerably shorter latencies of 7-10 sec than those observed in the initial fall after beginning locomotive exercise. Why did T_{ty} fall with such short latencies? Rossberg and Penaz [10] reported that blood pressure decreased at 7.1 ± 1.1 sec after a squatting-standing manoeuvre, due to activation of the baroreflex. After a squatting-standing manoeuvre the blood flow in the common carotid artery may be decreased by this reflex. We have found a decrease in blood flow in the common carotid artery of 36% and decrease in T_{ty} of 0.1°C (in press).

Impulses reaching the baroreceptors during KJEFE or squatting-standing manoeuvre should be considered. The medial nerve suppling the cat's knee joint contains a high percentage of group III and IV fibers. Joint movements except for extreme flexion, extension and, in particular, rotation of the joint, do not activate these afferent nerves [11]. It is possible that these polymodal afferent nerves projecting from the knee joint would also stimulate the baroreceptors in humans. Afferent impulses from joints play an important role in controlling body temperature during exercise [11,7]. The group III and IV fibers of the afferent nerve from skeletal muscles such as the gastrocnemius, relay to the hypothalamus [6,8]. Impulses from thermosensitive elements in the femoral veins [12] or intra-abdominal veins [4] also may reach the hypothalamus. These mechanisms could explain the decrease in T_{ty} after the onset of KJEFE or weight lifting exercises.

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IONIZING RADIATIONS AND THERMOREGULATION

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Ionizing radiations are defined as those types of particle and electro-magnetic radiations that inter-act with matter and, either directly or indirectly, form ion pairs. Consequently, ionizing radiations are divided into two general categories: directly ionizing and indirectly ionizing. Directly ionizing radiations are charged particles (electrons, protons, beta particles, positrons, alpha particles, etc.) that have sufficient kinetic energy to produce ionizations through direct coulombic interactions (or collisions) with the bound orbital electrons of an atom or molecule. Indirectly ionizing radiations (X rays, gamma rays, neutrons, etc.) are uncharged particles or photons that can liberate bound orbital electrons, but only indirectly, i.e., secondarily through a preliminary interaction [1]. Regardless of the type of ionizing radiation, the final common event in all modes of absorption of ionizing radiation is the ejection or excitation of bound orbital electrons. Exposure to ionizing radiation may lead to disruption of normal behavior and physiological mechanisms. They can induce nausea, vomiting, hypotension, hyperthermia and decrements of cognitive performance [1].

Changes in body temperature can be observed after radiation exposure [2], an effect that depends on the species being used. For example, radiation induces hyperthermia in cats, rabbits [3] and humans [4], a biphasic response in monkeys (a fall followed by a rise [5]), a dual effect in rats (low and high doses produced hyper- and hypothermia, respectively [6] and hypothermia in guinea pigs [7]. In this paper we provide a review of the work which is being done in our laboratory [6-9]. Radiation induces hyper- or hypothermia in rats or guinea pigs and is a result of a direct effect on the brain, since these temperature responses occurred only when after whole body or head only exposure, not when the head was shielded [6,7].

Experiments were undertaken to determine what mechanisms may underlie radiation induced changes in body temperature by comparing radiation to the effects of drugs with known actions and by determining if antagonists to these drugs could block the effects of radiation.

As can be seen in Table I pretreatment with indomethacin inhibited the hyperthermia induced by 1-15 Gy of radiation [6]. In addition, indomethacin given immediately after determining body temperature 15 min after irradiation rapidly reversed the hyperthermia [6]. Naloxone attenuated hyperthermia after radiation doses of 1-3 Gy but the hyperthermia due to 5-15 Gy of radiation was unaffected. These findings suggest that radiation induced hyperthermia may be mediated through the synthesis and release of prostaglandins (PGs) in the brain and, to a less extent, to the release of endogenous peptide opioids [6].

Radiation increases the release of ACTH and neurotensin into blood. The presence of ACTH in the hypothalamic region, its concentration in nerve fibers and synaptosomes and the production of hypo-thermia by central administration of this peptide suggest that it may take part in the control of body temperature. There are reports that some neuropeptides are several hundred to a thousand times more potent antipyretics than acetaminophen on a molar basis [10]. Lower, nonhypothermic doses of ACTH reduced febrile responses to endotoxin, PGE2 and radiation [9].

As seen in Table II the release of histamine acting on H_1 and H_2 receptors may be involved in radiation induced hypothermia, since both H_1 receptor antagonist mepyramine and H_2 receptor antagonist cimetidine, antagonized it [6,7]. Serotonin is not involved, since the serotonin receptor antagonist methysergide had no antagonistic effect on radiation hypothermia [6,7].

TABLE I   Effect of indomethacin or naloxone on radiation-induced hyperthermia in rats

Pretreatment	Dose of Radiation (Gy)	Antagonism
Indomethacin	1-15	Yes
Naloxone	1-3	Yes
Naloxone	5-15	No

TABLE II   Effect of mepyramine or cimetidine or methysergide on radiation-induced hypothermia in rats and guinea pigs

Pretreatment	Antagonism
Mepyramine	Yes
Cimetidine	Yes
Methysergide	No

A recent development in radiotherapy originating from radiobiological studies is the use of radioprotective drugs to reduce injury to normal tissues [11]. WR-2721 (S-2-(3-aminopropylamino)-ethylphosphorothioic acid), a thiophosphate derivative of cysteamine with the official generic name of ethiofos in the United States and gammaphos in the Soviet Union, exerts a radioprotective effect in several animal species. Because this drug protects normal tissues to a greater extent than most malignant tissues, its use in cancer radiotherapy is being investigated. Phase I and II clinical trials are currently underway [12]. Presumably this drug is dephosphorylated *in vivo* to yield the active thiol form WR-1065, N-(2-mercapto-ethyl)-1,3-diaminopropane [13]. We studied the radioprotectant effect of WR-2721 and WR-1065 in guinea pigs and they induced hypothermia when injected systemically or centrally [8]. Similar hypothermic responses have been noted to occur after administration of a variety of radioprotectants [14]. The mechanisms that explain chemical radiation protection are complex and as yet not completely understood. Although radioprotectants scavenge free radicals generated by radiation [11], it also has been suggested that the hypothermia may mediate radioprotection by two mechanisms [15,16]. The reduced metabolic activity that accompanies hypothermia may allow more complete and efficient repair of radiation damage [15,16]. Alternatively damage producing reactions following the absorption of energy may be slower and less complete, thereby resulting in reduced sensitivity [15,16]. This investigation was done to determine whether radioprotectants, such as WR-2721 or WR-1065, could antagonize hypothermia induced by exposure to ionizing radiation in guinea pigs [8]. Since oxygen tension plays a critical role in radioprotection [17], and anoxia is one proposed mechanism for protection by aminothiols in mammalian and aerated cells [18], the effect of WR-2721 and WR-1065 on oxygen uptake was measured in brain homogenates from guinea pigs [8].

As seen in Table III WR-2721 attenuated and WR-1065 accentuated radiation hypothermia, respectively. In brain homogenates, oxygen uptake was inhibited by WR-2721 but elevated by WR-1065. These results suggest that the antagonism of radiation induced hypothermia is due to direct actions and not to a dephosphorylated metabolite and that this effect may be correlated with inhibition, by WR-2721, of oxygen uptake [8].

TABLE III   Effect of WR-2721 and WR-1065 on radiation-induced hypothermia and oxygen uptake in
            guinea pigs

Pretreatment	Radiation-hypothermia	Oxygen Uptake
WR-2721	Attenuated	Inhibited
WR-1065	Accentuated	Enhanced

These studies suggest that the release of neurohumoral substances induced by exposure to ionizing radiation has different consequences on physiological processes, such as the regulation of body temperature. Furthermore, the antagonism of radiation induced hyperthermia by indomethacin and ACTH may have potential therapeutic implications in the treatment of fever resulting from accidental irradiations. Our long term goal in this project is to gain more information on the mechanism of radioprotectant induced hypothermia, to facilitate the development and evaluation of better radioprotectants using experimental hypothermia as an end point.

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CHOLERA TOXIN ALTERS PROTEIN EXPRESSION IN BROWN ADIPOCYTES DIFFERENTIATING IN CULTURE

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As a means of studying the growth, proliferation and differentiation of the brown adipocyte, primary cell culture systems have been developed. The precursor cells for the cultures have been isolated from the stromal vascular fraction of brown fat from fetal rats [1,2], weanling rats [3,4], human newborns [5], human fetuses [6], and juvenile mice [7]. Also a brown fat clonal cell line has been developed [8]. Under suitable conditions these cells grow in monolayer culture into cells which have many of the characteristics of the mature brown adipocyte.

In culture, the precursor cells accumulate lipid droplets in a multilocular fashion; the size of these droplets is consistently smaller than those in precursors from white fat developing under identical conditions. The brown fat precursors demonstrate higher cytochrome-c-oxidase activity than the white fat precursors, indicating a higher mitochondrial content [3]. During the first week of development, the mitochondria in the brown fat cells differentiating in culture have similar characteristics to those in the mature brown adipocyte, but later they tend to dedifferentiate indicating that optimal culture conditions have not yet been achieved for full differentiation [4]. The presence of the brown fat specific uncoupling protein (UCP or thermogenin) is yet to be confirmed in these cultures. The cells in culture demonstrate a functional β-adrenergic response [9], insulin response [2] and show indications of α_2-adrenergic inhibition of fatty acid release [9]. Taken together, these results indicate that precursor cells obtained from brown adipose tissue are determined for showing brown fat characteristics and rapidly become committed.

The β-adrenergic agonist isoprenaline has been shown to increase the expression of differentiation markers associated with adipocyte differentiation in mouse brown fat precursor cells and brown fat derived clonal cells grown in primary culture [7,8]. Similarly, Néchad et al. [10] have shown that chronic exposure of the rat brown fat precursor cells to norepinephrine stimulates mitochondriogenesis and that this effect could be mimicked by cholera toxin. These results indicate that β-adrenergic agents can influence the differentiation of the brown adipocyte in vitro.

The aim of the present study was to further analyse the effects of the cholera induced stimulation on differentiating brown adipocytes. As part of the approach, the effect of cholera toxin on overall protein expression in the developing precursor cells was investigated.

METHODS: Brown fat precursor cells were cultured essentially according to the method of Néchad et al. [3], except for the use of insulin (only 1 nM) in the culture medium. The cells were grown in parallel with and without the addition of cholera toxin (1 μg/ml) from day 1. At confluence, the culture medium was removed and the cells were rinsed three times with sucrose (250 mM) and then harvested by scraping into 2 ml of sucrose. The cells were lysed by freeze-thawing and the protein concentration of the whole cell lysate determined by the method of Bradford [11]. SDS-PAGE was performed according to Laemmli [12] with acrylamide (10%) and bisacrylamide (0.3%) and the protein bands were visualised by silver staining [13]. For semi-quantitative analysis the gels were scanned with a densitometer and the relative integrated peak areas compared.

RESULTS: The exposure of developing brown adipocytes to cholera toxin resulted in a number of changes in the pattern of protein expression (Fig. 1). Most notable was the change concerning a protein of approximate molecular weight 35 kDa. Preliminary results indicate that this protein increased in amount by approximately 75%, after cholera toxin treatment. The function of the cholera toxin inducible protein (CHIP) is at present unknown. This presumed effect of cholera toxin on gene expression in these cultures has been demonstrated in our laboratory as an increase in mRNAs coding for lipoprotein lipase and cytochrome-c-oxidase (unpublished).

67   43   36 molecular weight
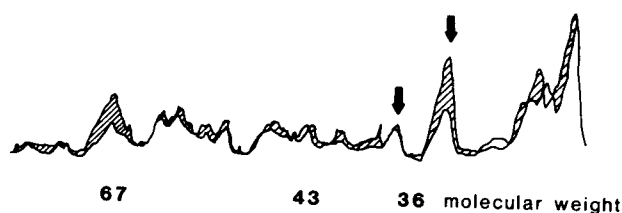
FIGURE 1: Densitometric scans of two tracks of a silver-stained gel. A scan of proteins from cholera toxin exposed cultures is superimposed on a control scan. Changes in protein levels are represented by the shaded areas. Right-hand arrow shows position of the 35 kDa cholera toxin inducible protein (CHIP) and the middle arrow points to an unaffected reference protein.

CONCLUSION: Chronic exposure of developing brown fat precursor cells to cholera toxin results in a change in protein expression of the cells, especially on the induction of the 35 kDa protein. This CHIP may be considered as an expression of increased differentiation, in being, for example, a mitochondrial protein or another protein essential for the thermogenic process.

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INFLUENCE OF HYPOTHYROIDISM ON BROWN ADIPOSE TISSUE

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The highly active thyroxine deiodinase within the brown adipose tissue, which may be necessary for the recruitment of the tissue in adaptive states, has renewed interest in the interaction between the "thyroid" and "adrenergic mechanisms" of thermogenesis. We have studied the effects of hypothyroidism on whole animal metabolism and on the recruitment state of brown adipose tissue.

To investigate this, rats were made hypothyroid by addition of methimazole to the drinking water, and their metabolism and brown adipose tissue were investigated.

Hypothyroidism led to the expected decrease in basal metabolic rate, and norepinephrine lost its ability to increase the metabolic rate.

The cause of this lack of response to norepinephrine was investigated at the brown adipose tissue level.

The number of both β-receptors (estimated with the ligand [^{3}H]CGP-12177) and of α-receptors (with [^{3}H]prazosin) was practically unchanged, with a small tendency to an increased ratio α_1/β. This is what would be expected in a recruited state.

The amount of the uncoupling protein thermogenin, estimated as GDP-binding to isolated mitochondria, was not markedly different.

The conclusion was that hypothyroidism had induced an ability of the tissue to couple the signal from the receptor to the mitochondria, but that the total thermogenic potential was essentially intact.

EFFECT OF MILD COLD ON XENOBIOTIC METABOLISM INDUCTION AND NADPH GENERATING CAPACITY IN MOUSE LIVER

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The liver plays an important role in the biotransformation of drugs and other exogenous chemicals [1,2]. The ability of the liver to metabolize these compounds is subject to genetic control and is influenced by various factors of which the role of animal species, strain, sex, age and nutritional status are well known [3]. Environmental temperature affects the liver xenobiotic metabolism activity, the change being dependent on the temperature, the duration of exposure and the measured parameters [4,5]. Mild cold (+14°C) exposure has been found to decrease liver xenobiotic metabolism ability [6].

Many drugs, insecticides, carcinogens and other chemicals induce liver xenobiotic metabolizing enzymes in animals and in man [1,2]. Most studies are, however, performed at room temperature, and little is known about enzyme induction in a cold environment. We investigated the effects of phenobarbital (PB), the best known enzyme inducer, on the amounts of two components of the microsomal electron transport chain, cytochrome P450 (cytP450) and NADPH cytP450 reductase, and on the activity of a cytP450 associated enzyme, 7-ethoxyresorufin 0-deethylase (ERDE) during a 1 week or 4 week period of exposure to mild cold (+14°C).

Liver cytP450 mediated xenobiotic metabolism requires NADPH as a reducing equivalent [7]. Kauffman and his co-workers [8] have demonstrated that PB treatment increases the use of cytocolic NADPH, and that this compound compensates for this increase in NADPH use by enhancing its production. We have shown previously that mild cold may affect the ability of the liver parenchyma to generate NADPH [9]. In this study we report on the effects of PB treatment on four hepatic NADPH generating enzymes, glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), malic enzyme (ME) and isocitrate dehydrogenase (ICDH) in mice exposed to mildly cold conditions.

METHODS: Thirty six male mice of the C57BL/6J strain were utilized. The animals were allowed tap water and standard rodent chow freely and maintained under controlled conditions of light, humidity and temperature (+23 or +14°C).

The mice were randomly divided into six groups of six animals each before the trial. Two groups were exposed to +14°C for 1 week and one of these groups simultaneously received PB (0.5 g/l) in their drinking water and the others received no other treatment. Two groups were exposed to +14°C for 4 weeks and one of these groups received PB in their drinking water during this time and the other group received no other treatment. Two groups were kept at room temperature (+23°C) and one of these groups received PB in their drinking water for a week and the other group received no other treatment. The last was used as a control group.

The liver samples for drug metabolism assays were homogenized in 4 vol of sucrose (0.25 M), pH 7.4 and centrifuged at 10,000 g for 20 min, and the supernatant obtained was subsequently centrifuged at 105,000 g for 60 min. The microsomal pellet obtained was resuspended in glycerol (30%) in a Na/K phosphate buffer (0.25 M), pH 7.25, in such a way that 1 ml of the suspension contained the microsomes from 1 g of liver.

Micrsomal cytP450 content, NADPH cytP450 reductase and ERDE activity were measured in the microsomal fraction according to the methods of Omura and Sato [10], Masters et al. [11] and Burke and Mayer [12], respectively. The protein content was determined according to Lowry et al. [13].

The activities of NADPH generating enzymes, G6PDH, 6PGDH, ME and ICDH were estimated by monitoring the liberation of NADPH as described by Marks [14,15] and Ochoa [16,17], respectively.

Statistical evaluation of the results was performed using variance analysis and Newman-Keul's test.

RESULTS: A 1 week period of exposure to +14°C, with no other treatment, lowered the liver xenobiotic metabolism capacity as compared to that observed in control mice kept at room temperature (Fig. 1). The microsomal cytP450 content decreased by approximately 30% and the activities of NADPH cytP450 reductase and ERDE by 35% and 58%, respectively. The liver weight and microsomal protein content remained unaltered in the animals. The activity of one NADPH generating enzyme, 6PGDH, was decreased by approximately 20% during a 1 week period of exposure to +14°C whereas the activities of G6PDH, ME and ICDH varied within the control range (Fig. 2).

Similarly, the liver xenobiotic metabolism capacity was also lowered in those animals exposed to such conditions for 4 weeks. The microsomal cytP450 content was reduced by approximately 47% and the NADPH cytP450 reductase and ERDE activities by 30% and 72%, respectively. The liver weight and microsomal protein content remained unaltered. The activities of two NADPH generating enzymes, G6PDH and ICDH, were increased by approximately 70% and 30%, respectively, during a 4 week period of exposure to +14°C, whereas 6PGDH and ME varied within the control range (Fig. 2).

Administration of PB for 1 week induced liver xenobiotic metabolism in mice kept at room temperature as indicated by an increased amount of microsomal cytP450 and enhanced activity of ERDE (Fig. 1). The liver weight was increased by approximately 12% (from 1.13 ± 0.08 g in controls to 1.27 ± 0.09 g in PB treated animals, p < 0.05) and the microsomal protein content increased by 21%. The activities of two NADPH generating enzymes, G6PDH and ICDH, were increased by approximately 20% and 30%, respectively, and the activities of 6PGDH and ME varied within the control range (Fig. 2).

Simultaneous +14°C exposure and PB treatment for 1 week increased the liver weight by approximately 65% (from 1.13 ± 0.08 g to 1.87 ± 0.3 g), whereas the liver microsomal protein content, cytP450 content and the activities of NADPH cytP450 reductase and ERDE varied within the control range (Fig. 1). The microsomal cytP450 content was, however, increased by approximately 80% as compared to the corresponding value in mice exposed to +14°C alone, and the activities of NADPH cytP450 reductase and ERDE were increased by 80% and 160%, respectively.

Furthermore, administration of PB enhanced all the NADPH generating enzyme activities in +14°C exposed animals, the extent of the increase being approximately 93% in G6PDH activity, and 34%, 85% and 34% in 6PGDH, ME and ICDH activities, respectively (Fig. 2). The activities of three NADPH generating enzymes, G6PDH, 6PGDH and ME, were also elevated in relation to the values obtained in mice exposed to +14°C. alone.

Simultaneous +14°C exposure and PB treatment for 4 weeks increased the liver weight by approximately 52% (from 1.13 ± 0.08 t 1.72 ± 0.27 g, p < 0.01) and the microsomal protein content by 60% (Fig. 1). The liver microsomal cytP450 content and NADPH cytP450 reductase activity varied within the control range and the activity of ERDE was lowered by approximately 40%. The amount of cytP450 was, however, increased by approximately 145% as compared to the corresponding value obtained in mice exposed to +14°C alone, and NADPH cytP450 reductase activity was increased 75% and ERDE activity 120%.

Furthermore, PB treatment increased the activity of one NADPH generating enzyme, G6PDH, by approximately 40%, but the activities of 6PGDH, ME and ICDH varied within the control range (Fig. 2). The activity of G6PDH in these PB treated animals did not, however, diverge from that induced by 4 week period of exposure to +14°C alone.

DISCUSSION: The liver xenobiotic metabolizing system is comprised of three integral components; a hemoprotein, cytP450, which catalyzes the terminal oxidation of the substrate; a flavoprotein, NADPH cytP450 reductase, which mediates electron transport from NADPH to cytP450; and membrane bound phospholipids [1,2]. Takano and Miyazaki [5] have demonstrated that 24 h exposure to +4°C increases the amount of liver microsomal cytP450 in rats, and that the enzyme content then returns to the control level during a 14 or 30 day period of such exposure. Mild cold (+14°C) has, in turn, been found to decrease the liver cytP450 content after a 1 or 4 week period of exposure to such temperatures [6]. The effect of cold on cytP450 linked xenobiotic metabolism activity *in vitro* depends on the measured parameter and both increased (p-hydroxylation of aniline and N-dealkylation of ethylmorphine) and decreased (NADPH cytP450 reductase and ERDE) activities have been described [4-6].
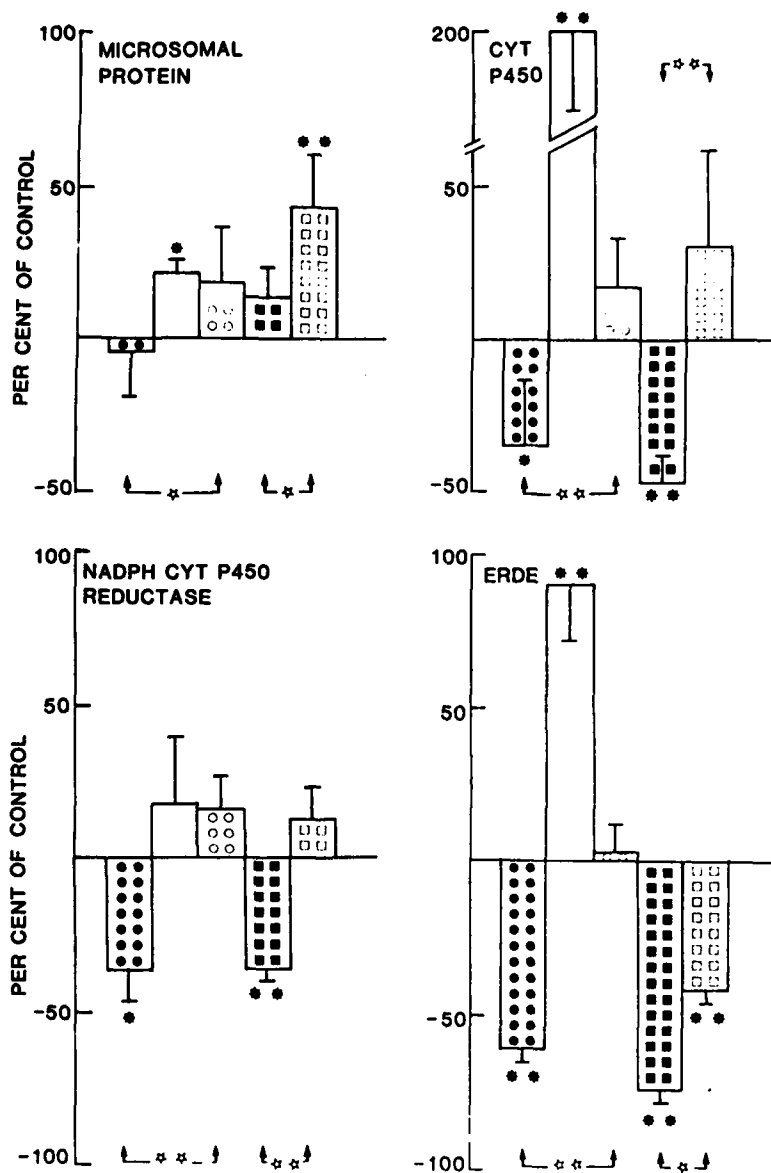
FIGURE 1: Liver xenobiotic metabolism capacity in +14°C exposed and/or phenobarbital (PB) treated mice. The liver microsomal protein content in the control mice was 15.28 ± 1.28 mg/g liver, the cytP450 content 0.75 ± 0.17 nmol/mg microsomal protein and the activities of NADPH cytP450 reductase and 7 ethoxyresorufin O-deethylase (ERDE) 73.83 ± 18.62 nmol/mg microsomal protein and 5.38 ± 1.07 pmol/mg microsomal protein, respectively. Symbols: (●), cold exposure alone for 1 week; ( ), PB treatment alone for 1 week (at room temperature); (O), simultaneous PB treatment and cold exposure for 1 week; (■), cold exposure alone for 4 weeks; (▢), simultaneous PB treatment and cold exposure for 4 weeks. * : $p < 0.05$ and ** : $p < 0.01$ as compared to the values obtained in control mice kept at room temperature. Accordingly, ☆ : $p < 0.05$ and ☆☆: $p < 0.01$ as compared to the values obtained in mice exposed to +14°C alone (for 1 or 4 weeks). Statistical evaluation of the data was performed using absolute values.
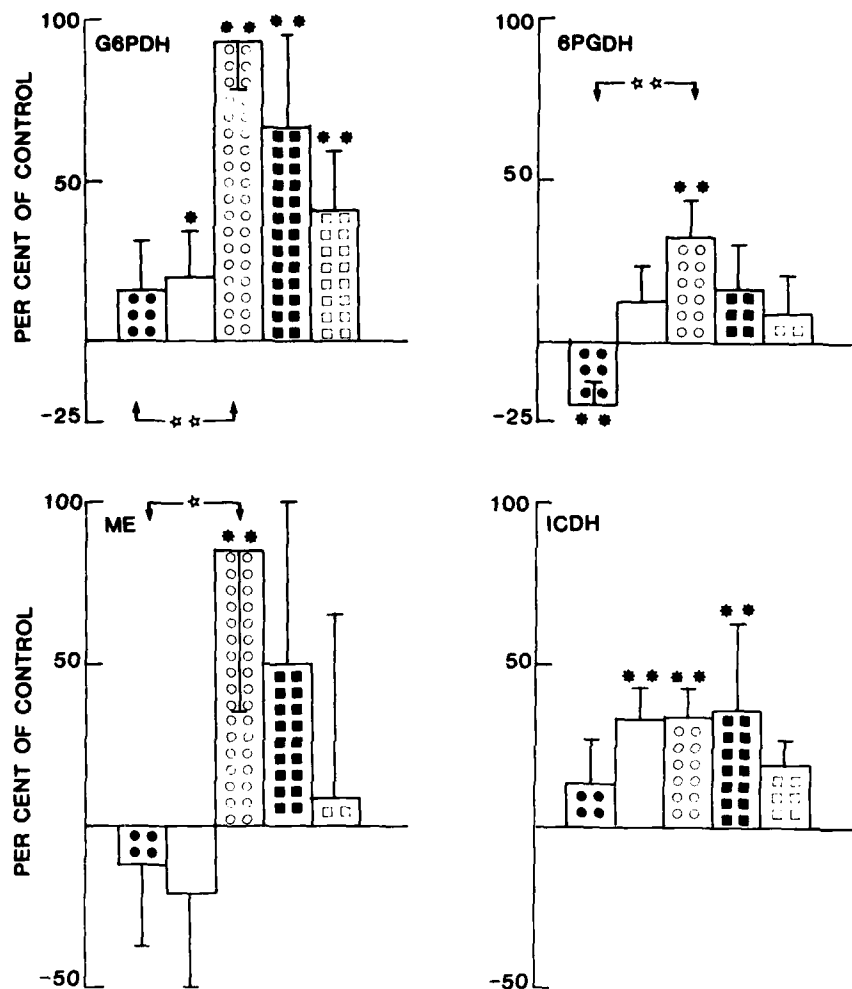
FIGURE 2: Liver NADPH generating capacity in cold exposed mice with or without phenobarbital (PB) treatment. The glucose-6-phosphate dehydrogenase (G6PDH) activity in the control mice was 8.45 ± 0.86 nmol/min/mg protein and the activities of 6-phosphogluconate dehydrogenase (6PGDH), malic enzyme (ME) and isocitrate dehydrogenase (IDH) 20.53 ± 2.08 nmol/min/mg protein, 19.75 ± 3.25 nmol/min/mg protein and 0.26 ± 0.30 μmol/min/mg protein, respectively. Symbols: (O), cold exposure alone for 1 week; ( ), PB treatment alone for 1 week (at room temperature); (O), simultaneous PB treatment and cold exposure for 1 week; ( ), cold exposure alone for 4 weeks; ( ), simultaneous PB treatment and cold exposure for 4 weeks. * : p < 0.05 and ** : p < 0.01 as compared to the values obtained in control mice kept at room temperature. Accordingly, ☆ : p < 0.05 and ☆☆: p < 0.01 as compared to the values obtained in mice exposed to +14°C alone (for 1 or 4 weeks). Statistical evaluation of the data was performed using absolute values.

A number of drugs and environmental chemicals induce liver cytP450 mediated xenobiotic metabolism. Characteristic of this induction phenomenon is growth of liver size, increased amount of microsomal protein, proliferation of smooth endoplasmic reticulum, increased amount of microsomal cytP450 and enhanced activities of one or more of the cytP450 linked enzymes [1-3], as seen also here in PB treated mice. Most of the factors that affect liver xenobiotic metabolism capacity may also affect induceability of the enzymes involved in xenobiotic metabolism, e.g., induction of aryl hydrocarbon hydroxylation (AHH) is subject to genetic control [19] and depends on sex [20]. The present data demonstrate that environmental temperature does not greatly affect the induceability of cytP450 linked xenobiotic metabolism in mice. The administration of PB increased the liver microsomal cytP450 content by approximately 220% in mice kept at room temperature and by 80-145% in +14°C exposed animals. Accordingly, NADPH cytP450 reductase activity was increased by 20% in mice kept at room temperature and by 75-80% in +14°C exposed animals and ERDE activity 90% and 120-160%, respectively. The absolute values of these enzymes varied, however, within the control range in +14°C exposed mice due to the initially low xenobiotic metabolism capacity of these animals.

The present study further demonstrates that PB treatment enhanced the liver NADPH generating capacity in the mice kept at room temperature and in the animals exposed to +14°C for a 1 week period but not in those exposed to this temperature for a 4 week period. In mice kept at room temperature PB treatment increased G6PDH and ICDH activities, and 6PGDH and ME activities remained unaltered. After 1 week of cold exposure the activities of three of these NADPH producing enzymes, G6PDH, 6PGDH and ME, were increased. Cytosolic NADPH is needed as a reducing equivalent for cytP450 mediated oxidative reactions [7], and Kauffman and his colleagues [8,18] have suggested that increased NADPH production in PB treated animals is an important part of induction process, which compensates for an increase in the use of the cofactor for enhanced xenobiotic metabolism. Our findings suggest that mild cold environment does not affect this ability of PB to compensate NADPH use at the beginning of the exposition. Prolonged exposure (4 weeks) to this temperature alone increased the activities of two NADPH generating enzymes, G6PDH and ICDH, and no further induction by PB treatment seemed to be needed.

The main function of liver cytP450 mediated oxidation processes is to convert lipophilic compounds to more water soluble metabolites, which can be easily secreted into bile or urine. Most of lipid soluble drugs are metabolized through this cytP450 mediated system, and changes in the cytP450 content and related enzyme activities may affect the fate of these drugs and the responses to them. On the other hand, cytP450 linked enzymes play an important role in the metabolic activation of precarcinogenic compounds to carcinogenic metabolites, and induction of these enzymes increases the rate at which those carcinogenic metabolites are formed [21]. Our finding that liver cytP450 mediated xenobiotic metabolism capacity was low, and that PB treatment did not induce cytP450 mediated xenobiotic metabolism above the (warm) control level in +14°C exposed mice may thus have pharmacological and toxicological importance when evaluating the therapeutic efficacy of drugs in Arctic populations. These findings may also partly explain the lower incidence of neoplasms in the Northern regions [22] and be of significance when assessing the carcinogenic risk of environmental chemicals in Arctic areas.

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IMMUNOLOGICAL DETERMINATION OF THERMOGENIN CONTENT IN BROWN ADIPOSE TISSUE: VALIDATION OF THE METHOD

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The limiting factor for the capacity for nonshivering thermogenesis in an animal (or in man) is the amount of the brown-fat specific, uncoupling protein thermogenin in the brown adipose tissue of that animal [1]. Thus, a simple method for estimation of the amount of thermogenin within an animal, or within a tissue sample (obtained, e.g., by biopsy), may be relevant as a tool in several different contexts, such as investigations concerning possible relationships between obesity and brown fat function etc. The presently available methods for estimation of thermogenin are rather cumbersome. The most widely used relies on the property of thermogenin that it is a mitochondrially localised GDP binding protein with the binding site exposed to the external environment of the mitochondrion. Thus, the amount of thermogenin in a mitochondrial preparation may be estimated by measurement of the specific binding of (^{3}H)GDP to isolated mitochondria, based on the method described by Nicholls [2] and modified in several respects when used by different groups. There are, however, both practical and theoretical limitations of this method. Practically, the main problem is that a mitochondrial preparation of several mg must be obtained from a sample before the measurement can be performed; this in reality makes the method unsuitable for biopsies. Theoretically, the problems are related to the degree of certainty with which the amount of specific binding sites for (^{3}H)GDP obtained can be considered as a true reflection of the amount of thermogenin. Besides the obvious questions related to the recovery of the mitochondria during the preparation, the exact conditions of the incubation medium, the separation of mitochondria from the incubation medium etc, it would seem that there are physiological conditions under which not all thermogenin is accessible to (^{3}H)GDP, i.e., thermogenin may be masked [3]. Although it has been possible to find methods which ensure that this masking effect can be overcome [4], the problem still remains to ensure that the (^{3}H)GDP binding really is to thermogenin, which means that it may be necessary to also perform functional tests such as to demonstrate GDP inhibited Cl$^-$ transport in the mitochondria etc. Thus, although widely used, the (^{3}H)GDP binding method has several limitations.

After the introduction of a method for isolation of pure thermogenin [5], the possibility of a development of an immunological method for thermogenin determination was opened. In several laboratories, antibodies against thermogenin have been obtained and tested for specificity etc. [6-8]. However, although these antibodies have provided good tools for scientific progress in brown adipose tissue research, the antibodies have been used only to a limited degree for quantitative studies of thermogenin content, and until now, only in mitochondrial preparations [9-13]. We have therefore endeavoured to develop and test a method for the use of thermogenin antibodies for quantitative analysis of thermogenin directly in untreated samples of brown adipose tissue. We present here results validating this immunological method for thermogenin determination.

METHODS: Male rats of 150 g starting weight were placed either in a cold room (4°C) for 3 weeks or remained in normal animal house conditions (21°C) for the same period. They had free access to a normal rat pellet diet and water. The rats were killed and the interscapular brown adipose tissue was quantitatively dissected out. Samples of other tissues (liver, epididymal white fat) were also excised. All tissues were transferred to preweighed vials containing 5 ml of ice cold sucrose and were homogenised. The homogenates were filtered through gauze and frozen and stored at -20°C until analysis. Diluted samples were applied to ELISA plates precoated with thermogenin, and the assay as such was performed exactly as described previously [12].

RESULTS: Due to the potential problems arising from the fact that thermogenin is a membrane protein and that the assay was to be performed with tissue homogenates with a high fat content, the main question to be investigated was as to whether the response was linear with the amount of tissue added. In

Fig. 1A, examples of ELISA curves obtained with increasing amounts of samples from different tissues are shown. It is immediately clear that there is more immunologically reactive material in brown adipose tissue from cold acclimated animals than in tissue from control animals. It is, however, difficult from curves like this to establish whether the response is linear with added amount, and the actual absorbance observed on each plate is not only a function of thermogenin amount but also of incubation time. Therefore, the data were normalized and linearized as shown in Fig. 1B. In this representation, the line should be linear if the assay fulfills criteria for linearity. It is seen that this criterion is met for the lower amounts of tissue homogenate added in this series; for higher amounts, the curves bend off. Thus, the content would be underestimated if these points were used for calculation.
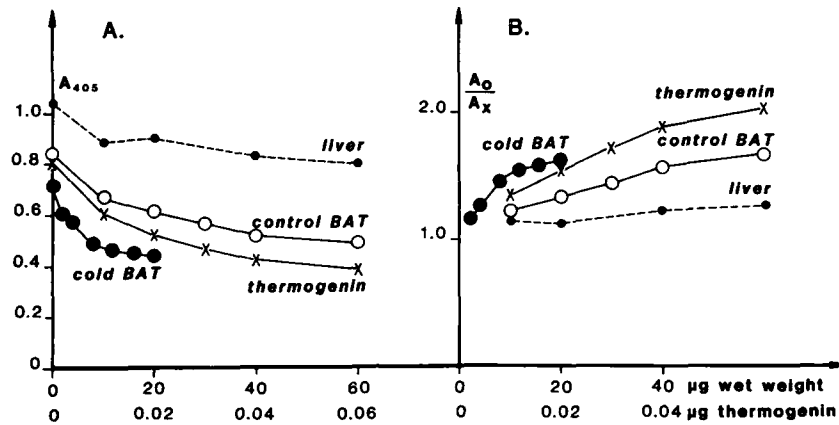
FIGURE 1: A: Absorbance at 504 nm as a function of added amounts of tissue samples or thermogenin in a thermogenin ELISA. B: Linearization of the data in A, obtained by division of the absorbance without added sample (A_0) with that obtained with each sample amount (A_x).

By division of the slope of the lines corresponding to tissue homogenates with that corresponding to thermogenin, the content of thermogenin in each sample is calculated. In Fig. 2, we have compiled the results from a series of such measurements. It is seen in Fig. 2A that the wet weight of the interscapular depot was increased more than 3 fold due to cold acclimation, in agreement with many earlier observations. In Fig. 2B it is seen that the concentration of thermogenin within the tissue was more than doubled due to cold acclimation and reached a value of about 1.5 mg thermogenin per gram wet weight. Thus, the total amount of thermogenin within that depot was increased by nearly one order of magnitude (Fig. 2C), in agreement with results of Sundin and Cannon [14], obtained with the (^{3}H)GDP binding method.

DISCUSSION: In this investigation we have demonstrated that it is possible to use an immunological method directly on tissue homogenates to obtain reliable values for thermogenin content in the tissue. It may especially be noted that the concentration of thermogenin estimated by this method is in remarkably good agreement with what can be calculated from known data on brown adipose tissue. Thus, based on the following assumptions: a binding of 0.6 nmol GDP per mg mitochondrial protein [14], a thermogenin molecular weight corresponding to 64,000 g per mol GDP bound [15] mitochondria being nearly 50% of total protein in the tissue [16] and total protein concentration being about 10% in cold acclimated rats [17], it can be calculated that the expected concentration of thermogenin in the brown adipose tissue of cold acclimated rats would be nearly 2 mg/g wet weight - a value which is in excellent agreement with that obtained in the experiments described here.

A. Wet weight　　B. Thermogenin concentration　　C. Total thermogenin content
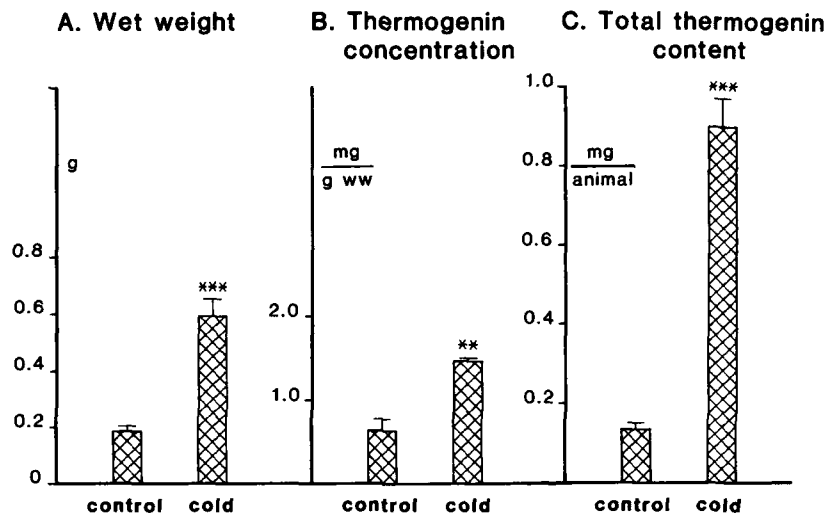
FIGURE 2: Effect of cold acclimation. Data in B are obtained from analyses performed as exemplified in Fig. 1. Means from 4 animals in each group. ** and *** indicate effects of acclimation to cold (P < 0.01 and < 0.001, respectively).

Of course, the demonstration of the validity of the method only shows that expected results concerning thermogenin concentration and relative increase due to cold acclimation can be obtained. The potential of the immunological method is to be found when it is used under circumstances where no alternative method is applicable.

Thus, it has been shown that there is good immunological cross reactivity between human and rat thermogenin [18]. As extremely small amounts of tissue are needed for this assay (as seen in Fig. 1, less than 50 μg tissue is needed even with tissue from non-cold acclimated animals) the method presented here would seem to be useful to estimate the thermogenin content of, e.g., human biopsies.

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DESENSITIZATION TO ADENOSINE IN BROWN ADIPOSE TISSUE

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In a variety of tissues, adenosine is an important regulator of metabolism. This is the case in both white [1-4] and brown [5-10] adipose tissue. The regulatory effects of adenosine in brown fat are thought to be mediated via its binding to extracellular adenosine (A_1) receptors, coupled to adenylate cyclase via an inhibitory guanine nucleotide binding protein (N_i-protein) [8]. Thus, in brown fat cells, adenosine attenuates the stimulatory effect of β-adrenergic agonists on adenylate cyclase activity, lipolysis and respiration [6,7].

Brown adipose tissue is highly specialized to produce heat [11]. During acclimation of mammals to cold, the tissue undergoes biochemical and metabolic changes in order to fulfil the extra thermogenic demands. We have investigated here whether the regulation of norepinephrine stimulated respiration (thermogenesis) by adenosine is different in cells isolated from control and from cold acclimated hamsters.

METHODS: The rate of oxygen consumption of isolated brown-fat cells was measured polarographically as described previously [12]. Brown-fat cells were prepared from control and cold acclimated hamsters and assayed in parallel.

Binding of (^{3}H)phenylisopropyladenosine [(^{3}H)PIA] to crude membranes was performed in principle as in [13]. After 15 min incubation at 37°C the binding reaction was stopped by dilution and filtration through Whatman GF/B filters in a cell harvester. Nonspecific binding was measured with an excess of unlabelled PIA.

RESULTS: Brown fat cells isolated from cold acclimated hamsters showed a lower maximal respiratory response, as well as a decreased sensitivity to norepinephrine, compared to cells from control hamsters (Fig. 1). This cold induced desensitization of β-adrenergic pathways is in agreement with previous results from this and other laboratories [14-16].

In order to examine whether the adenosine sensitivity of the cells was altered by acclimation of hamsters to cold, brown adipocytes were incubated in the presence of adenosine deaminase (an enzyme that inactivates endogenously formed adenosine), with or without the non-hydrolysable adenosine analogue 2-chloroadenosine. Adenosine deaminase in itself shifted the norepinephrine dose-response curve slightly to the left in control cells, but had no effect in cells from cold-acclimated animals (not shown). When 2-chloroadenosine was added there was a clear shift to the right of the norepinephrine dose-response curve in the control cells (Fig. 1). The EC_{50} value increased about 5 fold from 60 to 300 nM. In contrast, there was no effect of 2-chloroadenosine in cells from cold acclimated hamster (Fig. 1) (EC_{50} values were 220 and 260 nM, without and with 2-chloroadenosine, respectively).

From these experiments it was concluded that the cells from the cold acclimated hamster had become insensitive to adenosine.

The molecular mechanism behind this desensitization is under investigation in our laboratory. Preliminary experiments with (^{3}H)PIA binding to brown fat crude membranes show no difference in affinity (K_D value) or maximal number of binding sites between control and cold acclimated hamster (Fig. 2). In controls, a K_D value of 3.1 nM and a maximal number of binding sites of 23 fmol/mg protein were observed; in the cold acclimated state, these values were 3.8 nM and 23 fmol/mg, respectively.
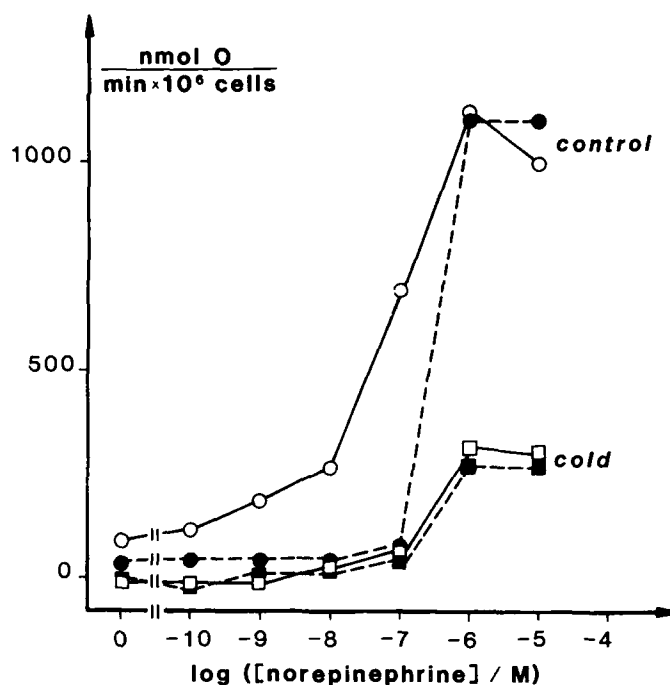
FIGURE 1: The effect of 2-chloroadenosine on norepinephrine stimulated respiration of brown fat cells incubated in the presence of adenosine deaminase. Brown fat cells were isolated and oxygen consumption measured in the presence of adenosine deaminase (2 μg/ml) and the indicated concentrations of norepinephrine, without (open symbols) or with (filled symbols) 2-chloroadenosine (10 μM). The respiratory response is expressed as the norepinephrine induced increase over the basal respiratory rate, i.e., in the absence of adenosine deaminase and 2-chloroadenosine. The figure shows a representative example with cells from control and cold-acclimated animals prepared and tested in parallel.

DISCUSSION: In these experiments we have confirmed that brown fat cells isolated from control hamsters are sensitive to adenosine. Acclimation of hamsters to cold apparently induced a desensitization to adenosine. It is likely that, due to the chronic sympathetic stimulation of the tissue in the cold, the cells are constantly exposed to adenosine released from the cells, and that the desensitization is due to this chronic exposure.

The molecular mechanism behind this phenomenon of physiological desensitization is now under investigation in our laboratory. Based on our preliminary observations (Fig. 2), our tentative conclusion is that the physiologically induced desensitization to adenosine is not due to a loss of adenosine receptors or to a decreased affinity to adenosine, but rather to an alteration in the molecular steps between binding of adenosine to its receptor and the inhibitory effect.
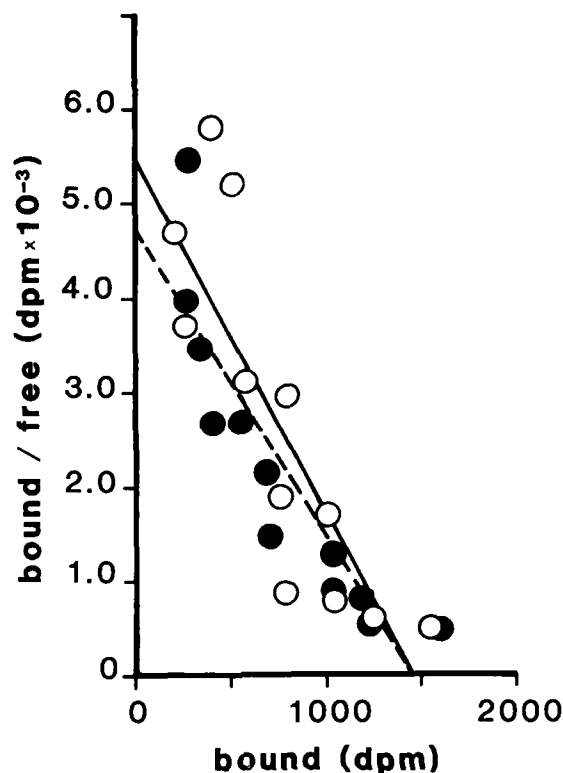
FIGURE 2: Scatchard plot analysis of specific (^{3}H)PIA binding to brown fat crude membranes. Brown fat crude membranes were prepared and incubated with different concentrations of (^{3}H)PIA. Open symbols represent control, and filled symbols cold acclimated hamster brown fat crude membranes. The figure shows one representative example.

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PEPTIDES OF THE CORTICOLIBERIN SUPERFAMILY PROTECT THE SKIN AGAINST THERMAL INJURY

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Extreme changes in temperature produce inflammatory responses in the skin which are manifested initially as increased vascular permeability and protein extravasation. Peptides belonging to the corticoliberin superfamily have the unusual property of making epithelial tissues resistant to these indices of injury [1]. For example, the 41-residue human/rat corticotropin-releasing factor (CRF, 28 μg/kg), sauvagine (2.5 μg/kg) or sucker fish urotensin I (10 μg/kg) injected iv 10 min before immersion of the anesthetized rat's paw in 58°C water for 5 min, reduced by 50 to 60% the heat-induced edema and Evans blue dye extravasation into the pawskin. Using CRF as the prototype agent, the time-course of the action of CRF was studied. Pretreatment with CRF (28 μg/kg sc) was effective for up to 4 h in reducing the swelling produced by heat (58°C for 30 sec, observation period of 1 h). The same dose of CRF injected iv 0, 10, 20 min after heat exposure immediately inhibited the progressive development of swelling.

Histological examination of the skin before and after CRF showed that CRF attenuated vesication, edema, epidermal necrosis and the disruption of tissue architecture produced by heat. The pharmacological specificity of CRF was revealed by the alpha-helical CRF(9-41) antagonist. The antagonist (92 μg/kg iv) administered alone did not affect heat injury; but it both prevented and reversed the inhibitory effects of CRF on the swelling produced by heat. Alpha-helical CRF(9-41) also antagonized the anti-inflammatory effects of sauvagine and urotensin I.

Overall, the results described here may provide new clues for interpreting the mysterious homology among peptides of the corticoliberin superfamily which are distributed in cells of the hypothalamus, the amphibian skin and the urophysis. Perhaps, these peptides have a common ancestry, not only as hypophysiotropic agents, but also as peptide agonists designed to enhance the survival of epithelial tissues against extremes in temperature. Their protective effects would be akin to the anti-inflammatory actions of the corticosteroids.

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EFFECTS OF ACUTE AND CHRONIC HYPERGLYCEMIA ON MORPHINE-INDUCED HYPOTHERMIA IN MICE

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Changes in body temperature are among the more widely studied responses to acute morphine administration. However, the predominant effect of this drug on core temperature in several species still remains unclear. The thermic response to morphine is sensitive to various exogenous and endogenous factors [1-4]. Furthermore, receptors involved in thermic response to morphine are not well defined [5-7]. In hyperglycemic states, both a decrease [8,9] and an increase [10] in the analgesic potency to morphine has been reported, but the effects of hyperglycemia on thermic responses to morphine are unknown. In the present study, the thermic response to morphine was assessed in normoglycemic controls and in glucose and streptozotocin induced hyperglycemic mice. The results indicate that glucose induced acute hyperglycemia attenuates, while streptozotocin induced chronic hyperglycemia augments, the thermic response to morphine and support the hypothesis that hyperglycemia is not the primary mechanism for the altered sensitivity to morphine in animal models of diabetes.

METHODS: Male Swiss Webster mice weighing 25-30 g were housed five per cage in a room with controlled temperature (22 $\pm$ 2°C) humidity and artificial light (06:30 - 19:00 h). The animals had free access to food and water and were used after a minimum of 4 days acclimation to the housing conditions. Chronic hyperglycemia was induced by injection of streptozotocin (200 mg/kg ip) dissolved in citrate buffer (1%) at a pH of 4.0-4.5, administered 7-8 days before the experimental day [11]. Control animals received vehicle alone. Acute hyperglycemia was induced by glucose (5.04 g/kg ip) with simultaneous injection of morphine. Blood samples were collected from the retro-orbital sinus to measure blood glucose levels [12]. Blood glucose was measured using Ames Dextrostix and a reflectance colorimeter. Morphine sulfate was dissolved in pyrogen free saline (0.9%) just before use. Drug doses refer to the salt. Animals in the control group received vehicle injections. The effect of morphine (3, 10, 30 and 100 mg/kg sc) was assessed in drug and naive animals. A minimum of 10 animals were used per group.

Colonic temperature was measured at an ambient temperature of 22 $\pm$ 0.2°C using a telethermometer and thermistor probe inserted 3.5 cm deep into the rectum until a constant reading was obtained. While the temperature measurements were made, the animals were gently restrained (45-60 sec). The temperature for each mouse was measured before and 30, 60, 90, 120, 150 and 180 min after morphine injection. The net change in temperature after drug administration was obtained by subtracting each post-drug measurement for each mouse from its initial (pre-injection) temperature. The mean temperature data are presented as an increase or decrease from the base' value. For each animal the area under curve between control temperature and each temperature over a 3 h period after drug administration was determined by linear trapezoidal rule and expressed as temperature index [13]. Since increasing doses of morphine prolong the peak effects, the complications arising from an arbitrary selection of a single time interval for assessment of effects after variety of doses are avoided. Analysis of variance (ANOVA) across groups was calculated by a computer program [14]. Student's t-test with two tailed probability was used where appropriate.

RESULTS: Streptozotocin treated animals (chronic hyperglycemic group) had blood sugar levels of 450 $\pm$ 15 mg/dl (Fig. 1). Following acute glucose administration hyperglycemia lasted longer than 120 min (Fig. 2). The mean pre-drug colonic temperatures in normoglycemic, acute-hyperglycemic and chronic-hyperglycemic groups were, 38.7 $\pm$ 0.08, 38.8 $\pm$ 0.1 and 37.2 $\pm$ 0.2°C, respectively. The basal hypothermia observed in the chronic hyperglycemic group was statistically significant ($p < 0.01$) when compared to their controls. After vehicle treatment (i.e., subcutaneous saline and intraperitoneal saline) the maximum net deviation in temperature was < 0.3°C.
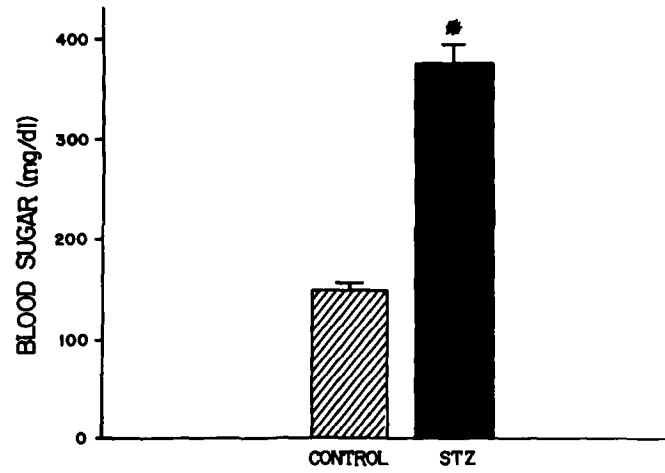
FIGURE 1: Blood glucose levels in citrate buffer treated (control; normoglycemic) and in streptozotocin treated (STZ; chronic hyperglycemic) mice. Data represent mean + SEM of 20-25 observations. *p < 0.001.
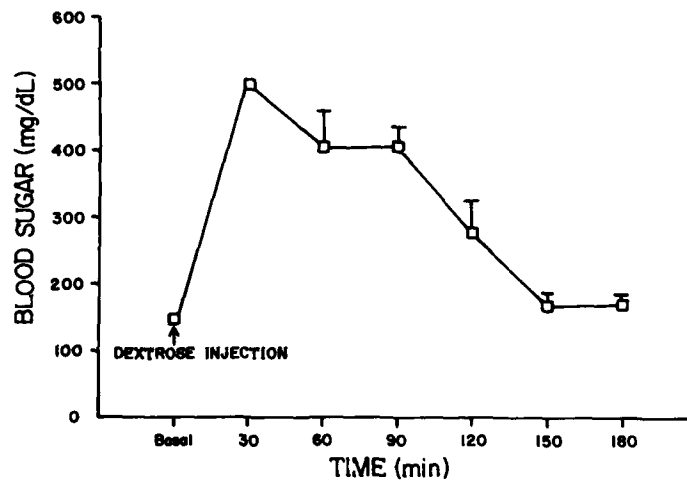
FIGURE 2: Time course of blood glucose levels after intraperitoneal glucose injection (5.04 g/kg ip; acute hyperglycemic groups). Data represent mean + SEM of 10-12 observations.
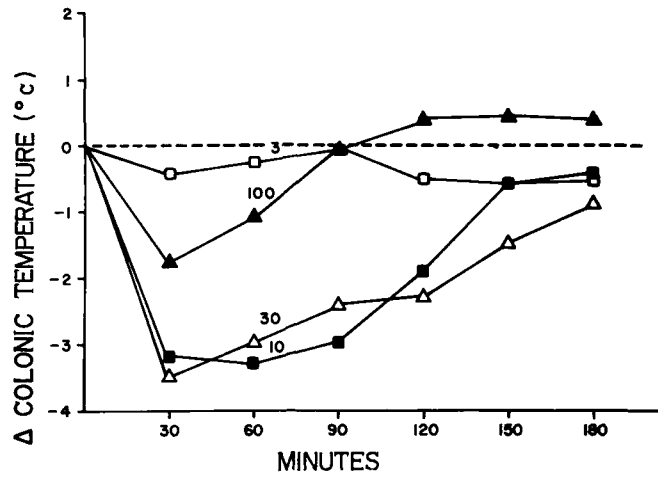
FIGURE 3: Effects of morphine (3, 10, 30 and 100 mg/kg sc) on colonic temperature in normoglycemic (citrate buffer treated) control mice. The data represent mean change in temperature from the pre-injection readings (N, 10-12).
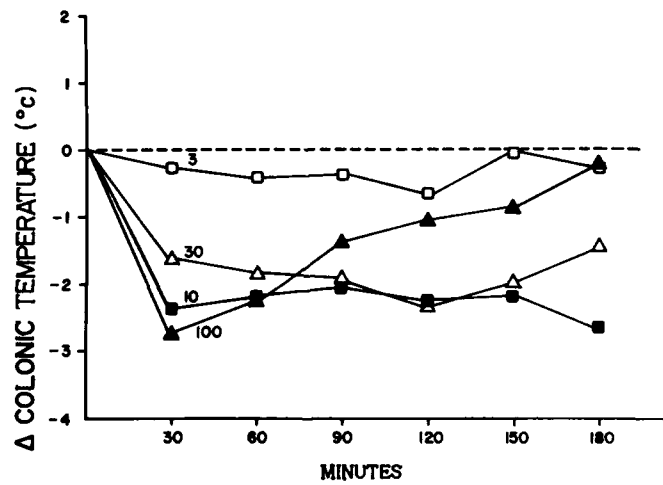
FIGURE 4: Effects of morphine (3, 10, 30 and 100 mg/kg sc) on colonic temperature in chronic hyper-glycemic (streptozotocin treated) mice. The data represent mean change in temperature from the pre-injection readings (N, 10-12).
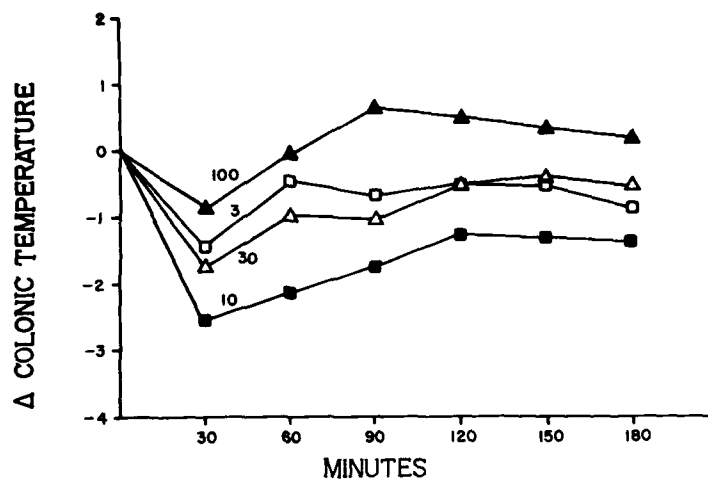
FIGURE 5: Effects of morphine (3, 10, 30 and 100 mg/kg sc) on colonic temperature in acute hyperglycemic (glucose 5.04 g/kg ip) mice. The data represent mean change in temperature from the preinjection readings (N, 10-12).
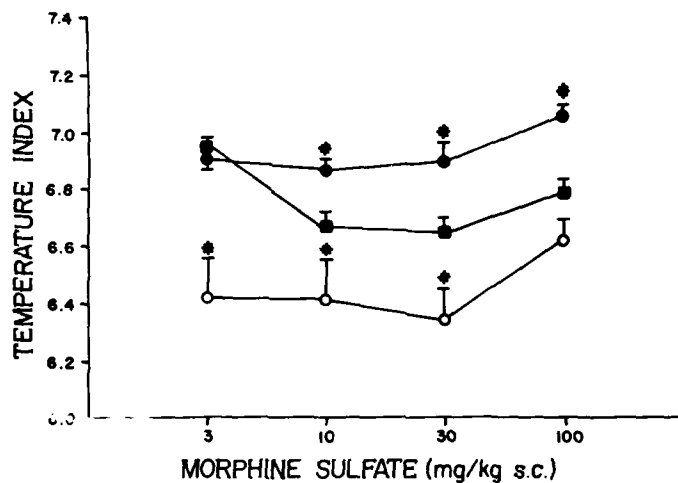
FIGURE 6: Thermic response to morphine expressed as temperature index (area under the curve) in normoglycemic (solid square) and acute (closed circle) and chronic (open circle) hyperglycemic groups. The data represent mean + SEM based on 10-12 observations. The ANOVA indicated that the dose of morphine ($F_{3,118}$ = 4.13, p < 0.01) as well as the glycemic state ($F_{2,118}$ = 39.8 p < 0.001) had significant effects. *p < 0.05 when compared to the respective normoglycemic controls.

The time course of thermic response to morphine as the net deviation from the basal temperature, in normoglycemic controls and in chronic and acute hyperglycemic groups are shown in Fig. 3, 4 and 5 respectively. Morphine produced a hypothermic effect in all groups which appeared to have a peak 30 min post-drug in all the groups. The colonic temperatures tended to return gradually to base line values in all groups. In normoglycemic controls and in acute hyperglycemic groups, the highest dose of morphine (100 mg/kg sc) produced a biphasic effect, a decrease followed by an increase in temperature (Fig. 3 and 5). When the intensity (peak) of hypothermic effects was compared a direct relationship between the dose of morphine and the hypothermic response was not apparent in any group. Furthermore, unlike acute hyperglycemia (Fig. 5), morphine induced changes on body temperature lasted longer in the chronic hyperglycemic groups (Fig. 4). The temperature indices for different doses of morphine in normoglycemia and in acute and chronic hyperglycemia groups are shown in Fig. 6. The ANOVA (morphine dose x glycemic state, 4 x 3) indicated that the dose of morphine ($F_{3,118} = 4.13$, $p < 0.01$) as well as the glycemic state ($F_{2,118} = 39.8$, $p < 0.001$) had significant effects. Furthermore, the temperature index indicated that acute hyperglycemia significantly attenuated, while chronic hyperglycemia augmented, the thermic response to morphine ($p < 0.05$).

DISCUSSION: The lower basal body temperature in streptozotocin treated mice observed in the present study confirms the earlier results in streptozotocin treated rats [15]. The results indicate clearly that acute hyperglycemia attenuates, while chronic hyperglycemia augments, the hypothermic response to morphine. Both *in vivo* [9,16,17] and *in vitro* [18] studies support the hypothesis that the decreased sensitivity to morphine in experimental models of diabetes is primarily due to the hyperglycemia associated with diabetes. However, recent results both *in vivo* [19] and *in vitro* [20] contradict this hypothesis. The present results indicate a dichotomy in the modulation of thermic responses to morphine, in acute versus chronic hyperglycemia. These results support earlier conclusions [19,20] that hyperglycemia *per se* may not be sufficient to explain the altered responsivity to morphine in experimental models of diabetes.

Morphine induced hypothermia has been related to changes in brain monoamines, such as serotonin and dopamine [21-23]. Brain monoamine content and metabolism are known to be altered in streptozotocin induced diabetes [15,24]. In addition, streptozotocin treatment has been shown to decrease β-endorphin content in hypothalamus and intermediate lobe of the neurohypophysis [25,26]. A change in the functional inter-relation of brain monoamine and β-endorphin systems in experimentally induced diabetes also has been observed [27]. Therefore, in streptozotocin treated animals, altered brain monoamines and/or endogenous opioid peptides may be the basis for the augmented thermic responses to morphine observed in this study.

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THERMAL RESPONSE TO THYROTROPIN RELEASING HORMONE IN DIFFERENT STRAINS OF MICE

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Earlier studies have shown that thyrotropin releasing hormone (TRH) induces hyperthermia in many species [1-3]. Peripheral administration, but not i.c.v. injection [4], of TRH usually induced a short lasting increase in the core temperature of mice [5]. During TRH induced hyperthermia, enhanced heat loss effector activity such as tachypnea, vasodilation, salivation and grooming have often been noted [6-8].

Our previous studies have shown that TRH induced hyperthermia is accompanied by an increase in norepinephrine (NE) and epinephrine (E) in the plasma. This hyperthermia could be abolished by hypophysectomy and adrenal demedullation [9]. Also, it has been reported that various strains of mice respond differently to TRH, but TRH binding to the brain membranes of different strains does not differ significantly. The mechanism of hyperthermia induced by TRH administration has not been clarified with the different strains. Therefore, the present studies were designed to examine the effects of TRH on rectal and tail skin temperature, and attempt to correlate them with changes in plasma catecholamines after TRH, in four inbred strains and one closed colony of mice.

METHODS: Four strains of male inbred mice, C57BL/6N (C57BL), DBA/2N (DBA), BALB/C (BALB), C3H/HeN (C3H) and a closed colony of the ICR strain were obtained. Each strain of 25 mice was 6 to 7 weeks old with a mean weight of 29.4 ± 0.2 g (ICR), 21.9 ± 0.3 g (BALB), 17.8 ± 0.2 g (DBA), 22.5 ± 0.3 g (C3H), and 17.6 ± 0.2 g (C57BL). The mice were conditioned in the animal room for 7 days under 14 h light and 10 h dark cycle (light from 06:00 to 20:00 h) with free access to food and water prior to experiments. All experiments were performed between 10:00 and 15:00 h at an ambient temperature of $22.4 \pm 0.5°C$. The mice were unrestrained except during measurements of rectal temperature (T_r), tail skin temperature (T_s) and the collection of blood sample from each mouse. The T_r was measured at selected time intervals by inserting a thermocoupled probe 2 cm into the rectum and T_s by a thermomex probe placed on the surface of the tail 4 cm distal to the root. The changes in T_r and T_s of each mouse were expressed as the difference from the 0 time value.

Blood samples were collected under light ether anesthesia from the jugular vein 15 min after TRH (10 mg/5 ml saline/kg ip) or saline (5 ml/kg ip). The concentration of plasma catecholamine was then determined using HPLC with an EC detector [10].

Analysis of variance (ANOVA), Sheffe's and Student's t-test were applied to statistically analyze the results.

RESULTS: The effects of the two doses of TRH (10 and 30 mg/5 ml/kg ip) and of saline on T_r were investigated in the five strains of mice. There was a significant difference in the basal level of T_r among the strains (F=49.0, df 4/124, P < 0.01). The DBA strain ($39.0 \pm 0.06°C$) showed the highest mean T_r followed by ICR ($38.5 \pm 0.04°C$), C3H ($38.3 \pm 0.05°C$), C57BL (38.1 ± 0.07 °C) and BALB strains ($38.0 \pm 0.06°C$) in decreasing order. TRH (10 and 30 mg/kg) caused hyperthermia except in the C57BL strain. The peak of hyperthermia was observed 15 min after TRH (Fig. 1). The mean rise in T_r 15 min after administration of TRH (30 mg/kg) was greatest in the ICR strain ($1.27 \pm 0.14°C$), followed by the BALB ($1.05 \pm 0.1°C$), DBA ($0.58 \pm 0.26°C$) and C3H strains ($0.46 \pm 0.10°C$) in decreasing order. The exception was the C57BL strain which showed significant hypothermia ($-0.92 \pm 0.22°C$). However, the difference between the hyperthermic effects of the two doses of TRH was not found in these four strains. Examination of the data collected 15 min after TRH (30 mg/kg) showed a significant strain difference (F=13.35 df 4/25, P < 0.01).
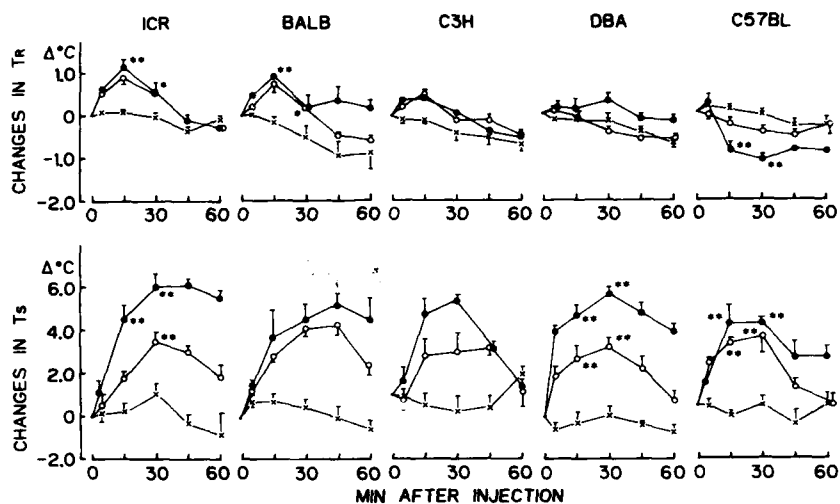
FIGURE 1: Time course of effects of saline (5 ml/kg ip (X)) or TRH (10 mg (O) and 30 (●) mg/kg) on rectal (T_r) and tail skin temperature (T_s) in five strains of mice. The change in T_r and T_s was expressed as the difference from the 0 time value, just before injection of saline or TRH. The data are depicted as mean ± SEM of 8 mice. *P < 0.05, **P < 0.01 (compared to the corresponding controls, Student's t test).

There was a significant difference in the basal level of T_s among the strains used (F=9.78, df 4/124, P < 0.01). Administration of TRH (30 mg/kg) elicited no significant difference in response of T_s among the five strains. The highest mean rise in T_s 15 min after administering TRH (30 mg/kg) was, in the case of C3H strain, (4.7 ± 1.0°C), and in the DBA (4.6 ± 0.5°C), C57BL (4.3 ± 0.8°C), ICR (4.2 ± 0.65°C) and BALB strains (3.68 + 1.3°C) in decreasing order. More specifically, in C57BL, in spite of a decrease in T_r after administration of TRH (30 mg/kg), T_s was elevated as high as that in other strains.

There was a significant difference in basal levels of NE and E among the strains examined: (F=10.61, df 4/24, P < 0.01) and (F=3.21, df 4/23, P < 0.05). But C57BL particularly showed the highest basal level of NE (1.95 ± 0.26 ng/ml) and E (2.51 ± 0.37 ng/ml). Also, a significant difference in the rise in NE and E after TRH administration (10 mg/kg ip) was observed: (F=7.91, df 4/25, P < 0.01) and (F=4.21, df 4/25, P < 0.05). The greatest effect of TRH on the basal level in plasma catecholamine was in the C57BL strain, NE (6.10 ± 0.65 ng/ml) and E (7.17 ± 0.48 ng/ml) respectively. However, in C57BL, there was no correlation between changes in T_r and plasma catecholamines after administration of saline or TRH (10 mg/kg ip).

DISCUSSION: We agree with previous findings [11] that different strains of mice produce different TRH induced core temperature rises at normal ambient room temperatures. Under these experimental conditions, there was a clear difference in the effects on rectal and tail skin temperatures and plasma catecholamines 15 min after TRH (10 mg/kg): (F=22.29, df 4/55, P < 0.01), (F=5.92, df 4/53, P < 0.01) and (F=7.90, df 4/25, P < 0.01) respectively. Following administration of TRH (10 and 30 mg/kg), the ICR and BALB strain displayed marked hyperthermia, although a slight rise in rectal temperature was seen in the C3H and DBA strains. On the other hand, a significant decrease in rectal temperature was observed in the C57BL strain. The results have been ranked by the order of the rectal temperature, but T_s is not ranked by temperature variations. Figs. 1 and 2 highlight the effects of TRH. The strains of mice have been arranged according to the increase in rectal temperatures in Fig. 1, while the tail skin temperature rises of these strains showed no significant pattern. Bansinath et al. [11] showed significant differences in hyperthermia in the strains of mice after a single injection of TRH. However, [3]H-(3-MeHis)TRH bound to brain membranes at a single high affinity site with a B_{max} of 53.72 fmol/mg protein and K_d of 3.77 nM and these did not differ between strains. Thus, the nature of TRH induced changes in

rectal temperature depends on the strain of mice and factors other than the brain receptors for TRH may be responsible for the variable hyperthermic effect. In this study, we noticed that the response to TRH by the C57BL strain was much more specific than that of the other strains examined for thermic and plasma catecholamines response. Therefore, we assume that TRH induced effective sympathetic outflow may be the most important factor responsible for potentiation of TRH induced hyperthermia in mice.
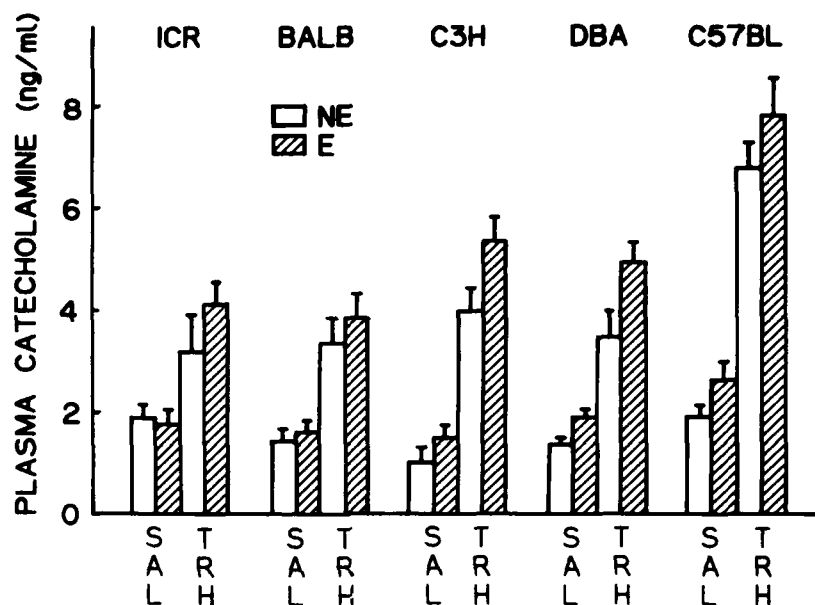
FIGURE 2: Effects of TRH on plasma catecholamines 15 min after injection. SAL: saline (5 ml/kg ip), TRH (10 mg/kg ip).

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A STUDY OF THE ANTIMETABOLITES (DIVICINE) ISOLATED FROM FABA BEANS (*VICIA FABA L.*) ON SOME PHYSIOLOGICAL RESPONSES WITH SPECIAL REFERENCES TO ITS THERMOREGULATORY EFFECTS

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Divicine is an active aglycone prepared by hydrolysis of β-glycoside vicine which is isolated from beans (*Vicia faba*) by microflora in the caecum and large intestine under anaerobic conditions [1,2]. It can induce oxidative changes in erythrocytes, such as rapid depletion of GSH, and generally is thought to be the toxic constituent of the beans [3,4] which causes favism, an acute haemolytic crisis by the individuals deficient in glucose-6-phosphate dehydrogenase [3,5]. Recent studies [6] have demonstrated that divicine administered intravenously to rats causes a fall in blood glutathione concentration and haematocrit and an increase in adrenal and spleen weights. Higher doses of divicine results in severe cyanosis, followed by death within a relatively short time.

The objectives of this study were to demonstrate the effect of intraperitoneally injected divicine on body temperature and some physiolgoical responses on white rats.

METHODS: Male albino rats of the Sprague-Dawley strain were chosen from the breeding station of National Research Centre, Cairo, Egypt. The animals of about the same age and average weight (100-120 g) were kept in special plastic metabolic cages.

Divicine was prepared by acid hydrolysis according to the method of Arbid and Marquardt [7].

Three groups were used, each of 15 rats. The control rats were injected with saline solution (ip). The second group was injected with a single lethal dose of divicine (28 mg/100 g ip). The third group was injected with a single sublethal dose of divicine (20 mg/100 g ip) in order to determine the time for collection of urine 24 h before the lethal effect.

Rectal, skin and tail temperatures were measured with digital thermistors of two types: one long probe inserted in the rectum for 3 min; the other a disc probe placed in direct contact with skin or tail for 3 min.

Blood was obtained from the retro-orbital venous plexes [8]. Samples were subjected to the following haematological estimations: haemoglobin percentage was determined by using Sahli's apparatus; haematocrit was determined using heparinized micro-haematocrit tubes; erythrocyte and leucocyte counts were carried out according to the method of Harris *et al.* [9].

Urine samples were collected from the third group 1 and 24 h after injection. The haemoglobin percent in urine was estimated by Wong's method [10].

Liver glutathione determination was according to the method of White [11].

RESULTS: The thermoregulatory effect of a lethal dose of divicine is shown in Table I. There was highly significant decrease (p < 0.01) in rectal, skin and tail temperature 45 and 60 min after injection, compared with control group. The decrease in tail temperature started after 15 min and continued until 60 min.

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The effect of lethal dose of divicine on haematological patterns are recorded in Table II. There were insignificant increases in erythrocytes, leucocytes and the haematocrit while the haemoglobin percent showed an insignificant decrease.

TABLE I   The thermoregulatory effect of a lethal dose of divicine (28 mg/100 g) on rectal, skin and tail temperatures of rats

Time (min)	Temperature (°C)		
	Rectal	Skin	Tail
0[a]	37.8 ± 0.2	37.0 ± 0.1	34.2 ± 0.2
15	37.0 ± 0.3	36.9 ± 0.2	34.2 ± 4
30	35.8 ± 0.3[1]	36.4 ± 0.3	33.5 ± 0.6[2]
45	35.8 ± 0.2[2]	36.2 ± 0.2[2]	33.0 ± 0.8[2]
60	35.1 ± 0.3[2]	35.6 ± 0.2[2]	33.5 ± 0.2[2]

[a]Zero time and acts as a control for each route of determination of temperature.

[1]$P < 0.05$
[2]$P < 0.01$

TABLE II   Effect of divicine (28 mg/100 g ip) on erythrocyte, leucocyte counts, haemoglobin and haemotocrit values

	1 h	
	Control	Treated
Erythrocyte ($10^6/\mu l$)	7.06 ± 0.4	7.52 ± 0.9
leucocytes ($10^3/\mu l$)	12.07 ± 0.4	16.89 ± 6.4
Haematocrite (%)	41.30 ± 1.4	46.10 ± 3.5
Haemoglobin (%)	86.20 ± 2.2	78.20 ± 4.4

The effect of divicine on liver GSH and haemoglobinuria is represented in Table III. There was a highly significant decrease in liver GSH after 1 h (P<0.01) and 24 h (P < 0.001) and a significant increase in haemoglobin in the urine.

TABLE III   Effect of divicine (20 mg/100 g ip) on liver GSH and haemoglobin in urine

	Liver GSH		Haemoglobin (% in urine)	
	1 h	24 h	1 h	24 h
Control	175.4 ± 1.3	177 ± 1.1	–	1.81 ± 0.2
Treated	97.7 ± 5.5[1]	25.26 ± 2.9[2]	–	1.97 ± 0.2[1]

[1]P < 0.01
[2]P < 0.001

DISCUSSION: The decrease in body temperature of rats injected with lethal dose of divicine may be due to the decrease in oxygen content of the blood [7] or to a decrease in blood glucose as reported by Kaneko [12] who attributed the fall to liver dysfunction or kidney tubular damage. The slight increase in erythrocyte, leucocyte and haematocrit values may be attributed to the loss of body fluids [7]. Yannai and Marquardt [6] reported a decrease in haematocrit values of rats given a single intravenous injection of divicine. The decrease in haemoglobin content was similar to that obtained by Lin and Ling [13] who noted transient haemoglobinuria in puppies 3 h after oral administration of vicine.

Liver glutathione was highly significantly decreased in treated rats. Divicine causes rapid oxidation of glutathione [14,15]. Blood glutathione was decreased in rats injected with vicine, convicine or divicine [6,7].

It is concluded that the lethal dose of divicine in rats may be due to the decrease in body temperature and liver glutathione.

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HYPOTHERMIC EFFECTS OF "PYROGENS" IN THE MOUSE AND INTERACTION WITH LYSINE ACETYLSALICYLATE

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Previous studies from our laboratory were concerned more with thermoregulation than with fever. Thus, we have demonstrated the α-adrenergic mechanism of hyperthermia induced by TRH [1], its potentiation by amphetamine [2] and the possibility of using enhancement of this hyperthermia by antidepressants to screen the latter [3]. The mechanism of action of this hyperthermia involves the adrenal medulla, pituitary and thyroid [4,5]. It is reported elsewhere in this volume [6] that the temperature lowering effect of neuroleptics is not, as has been thought, of central origin. The study reported here began with an attempt to induce fever in the mouse. Curiously enough, this has been the subject of very few studies.

METHODS: Male CD1 mice, aged 5 weeks and weighing 25-30 g on the day of the experiment, were maintained at 22 ± 1°C. Rectal temperature was measured with a thermocouple probe. Readings were taken 30 min and just before injection and then every 10 min for 1 h and at 90 and 120 min. For intracerebroventricular injections a cannula was implanted into the right lateral ventricle using a method described previously [7]. Solutions were filtered through a 0.22 μm Millipore filter before perfusing 0.5 μl over a 50 sec period. For intravenous route injections were made in one of the two lateral veins of the tail. Intraperitoneal administrations were made with a volume of 0.1 ml for 10 g. Lipopolysaccharides of *Escherichia colli* (LPS) and sodium arachidonate (AA) were dissolved in sterile pyrogen free saline (0.9%). Prostaglandins PGE_1 and PGE_2 were solubilised according to the technic of Stiff [8]. Statistical analysis was performed using the Mann-Whitney U-test to compare drug treated groups and the appropriate control groups.

RESULTS: LPS reduces temperature by whatever route it was administered. PG_E exhibited inverse effects, depending on whether a peripheral route (hypothermia) or a central route (hyperthermia) was used (Table I). AA, which decreased the temperature when injected peripherally produced a hyperthermic effect when injected icv. However, it precipitated in the brain ventricle. Therefore, the results obtained with 50 μg/mouse : control 36.1 ± 0.19°C, treated 37.9 ± 0.21°C should be considered cautiously. We then examined whether TRH restored to normal the temperature lowered by LPS, as it does in the case of barbiturates [9]. Simultaneous injection of TRH (1 mg/kg ip) and LPS (2 mg/kg iv) produced hypothermia 57% below that produced by LPS alone at the same dose.

Lysine acetylsalicylate (ASL) (200 mg/kg ip) administered during hypothermia caused by LPS (5 mg/kg ip), raised the temperature by 64.5%, and during hypothermia following AA (20 mg/kg ip), the temperature was raised by 62%. In both cases, ASL was injected simultaneously with the inducers. The reference temperature was that existing 90 min later.

DISCUSSION: The significance of the effect of the acetylsalicylic acid derivative on hypothermia caused by "pyrogenic" substances is unclear. Several possible explanations can be envisaged. Fever results from an upward displacement of the thermoregulatory set point. Conversely, there could be a downward displacement. Depending on the species, there would be, under the influence of a given substance, a displacement of the setting of the thermostat in either direction. This cannot, however, explain the effects of PGE_1, which are opposite, in a given species, depending on the route of administration. For PGE_1, it could be an inability to cross the blood-brain barrier. There would then have to be a direct effect of PGE_1 on the heat loss mechanisms, vasodilatation, for example. Accordingly, it is also essential that ASL has a peripheral action and act in the other direction. However, such an effect has never, as far as we know, been established. Could the specific effect of antipyretics, by inhibition of synthesis of the PG responsible for fever, not be extended, in this case, to substances responsible for hypothermia? Cremased-Campos and Milton [9] have described the parallel existence of two isomers arising from the unstable

TABLE I   Effect of various "pyrogenic" substances in the *mouse*

Prod.	Mice Number Control	Treated	Route	Dose	Time to Maximum Effect (min)	Control (°C ± S.E.M.)	Treated (°C ± S.E.M.)	p
LPS	10	10	i.p.	5 mg/kg	120	36.55 ± 0.12	35.0 ± 0.27	<0.01
	8	8	i.v.	2 mg/kg	90	37.40 ± 0.2	36.0 ± 0.23	<0.01
	8	9	i.c.v.	10 µg/mouse	100	37.35 ± 0.21	36.0 ± 0.14	<0.01
AA	10	10	i.p.	40 mg/kg	30	37.50 ± 0.19	33.8 ± 0.62	<0.01
	8	8	i.v.	30 mg/kg	10	37.60 ± 0.19	35.7 ± 0.14	<0.01
PGE$_1$	10	10	i.v.	1 mg/kg	10	36.80 ± 0.33	35.6 ± 0.22	<0.01
	10	10	i.p.	10 mg/kg	60	36.70 ± 0.16	32.5 ± 0.32	<0.01
	9	8	i.c.v.	0.1 µg/mouse	20	37.40 ± 0.2	39.2 ± 0.2	<0.01
	9	10	i.c.v.	0.2 µg/mouse	20	37.70 ± 0.19	39.0 ± 0.16	<0.01
	10	10	i.c.v.	0.5 µg/mouse	20	37.25 ± 0.12	39.7 ± 0.22	<0.01
PGE$_2$	10	10	i.p.	5 mg/kg	40	37.60 ± 0.08	34.3 ± 0.35	<0.01
	10	7	i.c.v.	0.1 µg/mouse	10	36.90 ± 0.2	38.6 ± 0.2	<0.01

endoperoxide PGH_2. The isomers are 9 α- and 11 α-PGM_2, 11 α-PGM_2 in fact increases temperature but 9 α-PGM_2 lowers temperature. On this basis the following hypotheses may be advanced : depending on the species, formation of one or other of the isomers is favoured. Since the inhibition of the temperature lowering effect is only partial, there could be modification of the physiological balance between the two prostaglandins, one with a temperature lowering effect, the other with a temperature elevating effect. Hyperthermia or hypothermia would result from breakdown of the equilibrium. Thus, in this case ASL would favour synthesis of one isomer at the expense of the other. This hypothesis of two substances with *opposite actions, present at the same time and in the same place*, has already been advanced for prostaglandins. Moncada and Vane [10] have demonstrated the simultaneous existence of thromboxane A_2, a platelet aggregation agent and prostacycline I_2, which, inhibits aggregation.

In fact, the phenomenon may in our case be simpler. Various PG acting on the vascular system are known. In the mouse, the effects could be mainly peripheral and independent of any central action. This would be inhibited little by little by interruption of synthesis of the agent responsible.

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INADEQUATE CUTANEOUS CIRCULATION AND DISTURBED SWEAT SECRETION IN PATIENTS WITH DISORDERS OF BODY TEMPERATURE REGULATION

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We have investigated 4 patients (age 28-37 yr) with severe disorders of body temperature regulation, most probably of hypothalamic origin. The purpose of the study was to determine to what extent the marked variations in core temperature could be attributed to inadequate function of the cutaneous circulation and the sweat glands. In a climate chamber, adaptation of the body to low (16.5°C) and to high (40°C) ambient temperatures was studied in 4 female patients with poikilothermia and in 4 normal women of similar age. During the experiment the following parameters were recorded: ambient temperature, rectal temperature, skin temperature, blood pressure, heart rate, blood flow in the right forefinger, sweat secretion and shivering. During cooling (ambient temperature 16.5°C) body temperature of normal subjects decreased only marginally (from 36.5°C ± 0.2°C, mean ± SD, to 36.3°C ± 0.1°C after 80 min in the supine position) whereas at 40°C core temperature stabilized at 37.0°C ± 0.4°C. In the 4 patients, cooling resulted in a decrease in body temperature, down to 32.3; 33.0; 33.5; 34.5°C, respectively. Once heating of the environment had started, an "after drop" in body temperature was observed in 3 patients. Thereafter, core temperature gradually increased up to a level of 38.5°C, at which point the experiment was terminated. In one patient the recording had to be interrupted at a body temperature of 38.0°C. In contrast to normal subjects, none of the patients showed any sweat secretion at an ambient temperature of 40°C. In the normal subjects transfer to the cold environment resulted in marked vasoconstriction in the skin; at 40°C ambient temperature obvious vasodilatation occurred. In 3 patients a disorder of both vasoconstriction and vasodilatation in the skin has been demonstrated at low and high ambient temperature. In one patient the regulation of skin circulation in relation to environmental temperature appeared to be normal. The preliminary results of the present study show that in these patients poikilothermia is accompanied by disorders in both cutaneous circulation and sweat secretion in relation to ambient temperature. It remains to be established to what extent poikilothermia in the patients can be attributed to these or other thermoregulatory mechanisms.

PHYSIOLOGICAL CHANGES IN CATIONS AND WATER BALANCE DURING STABLE LONG TERM HYPOTHERMIA IN RATS

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Hypothermia occurs in mammals when the rate of heat loss exceeds thermogenic capacity. Once hypothermic an animal's most pressing problem is the maintenance of homeostasis. To date, there is no consensus as to what physiological changes occur during prolonged severe hypothermia or on the quantitative importance of the various observed changes to hypothermic survival.

The cations sodium (Na^+), potassium (K^+) and calcium (Ca^{++}) are known to influence a variety of physiological processes. Because of the relatively large quantities of these ions, especially Na^+ and K^+, they are considered an important factor influencing the distribution of body water. Further, they can have profound effects on membrane excitability (K^+) and enzyme activity (K^+, Ca^{++}). Observations of general tissue edema tend to support reports of loss of ion regulation in hypothermia. Indeed, Brendel [1] suggests that cold induced swelling of the brain is one of the primary factors limiting survival in hypothermia. However, reported changes in plasma cations during hypothermia include increases [2], decreases [3,4], or no change [5,6] for Na^+, K^+ and Ca^{++}. The disparity of results may be due to the methods used for cooling as discussed by Swan [7] or possibly to the timing of observations. For example, observations made during the cooling or warming phases of hypothermia most likely reflect the perturbation of the system by changing temperature. However, observations during the stable phase of hypothermia should reflect the regulatory activity, or lack of activity, at that specific temperature.

Recently, we have developed a system [8] in which rats can successfully recover from deep hypothermia after being maintained at a stable low T_b (19°C) for 24 h. This has enabled us to re-evaluate the effects of hypothermia on metabolic homeostasis over time. We therefore, investigated cation concentrations and the occurrence of tissue edema in hypothermia as a function of time in hypothermia.

METHODS: Male Sprague-Dawley rats were housed individually under 12:12 light/dark photoperiod at 23°C. The rats were fed a ration designed to maintain weight constant at 400 g and provided with water *ad libitum*.

Hypothermia was induced by the method of Jourdan and Wang [8]. Briefly, animals were anaesthetized with halothane (5%) in Helox (21% O_2; 79% He: HeO_2) at 1.5 litres/min. A rectal thermocouple positioned 5-7 cm deep was secured to the tail with tape and needle ECG electrodes were attached. The animal was then cooled in a water jacketed plexiglas chamber at 0°C under halothane (1.5%) and Helox. During the initial rapid cooling halothane was decreased by approximately 0.5%/3°C drop in body temperature (T_b). When T_b dropped to 28°C, halothane was discontinued and cooling was continued under Helox to the desired T_b (19°C). Stabilization at 19°C from the onset of cooling generally required 60 min. Hypothermia was maintained via a feedback loop using rectal temperature as the reference for the adjustment of the circulating water bath temperature.

Blood sampling was via an arterial cannula (.040 O.D. X .025 I.D.) implanted 3 cm into the left carotid artery, under halothane anaesthesia, just prior to hypothermia induction. Blood samples (0.5 ml with replacement) were taken immediately (initial) after surgery and at intervals over the hypothermia period. An aliquot was used for hematocrit determination and the rest centrifuged at 10,000 RPM's for 3 min at 2°C, the plasma was then frozen (-70°C) for later analysis of Na^+, Ka^+ and Ca^{++}.

Tissue samples for evaluation of tissue edema were collected following decapitation of animals which had spent 2, 6, 16, 20 and 24 h in hypothermia at 19°C. Tissues were minced, weighed, and placed in a drying oven at 100°C for 48 h. Following drying the samples were placed in a vacuum desiccator to allow them to cool before weighing.

Statistical analysis included analysis of variance for time-dependent changes and unpaired t-tests for differences between times for each variable.
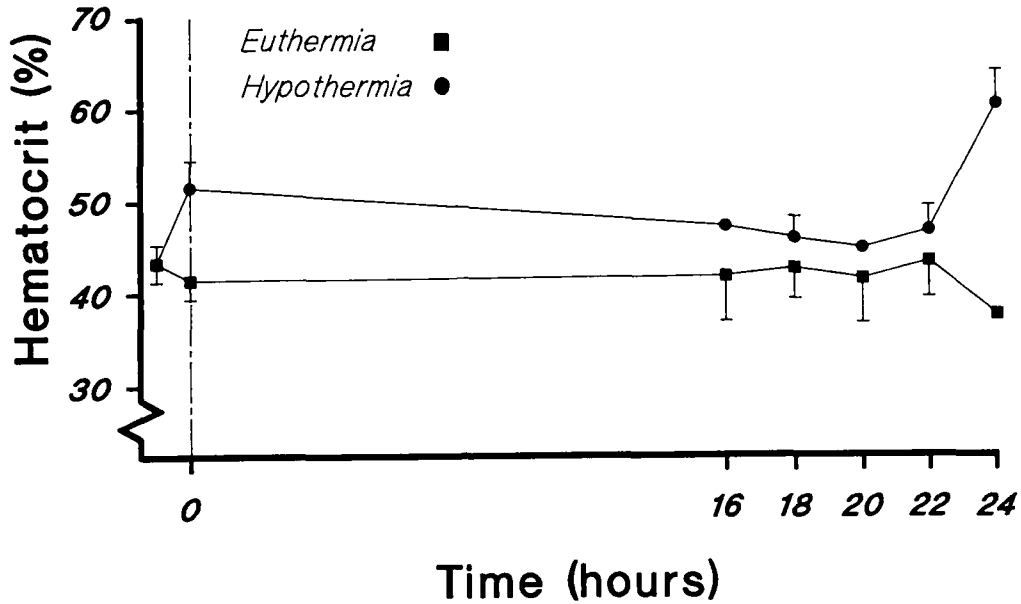
FIGURE 1: Changes in hematocrit ratio of euthermic (T_b, 37°C) and hypothermic (T_b, 19°C) rats with time. Means ± sem; N, 5 for all times except 24 h.

RESULTS: In euthermic animals, hematocrit was relatively constant at 42% over the entire sampling period (24 h) (Fig. 1). In hypothermia animals hematocrit values increased from 42% to 51.5% immediately after T_b reached 19°C. By 16 h it had returned to initial levels and remained relatively constant to about 22-23 h; after that, it increased sharply to 60% (Fig. 1).

As shown in Fig. 2, the levels of Na^+, Ka^+ and Ca^{++} of the euthermic rat were relatively constant throughout the sampling period. In the hypothermic animals the Na^+ level was about the same as that observed in the euthermic rat except the final sample which was significantly lower than the initial level (136.1 ± 2.15 vs 141.1 ± 1.0 mmol/l; Fig. 2). After exposure to hypothermia for 2 h, the plasma level of K^+ was significantly lower than the initial value and the euthermic value. Hypothermic K^+ levels remained stable but significantly lower than the euthermic values throughout the hypothermic period (Fig. 2). In contrast to the euthermic animal, the Ca^{++} levels of the hypothermic rat fluctuated significantly about the baseline value (7.61 ± 0.33 mg/dl) (Fig. 2). Its level was significantly lower (6.26 ± 0.69 mg/dl) at 6 h but significantly higher (8.95 ± 0.77 mg/dl) at 10 h after hypothermia. However, no significant difference in the Ca^{++} levels was observed between the euthermic and hypothermic animals and by 15 h the hypothermic levels had stabilized and remained so thereafter (Fig. 2).

The water content of the kidney, heart, lung and brain from the euthermic animals was about 76%, while that for the liver averaged slightly lower at about 70%. As illustrated in Fig. 3, prolonged exposure to hypothermia at 19°C had a significant effect on the water content of all tissues examined except the liver. All tissues tended to lose water after 6 h in hypothermia. In contrast, the water content of the kidney increased significantly by 2 h and decreased steadily thereafter. By the end of 24 h in hypothermia, no significant difference in kidney water content was observed between the euthermic and hypothermic animals (Fig. 3).
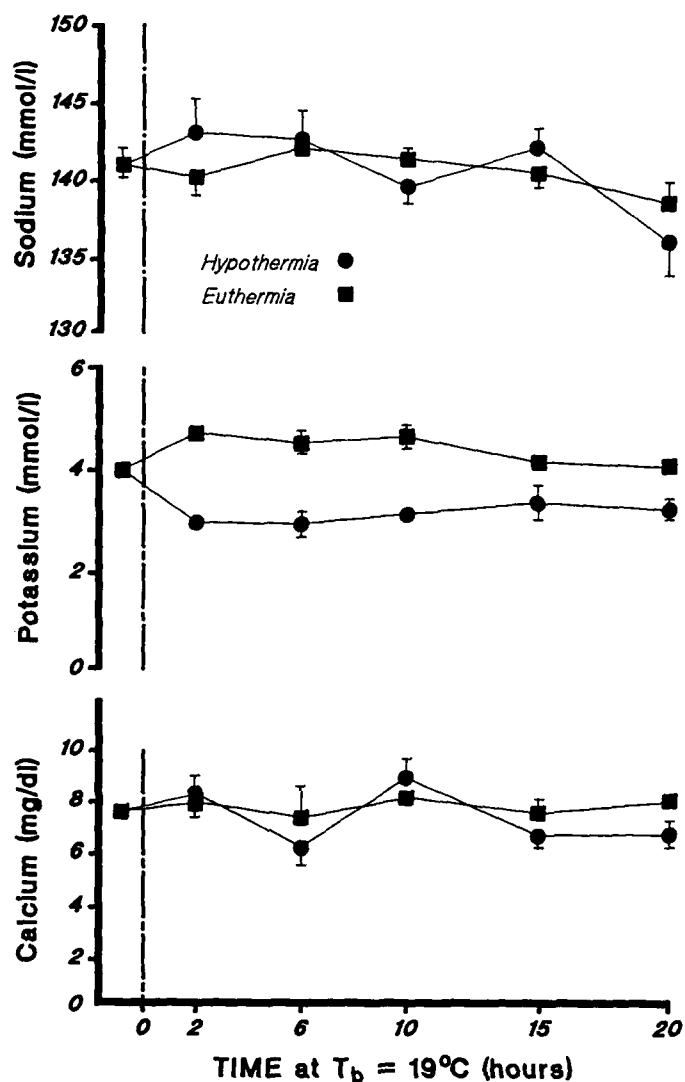
FIGURE 2: Plasma concentrations of Na^+, K^+ and Ca^{++} in euthermic (T_b, 37°C) and hypothermic (T_b, 19°C) rats at time. Means ± sem; N, 8.

DISCUSSION: Using our protocol for hypothermia induction and maintenance we have shown that rats can survive hypothermia at 19°C for up to 24 h [8]. However, the survival rate decreases from 100% at about 18 h to 90% at 24 h. Thus, any perturbation of the physiological system critical to survival should be evident by about 20 h.

Popovic [9,10] observed a continuous increase in hematocrit ratio from 42 to 70% in rats cooled to 15°C. Our hematocrit ratio increased significantly, as well, initially. However, it then returned to control levels and remained relatively constant until late in the hypothermic bout. Since an increase in hemoconcentration has also been observed in normothermic cold exposed animals [11], this suggests that the initial

rise seen in our rats may be a typical cold shock response. The rather dramatic increase to 60% by 24 h comes at a time when remedial measures aimed at reviving the animal are relatively ineffective, and may be one manifestation of a general failure of the regulatory system due to a decrease in energetic efficiency [12-14].
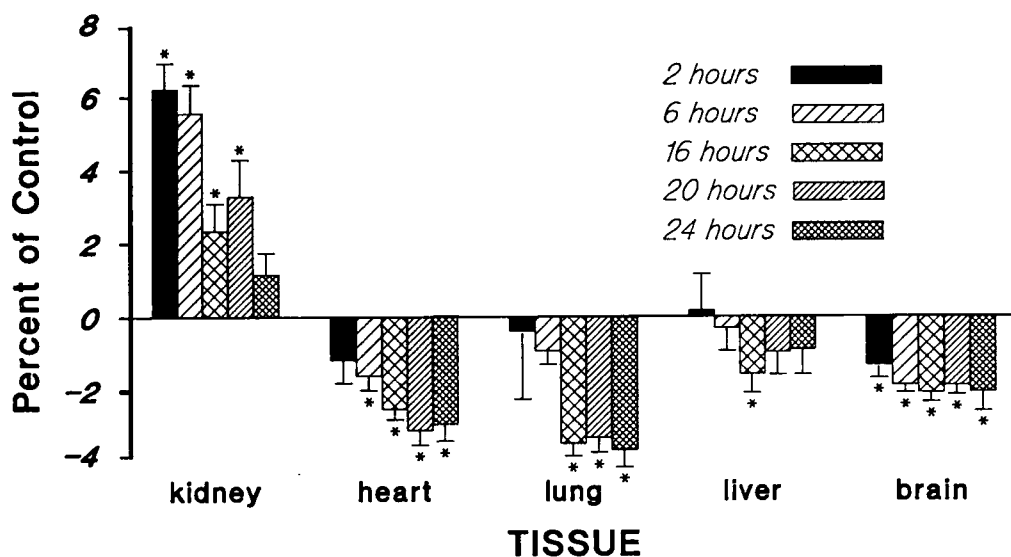
FIGURE 3: Tissue water content of hypothermic (T_b, 19°C) rats at different times in hypothermia. Means ± sem; N, 8. Asterisks (*) signify significant differences from euthermic control (0%).

In contrast to the general failure in homeostatic balance during hypothermia reported in the literature [1], the rat appears to be capable of maintaining its regulatory control of cation balance up to the latest stage of hypothermia under our protocol. Even though there was an early and significant decrease in plasma K^+, its level was maintained relatively constant throughout the hypothermic bout. Since glucose administration tends to decrease K^+ levels in hypothermic animals [1], the decrease in K^+ may be secondary to the observed increase in plasma glucose in hypothermia [15]. Further, this decrease may have the beneficial effect of reducing the tendency for ventricular fibrillation in hypothermia [1]. No significant difference was noted in the trend of Na^+ or Ca^{++} ions in hypothermia as compared to euthermic animals, indicating again that regulatory functions are still operative even at reduced T_b. The increase in fluctuation over time of Ca^{++} early in the bout suggests some instability in the control of Ca^{++} as compared to euthermic animals. However, this may not be a crucial factor for survival as its level was relatively stable by 15 h in hypothermia.

The finding of a decrease, rather than an increase, in water content of the tissues examined is somewhat contradictory to those reported earlier [1]. Other than differences in the hypothermic protocol used and the time of sampling, the discrepancy may also be due to the fact that water lost from one tissue may have been accumulated in other tissues not examined in the present study. Evidence of this redistribution of water has been noted in the gastrointestinal tract, which normally excludes water, tends to accumulate fluid in hypothermic animals (Jourdan, personal observation). The reason for this shift in water distribution is not well understood at this time, but may be due to changes in membrane permeability [16,17]. However, as no positive correlation was observed between tissue water content and the survival rate of the animal after prolonged hypothermia, it is evident that the changes in water content within the vital organs examined may not be the limiting factor for survival in long term hypothermia.

Taken together, these data indicate that hypothermia is not the completely uncontrolled state typically depicted in the literature. Under our protocol, which maintains a constant stable temperature (±0.3°C) for long periods of time, the cation levels and water balance appear to be well regulated and maintained relatively stable throughout the hypothermic bout. Further, the gradual changes in plasma cation levels and tissue water contents observed in this study are unlikely to be the crucial factors for survival of the animal under long term hypothermia, whether other factor(s) (e.g., plasma pH, substrate utilization etc.) are involved in limiting survival remains to be investigated.

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TRACHEOSTOMY AND BRAIN TEMPERATURE IN SHEEP EXPOSED TO COLD

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In four adult Dorper sheep, we implanted a tracheostomy valve which allowed reversible bypass of the upper respiratory tract. After recovery, the sheep were exposed for 2 h to 5°C ambient temperature, breathing normally and through the tracheostomy.

Hypothalamic temperature (T_{hy}) was significantly lower than rectal temperature (T_{re}) throughout the cold exposure in the sheep breathing normally, and the apparent brain cooling was not affected by upper respiratory bypass. Hence, the cooling presumably did not depend on the mucosal evaporation/carotid rete mechanism. The magnitude of the cooling ($T_{re}-T_{hy}$ = 0.5°C approximately) was similar to that evident in sheep exposed to neutral and hot environments [1], where it was abolished by upper respiratory bypass, and was therefore rete-dependent.

Sheep breathing normally showed a transient rise in T_{re}, reaching 0.2°C after 30 min of cold exposure. This rise was abolished during tracheostomy breathing, indicating that upper respiratory bypass also either increased general body heat loss, or reduced heat conservation, or both.

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INHIBITION OF ENDOGENOUS PYROGEN RELEASE BY N^6-2'-O-DIBUTYRYL ADENOSINE 3', 5'-MONOPHOSPHATE AND ISOBUTYL-METHYLXANTHINE

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The endogenous mediator of fever described by Bennett and Beeson [1,2] is indistinguishable from the cytokine, interleukin-1 [3], although interferon-α [4] and tumor necrosis factor [5] are reported to be intrinsically pyrogenic and may contribute to the pathogenesis of fever. In addition to being a mediator of fever, endogenous pyrogen (EP)/interleukin-1 (IL-1) has many other biological actions [3], therefore, the possible usefulness of drugs modulating the production of EP is extensive.

Numerous studies on the synthesis and release of EP have been made, but relatively little is known about the intracellular events mediating EP production [6,7]. Adenosine 3',5'-monophosphate (cyclic AMP) appears to be an intracellular or second messenger inhibiting several principal functions of cells producing EP, including mononuclear phagocytes and polymorphonuclear leucocytes [8]. The purpose of the present study was to determine the effect of raised concentrations of cyclic AMP on EP production by activated leucocytes *in vitro*.

METHODS: Blood was collected from the medial ear artery of large albino half lop rabbits (3.5-5 kg) using an aqueous solution (pH 7.4) of ethylenediaminetetra acetic acid disodium salt (EDTA, 10%) as an anticoagulant (1 ml EDTA: 9 ml blood). Blood was centrifuged at 280 RCF for 20 min at 6-8°C in silicon coated glass tubes and the platelet rich plasma discarded. Blood cells were further centrifuged at 2414 RCF for 20 min at 6-8°C to form a layer of buffy coat leucocytes for collection. Leucocytes were incubated (10^8 cells/ml) in phosphate buffered saline containing glucose (30 mmol/l; pH 7.44; 293 mOsm/l). The drugs used were N^6-2'-O-dibutyryl adenosine 3',5'-monophosphate sodium salt (dibutyryl cyclic AMP) and 3-isobutyl-1-methylxanthine (IBMX). These drugs were dissolved in the incubation medium before the addition of either *Sa!* ~ *nella abortus equi* lipopolysaccharide (LPS, 10 ng/10^8 cells) or saline (0.9%, 10 μl/10^8 cells). Leucocytes were incubated with continuous shaking for 16-18 h at 37°C then centrifuged at 2414 RCF for 20 min at 6-8°C. Cell free supernatant solutions were stored at -20°C until bioassay for pyrogen content. Each drug treatment was applied to a number of different leucocyte harvests (b). Procedures minimising contamination by extraneous pyrogens were used for the preparation, transfer and injection of solutions [9].

Sterile batches of leucocytic products and other drug solutions were injected i.v. into either D⋯r or albino half lop rabbits (2-3 kg, both sexes) using a dose volume of 1 ml (equivalent to 10^8 cells)/rabbit. Core temperature was measured with a thermistor inserted about 10 cm past the anus while each rabbit was restrained in stocks at an ambient temperature of 20-22°C. Temperature effects were assessed as the maximum change in core temperature from preinjection values (Δ°C) and as a temperature response index (TRI) integrating Δ°C against time for 4 h after injection. Results for EP samples are presented as the mean ± 1 s.e.m. of the average values for the batches of leucocytes (b), each batch being assayed in n rabbits (mode 2, range 1-3) For drug solutions, the mean ± 1 s.e.m. is for responses in n animals. The probability (P) of the significance of the difference between means was evaluated by a 2-tailed Student's t-test.

RESULTS: EP produced by buffy coat leucocytes in the presence of LPS induced a monophasic fever with a maximum rise in colonic temperature of 0.68 ± 0.05°C at 55 ± 3 min (b,9; n,17). This was a submaximal pyrogenic response. LPS (1 μg/rabbit i.v.) caused a biphasic fever with a maximum temperature rise of 1.30 ± 0.16°C at 157 ± 23 min (n,9). There was no significant difference between the responses of Dutch and albino half lop rabbits to either EP or LPS in these doses. The TRI values for EP (1.67 ± 0.15°C.h) and LPS (3.53 ± 0.47°C.h) were both greater (P < 0.05) than the TRI for saline (0.9%, 1 ml/rabbit iv) (-0.02 ± 0.30°C.h; n,15). Supernatant incubation medium from leucocytes not exposed to

LPS had no effect on temperature (0.35 ± 0.08°C.h; b,6; n,10). Similarly, phosphate buffered saline with or without LPS (10 ng/ml) had no effect on body temperature (0.50 ± 0.31 and 0.61 ± 0.34°C.h respectively; n,5).

The effects of dibutyryl cyclic AMP and IBMX on LPS stimulated EP production are shown in Table I. The TRI for EP (1 ml/rabbit iv) produced in the presence of dibutyryl cyclic AMP (1 mmol/l) was reduced (P < 0.05). Incubation of buffy coat leucocytes with LPS in the presence of a higher concentration of dibutyryl cyclic AMP (10 mmol/l) did not result in any further decrease in EP induced fever. IBMX (0.1 and 1 mmol/l) caused a dose-related decrease in EP activity, measured both as the maximum change in colonic temperature and the TRI (Table I). The inhibitory effects of dibutyryl cyclic AMP (1 mmol/l) and IBMX (0.1 mmol/l) were additive. The TRI for EP produced in the presence of both drugs was determined experimentally to be 41% of the control value, the same as the theoretical value for additive inhibitory effects.

TABLE I   Effect of N^6-2′-O-dibutyryl adenosine 3′-5′-monophosphate (DCAMP) and 3-isobutyl-1-methylxanthine (IBMX) on endogenous pyrogen (EP) production

Stimulus for EP Production	Drug Treatment (mmol/l)	Maximum Δ°C	TRI (°C.h)	b	n
Saline	–	0.28 ± 0.07[2]	0.35 ± 0.08[2]	6	10
LPS	–	0.68 ± 0.05[1]	1.67 ± 0.15[1]	9	17
LPS	DCAMP (1)	0.55 ± 0.06[1]	1.11 ± 0.06[1,2]	6	11
LPS	DCAMP (10)	0.61 ± 0.05[1]	1.19 ± 0.16[1]	5	9
LPS	IBMX (0.1)	0.47 ± 0.05[2]	1.27 ± 0.26[1]	5	6
LPS	IBMX (1)	0.32 ± 0.02[2]	0.45 ± 0.09[2]	5	9
LPS	DCAMP (1) + IBMX (0.1)	0.41 ± 0.06[2]	0.89 ± 0.23[2]	5	10

EP (1 ml/rabbit iv) was assayed by the pyrogenic response, measured as the maximum change in colonic temperature (Δ°C) and the area under the fever curve (TRI, °C.h) for 4 h after injection. See Methods for details of EP production and drug treatment.

Values are means ± 1 s.e.m. for batches (b) of buffy coat leucocytes.
The number of recipient rabbits is denoted by n.
[1]P < 0.05 compared with response to EP produced in presence of saline.
[2]P < 0.05 compared with response to EP produced in presence of lipopolysaccharide (LPS 10 ng/10^8 cells).

Injection (1 ml/rabbit iv) of dibutyryl cyclic AMP (10 mmol/l; n,3) or IBMX (1 mmol/l, n,9) had no effect on normal body temperature in rabbits (TRI 0.07 ± 0.07 and -0.16 ± 0.23°C.h respectively; P > 0.6).

DISCUSSION: Mononuclear phagocytes and polymorphonuclear leucocytes stimulated by an agent such as bacterial endotoxin (lipopolysaccharide, LPS) produce immune response modifiers of which some (IL-1, tumor necrosis factor, interferon-α) are pyrogenic [3-5]. These mediators and possibly others, either alone or in combination, probably constitute the EP described by Bennett and Beeson [1,2]. In view of the multiplicity of pyrogenic mediators and the absence of specific assays for cytokines of animal origin, it remains appropriate both to assay the product of mixed leucocyte populations by its ability to induce pyrexia in recipient animals, and to retain the term 'endogenous pyrogen'.

Cyclic AMP or drugs affecting endogenous concentrations of the cyclic nucleotide inhibit several proinflammatory activities in mononuclear phagocytes and polymorphonuclear leucocytes [8]. However, there are few detailed reports in the published literature concerning the effect of cyclic AMP on EP production. Sigal et al. [10] have reported that both adrenaline and prostaglandin E_1, agents stimulating cyclic AMP formation, have no effect on EP production by rabbit buffy coat leucocytes. In addition, dibutyryl cyclic AMP, a direct activator of cyclic AMP dependent protein kinase, was stated by these workers [10] to have no effect on the production of EP in response to LPS. However, the dose of dibutyryl cyclic AMP used in the experiment was not disclosed and the experimental data were not presented. In the present study, incubation of rabbit buffy coat leucocytes with dibutyryl cyclic AMP (1 mmol/l) did reduce the amount of EP produced in response to LPS when the pyrogen was assayed by the area under the fever curve. The corresponding maximum rise in body temperature ($\Delta°C$) was not reduced (Table I). This is consistent with the report by Sigal et al. [10] who used $\Delta°C$ to assay EP. Dibutyryl cyclic AMP appears to have a limited effect on EP production, the high dose of nucleotide (10 mmol/l) being no more inhibitory than the low dose (1 mmol/l). The reduction in the TRI for EP was not due to a hypothermic effect of dibutyryl cyclic AMP.

IBMX, an inhibitor of cyclic AMP phosphodiesterase [11], produced a dose-related inhibition of EP production by rabbit leucocytes in response to LPS, assessed by both the $\Delta°C$ and the TRI. Although potentiation of a response by methylxanthines is presumptive evidence that the response is mediated by cyclic AMP [12], it is not conclusive evidence, because the drugs have effects unrelated to cyclic AMP. In contrast, Bernheim and Wenc [13] found IBMX (unspecified dose) had no effect on EP production by human monocytes stimulated by endotoxin, and they concluded that cyclic AMP is probably not involved in EP production.

The conclusion of this study is that dibutyryl cyclic AMP and IBMX can inhibit rabbit EP production. These pharmacological data alone are insufficient for the proposal that cyclic AMP has a role in EP production. However, these results do challenge the hypothesis that cyclic AMP does not control EP production [10].

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ANTIPYRETIC AND ANALGESIC EFFECT OF SOME WILD EGYPTIAN PLANTS

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Ten wild Egyptian plants belonging to seven families were collected from their respective habitats in Desert areas. The air dried plants were powdered and extracted with ethanol and subjected to tests for active principles. Aliquots of the ethanolic extracts were evaporated to dryness and residues dissolved in Tween-80. Ethanolic extracts of *Zygophyllum album, Lycium shawii, Salvia aegyptiaca, Crotaleria aegyptiace, Centaurea aegyptiaca* and *Nitraria retuse* produced marked analgesic activity in mice. The reaction time for pain was significantly longer than control values. Their activity resembled the analgesic activity of standard paracetamol at a dose of 5 mg/100 g mice. The antipyretic effect of the plants studied showed that extracts of *Zygpophllm album, Salvia aegyptiaca* and *Centaurea aegyptiaca* produced marked antipyretic activity in experimentally hyperthermic rats. Rectal temperature was significantly decreased in a manner similar to that induced by aspirin in a dose of 50 mg/kg.

EFFECTS OF AMITRIPTYLINE HYDROCHLORIDE ON FEVER IN RABBITS

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The effect of tricyclic antidepressant drugs on normal thermoregulation in various animal species and man has been studied [1] but the effect of the drugs on fever is not well established. We carried out two series of experiments to study the effects of chronic and acute administration of amitriptyline hydrochloride on endotoxin fever in rabbits at 21-22°C ambient temperature.

In the first series amitriptyline hydrochloride (AmHCl) was administered ip in a dose of 1 mg/kg or 10 mg/kg to groups of six rabbits daily for three weeks. The rabbits' response to injection of *Salmonella typhosa* (0.1 μg/kg iv) endotoxin or sterile saline was tested on the first day of AmHCl administration and weekly thereafter. In the second series of experiments six rabbits were given AmHCl (either 1 or 10 mg/kg iv) simultaneously with endotoxin (0.1 μg/kg iv) or sterile saline. Control groups of animals in each series of experiments received an equal volume of sterile saline instead of the AmHCl.

After two weeks of daily injections AmHCl (10 mg/kg) and for the remainder of the experimental period the rabbits' response to endotoxin was significantly attenuated ($p < 0.05$, Student's t-test) compared to that of control animals; the lower daily dose of AmHCl had no significant effect. When AmHCl was administered simultaneously with endotoxin a significant dose-dependent antipyresis was observed from 50 min after injection of either dose of AmHCl compared to the response in control rabbits. AmHCl had no effect on the body temperature of afebrile rabbits.

We conclude that among its other central nervous system actions amitriptyline hydrochloride affects the development of the normal febrile response in rabbits. Our results indicate that the antipyretic effect is present after short or long-term administration, and is not due to any inherent hypothermic effects of drug.

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ANALYSIS OF BODY TEMPERATURE AT DIFFERENT SITES IN PATIENTS HAVING SLIGHT FEVER CAUSED BY PSYCHOGENIC STRESS

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The aim of this study was to investigate the correlation between psychogenic stress and prolonged slight fever.

METHODS: Among 70 patients having slight fever, 15 were diagnosed as cases of psychogenic fever. Diagnostic criteria were based on the following: 1. Those who had axillary temperature of 37.3°C–37.6°C through the year without organic diseases; 2. Those who were diagnosed as neurotics or psychosomatics without any psychiatric disorders. As a control group, 178 healthy subjects were examined.

Thermistors were applied to measure simultaneously tympanic temperature (T_t), oral temperature (T_o), rectal temperature (T_r), axillary temperature (T_a) and finger temperature (T_f) in a room at 20°~23°C and 50% humidity. The measurements were performed at rest. The circadian rhythm of body temperature of 18 young normal subjects was determined.

A heat loading test was performed by the immersion of right hand in a hot water bath (45°C) for 3 min. The changes in the temperature of the right hand and the left hand were recorded simultaneously with an electronic recorder and by thermography.

The relationship between T_a and the temperature of other sites was checked in 12 healthy male subjects wearing only light undershirts and slacks in a room at 20°C with 60% humidity for 30 min. Then, the room temperature was raised rapidly to 40°C for about 1~2 min. After that period, the room temperature lowered again to 20°C.

RESULTS: Basal T_r, T_t, T_o, T_a and T_f in males were 37.23 ± 0.21, 36.92 ± 0.24, 36.77 ± 0.24, 36.50 ± 0.43, 35.11 ± 0.88°C, and in females 37.36 ± 0.27, 36.99 ± 0.24, 36.88 ± 0.27, 36.53 ± 0.37, 32.86 ± 3.56°C respectively. Forehead, cheek, nose, lips, neck, chest, abdomen, waist, upper and lower thigh, leg and finger temperatures in males were 34.3 ± 0.6, 33.8 ± 0.8, 33.4 ± 1.8, 34.7 ± 1.1, 33.8 ± 0.5, 32.9 ± 0.8, 32.8 ± 0.8, 33.4 ± 1.8, 33.6 ± 0.4, 32.8 ± 0.5, 31.9 ± 0.7, 30.5 ± 1.6°C, and in females 32.6 ± 1.3, 33.2 ± 1.0, 30.9 ± 4.0, 34.6 ± 0.6, 34.0 ± 0.3, 32.9 ± 0.6, 33.8 ± 0.6, 34.3 ± 0.6, 31.5 ± 0.7, 31.9 ± 1.1, 31.0 ± 1.5, 30.0 ± 1.0°C respectively. The duration of circadian rhythm was 23 ~24 h in all subjects. Between 1:00 h and 11:00 h, male T_r was significantly lower than that of females during the luteal phase. Between 1:00 h and 16:00 h, female T_r in luteal phase was higher than in the follicular phase.

Immediately after immersion of the right hand in hot water, left T_f was slightly lowered, followed by a transient elevation.

The effects of high room temperature on T_t, T_o, T_a and T_f were: T_a was slightly higher than T_o, which showed a reversed value compared with the normal temperature distribution. If the room temperature was gradually raised from 30° to 35°C, T_a and T_o reached approximately at the same level, then T_o became higher than T_a. During the period of highest room temperature, 35° to 40°C, T_t was the highest, T_a was the lowest, and T_o was in between.

Patients with slight fever due to psychogenic stress had T_t, T_o and T_r within the normal range, except a slightly higher T_a than T_o and clearly lower in T_f.

Estimated temperatures at different sites by thermography showed that T_f was generally depressed, and nose temperature was often lower in patients with slight fever due to psychogenic origin. Among patients with psychogenic fever, one had an abnormal circadian rhythm. A 39 year old businessman, who was very nervous, with anxiety and fear, had a higher T_a between 37.3~37.6°C, which was higher than T_o. T_r, T_t, T_o, T_a and T_f were: 37.13~37.68, 36.81~37.49, 36.69~37.18, 37.18~37.60, 22.03~34.78°C, respectively.

Circadian T_r rhythms, examined at different seasons, were considerably variable and the lowest point distributed widely between 3:00-6:00 h. The lowest BRM was -28% in spite of slight fever; however, it rose to normal sooner after a meal. The patient underwent psychological treatment for 3 months and once again body temperature has been checked, and was somewhat lower than previously.

Loading tests by immersion of the hand in a hot bath showed that patients with a finger temperature above 30°C responded well. However, those with a finger temperature less than 30°C responded poorly.

DISCUSSION: These results demonstrate in 12 healthy male subjects that the relationship between T_a and T_o was influenced by the ambient temperature. The fact that T_a was higher than T_o at 20°C might be due to finger arterio-venous counter-current heat exchange.

In patients with prolonged slight fever due to psychogenic origin higher T_a than T_o with a lower T_f was observed. This might be related to our finding that environmental temperature as well as psychogenic stress would be factors which affect the relationship between T_a and T_o in terms of arterio-venous counter-current theory.

POLYMYXIN ATTENUATE⸍ STRESS INDUCED HYPERTHERMIA

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Psychological stress results in a rapid rise in body temperature ("stress-induced hyperthermia") in rats [1-9], rabbits [10,11], and humans [12,13]. During infection the fever that develops is attributable to the release of endogenous pyrogens from stimulated macrophages. These endogenous pyrogens (e.g., interleukin-1, tumor necrosis factor, etc.) elevate the thermoregulatory set point in the hypothalamus by causing the synthesis of prostaglandins [see reviews in 15 and 16]. Antipyretic drugs such as sodium salicylate and indomethacin are thought to reduce fevers by blocking the synthesis of prostaglandins. Much of the rise in temperature that occurs during psychological stress can also be blocked by systemic [3,6] or intracerebroventricular [14] injection of antipyretic drugs. Based on these data, we have concluded that part of stress induced hyperthermia is a prostaglandin mediated fever.

What is the signal for this rapid prostaglandin mediated rise in body temperature? Endotoxin, a component of the cell wall of gram negative bacteria, is one of the most potent stimuli for initiating prostaglandin mediated fevers. It is known that stress results in the rapid redistribution of blood such that blood flow to the gastrointestinal tract is markedly decreased [see review in 17]. Could the signal for stress induced hyperthermia come from the leakage of endotoxin across the gastrointestinal tract? If this hypothesis were correct, then oral administration of polymyxin, a cationic antibiotic that binds to and inactivates a large percentage of endotoxin, should result in an attenuation of stress induced hyperthermia.

METHODS: Specific pathogen free male Sprague-Dawley rats weighing 180 g at the start of the experiment were housed individually in a temperature controlled room at 27 ± 1°C, the thermoneutral zone for rats. A photoperiod of 12 h light (06:00 to 18:00 h) and 12 h dark (18:00 to 06:00 h) was maintained throughout the experiment.

Body temperature was measured using battery-operated biotelemetry devices implanted intraperitoneally into rats at least 3 days prior to the start of the experiment. Each transmitter was calibrated prior to implantation. Both deep body temperature and activity were monitored by signals received by a mounted antenna placed under each animal's cage and fed into a peripheral processor connected to a microcomputer. Temperature counts were recorded at 30 min intervals for 3 control days. On the fourth day of the experiment, temperature counts were recorded at 5 min intervals from 30 min prior to the rats being put into the stress box or "cage-switched" until the end of the experiment. Rats were fed rodent chow and tap water *ad libitum* prior to the start of the experiment and for the first two days of the experiment. At 12:00 h each day the rats and their water bottles were weighed. At 12:00 h on the third day, rats were given autoclaved rodent chow and either autoclaved distilled water ("control" group) or autoclaved distilled water containing polymyxin B sulfate (4.0 mg/ml) ("polymyxin" group). In preliminary experiments we found that control rats (n, 9) drank 46.4 ± 3.1 ml/day and that the polymyxin group (n, 12) drank only 22.3 ± 1.7 ml/day. To control for any possible effect of dehydration on stress induced hyperthermia, we had a second control group of rats that had access to only 25 ml of water. The polymyxin group also had access to 25 ml of water (containing 4.0 mg/ml polymyxin).

Three separate experiments were run:

One procedure we have used to induce acute psychological stress in rats has been to expose them to an open field [6,14,18]. Between 9:30 h and 11:30 h on the fourth day after implantation, each rat was taken to the open field chamber for its 30 min stress period. The open field used in these experiments consisted of a 150 x 97 x 200 cm temperature controlled chamber spray finished with white acrylic and illuminated by both fluorescent lights and a 200W white light suspended above. The temperature within the open field box was the same as that in the rats' home cages. The experimental protocol for the stress involved transporting each rat from the home room to the stress room, removing the rat from the cage and

placing him into the open field. After 30 min, the rat was returned to his cage and taken back to the housing area. Care was taken not to disturb the rats either before or after the stress exposure. Control animals were not placed in the open field box. All animals were conditioned to handling for at least 3 days prior to the experiments. To minimize possible circadian variability, all exposure to the open field occurred between 09:00 h and 14:00 h. Three groups of rats were studied: a control group (n, 5), a control with limited water group (n, 6), and a polymyxin group (n, 5).
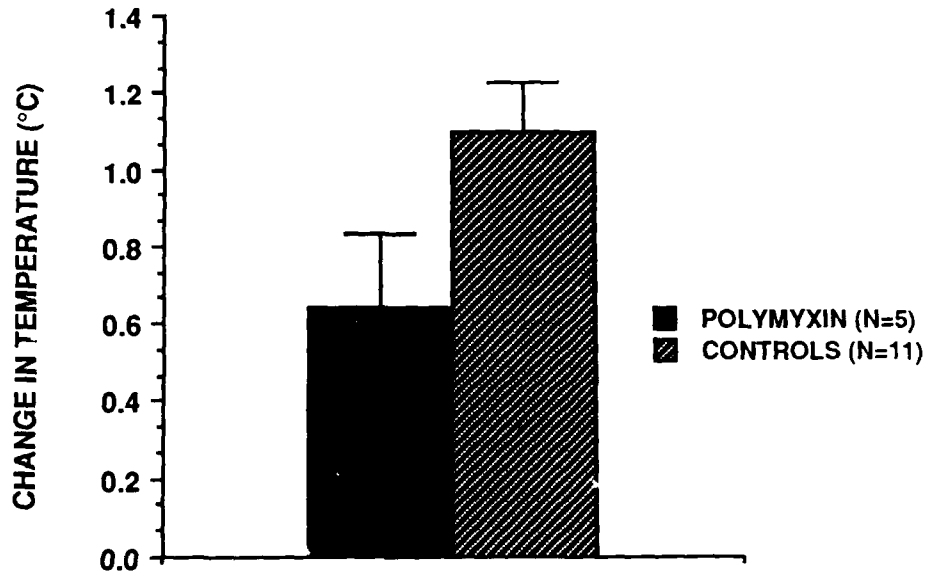
FIGURE 1: Effects of addition of polymyxin to drinking water on average change in body temperature of rats following 30 min of exposure to an "open field." The changes in body temperature for the polymyxin and control groups are for the period immediately prior to exposure to the open field subtracted from the temperature immediately after removal from the open field, i.e., the first temperature measured after the stress. Numbers in parenthesis = sample size.

The second procedure we have used (unpublished data) to induce stress hyperthermia is "cage switch." This simply involves switching the cages of two rats. Exposure to the olfactory and visual stimuli associated with this new environment results in a somewhat milder stress hyperthermia than that seen during exposure to the open field described above. As in the first experiment, three groups of rats were studied: a control group (n, 8), a control group with limited water group (n, 6), and a polymyxin group (n, 15). Rats were cage switched at 12:00 h on the fourth day of the experiment.

The rationale for the third experiment is two-fold. One is to determine whether the reduction of stress hyperthermia in polymyxin treated rats is attributable to an inability to raise body temperature in response to other factors known to raise thermoregulatory set point. The second is to determine whether polymyxin given orally becomes distributed systemically; this would be evidenced by a blockade of fever due to the administration of exogenous lipopolysaccharide (LPS). Although it has been reported that polymyxin B is not absorbed when ingested orally [19], it is possible that at the doses used in this experiment there could be some elevation in systemic concentrations. This might result in both a reduction in LPS induced fever and in other unknown side effects associated with this antibiotic. At 12:00 h on the fourth day, control rats (n, 4) and polymyxin treated rats (n, 6) were injected with E. coli LPS 0111:B4 Sigma phenol extract (10 μg/kg ip).
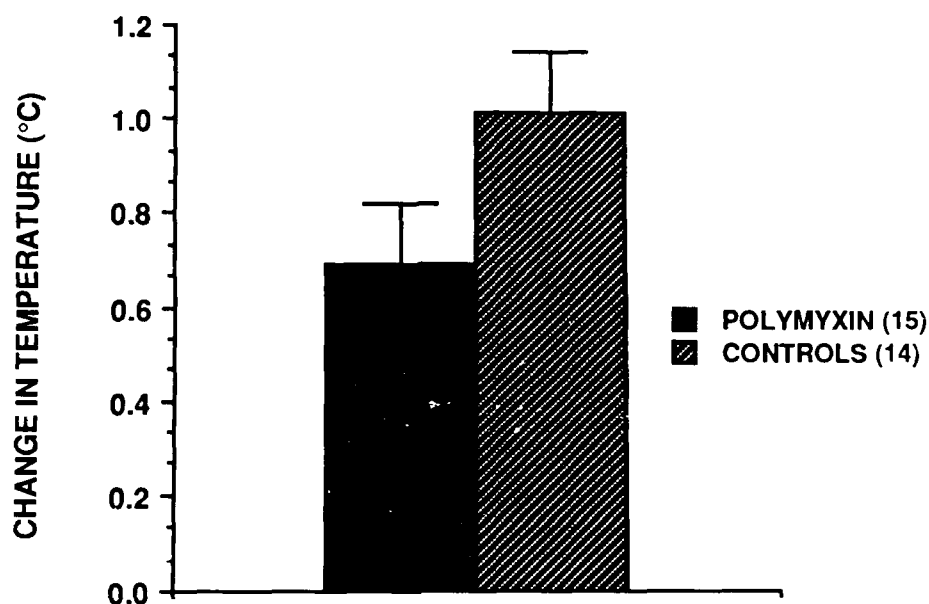
FIGURE 2: Effects of addition of polymyxin to drinking water on the average change in body temperature of rats following 30 min of exposure to new cages. The changes in body temperature for the polymyxin and control groups were obtained by subtracting values for the period immediately prior to "cage-switch" from the temperatures at the end of 30 min of "cage-switch." Numbers in parenthesis = sample size.

The rise in temperature due to stress or to LPS was measured by subtracting all temperatures following or during the stress period (or post-LPS injection) from an average of the 3 time points immediately prior to the animal being put into the open field, being "cage switched," (or given LPS). Statistical differences were determined using a one-tailed Student's t test. All ± refer to standard errors of the mean.

RESULTS: Limiting water was found to have no effect on open field stress hyperthermia; therefore, we have combined the data for these two control groups. The average starting temperature for the control animals was 36.8 ± 0.16°C; that for the polymyxin group was 36.6 ± 0.08°C. The polymyxin group had a significantly smaller rise in body temperature in response to exposure to the open field (0.64 ± 0.19°C vs. 1.10 ± 0.13°C; P < 0.03) (Fig. 1). We also evaluated the mean temperature change for the first 30 min following the stress period and found it, too, was significantly lower in the polymyxin treated group (0.70 ±0.12°C vs. 0.98 ± 0.07°C; P < 0.04).

As in experiment 1, limiting water had no effect on cage switch stress hyperthermia, and once again we have combined the data for these two control groups. The average starting temperature for the control animals was 37.1 ± 0.08°C; that for the polymyxin group was 37.0 ± 0.09. The average rise in body temperature during the first 30 min of exposure to the new cage for the polymyxin group was significantly smaller than that for the control group (0.69 ± 0.13°C vs. 1.01 ± 0.13°C; P < 0.02) (Fig. 2).

The patterns of fever for the control and polymyxin groups were virtually identical (Fig. 3), as were the average changes in temperature for the 6 h following injection of endotoxin (0.75 ± 0.14°C for the polymyxin group vs. 0.81 ± 0.14°C for the control group).
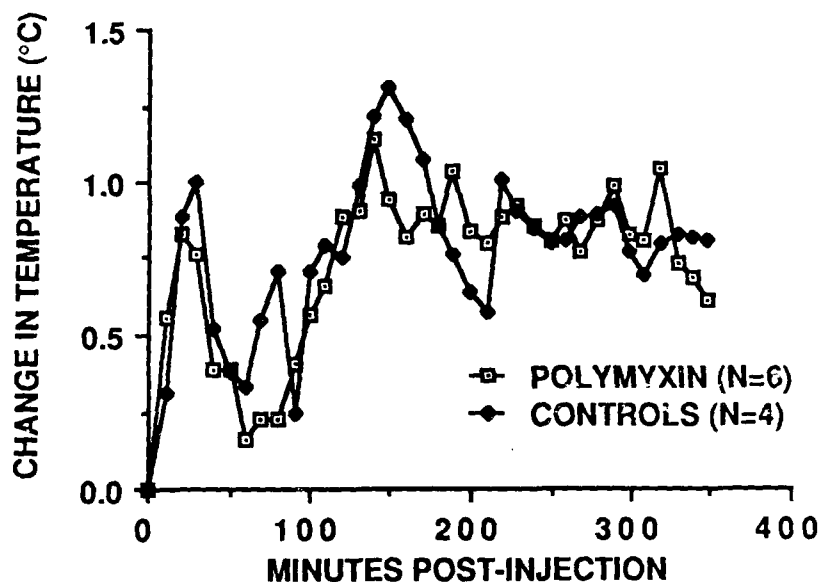
FIGURE 3: Effects of addition of polymyxin to drinking water on changes in body temperature of rats caused by injection of *E. coli* LPS (10 μg/kg ip). Numbers in parenthesis = sample size.

DISCUSSION: The addition of polymyxin to the drinking water of rats results in a significant attenuation in the stress fever that results from exposure to an open field or to a cage that was formerly occupied by another male rat. For two reasons it is unlikely that this reduced stress fever is due to an inability to thermoregulate normally: (1) the starting body temperatures of the polymyxin treated rats were virtually identical to the control rats, and (2) the fevers in response to injection of LPS were indistinguishable for the two groups.

These data are consistent with the hypothesis that psychological stress, whether it be the result of exposure to an open field or to a new cage, results in an acute release of endotoxin from the gastrointestinal tract. Also consistent with this hypothesis is the fact that the fraction of stress hyperthermia blocked by polymyxin is quantitatively similar to that blocked by antipyre drugs [3,6,14]. However, polymyxin ingestion may well have a variety of effects other than binding ᵤ ᵤotoxin, which could alter the response to stress, and so we are presently evaluating this hypothesis more directly by measuring plasma endotoxin concentrations in control and stress situations.

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EFFECT OF INDOMETHACIN ON HYPERTHERMIA INDUCED BY LIPOPOLYSACCHARIDES OR HIGH AMBIENT TEMPERATURE IN RATS

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Injection of lipopolysaccharide (LPS) to animals produces fever which is a highly integrated biologic response. It includes among other things elevated body temperature, increase in the release of arachidonic acid in the brain, metabolic changes and several alterations in neural as well as non-neural tissues. Prostaglandin E_2 (PGE_2) is believed to be the main arachidonic acid metabolite which mediates fever. It seems likely that prostaglandins (PG) modify the function, by a yet unknown mechanism, of the thermoregulatory neurons in the hypothalamus to produce fever. That is, a rise in set-point from the normal level to that of the febrile state. Such temperature elevations can be inhibited or reversed by non-steroidal anti-inflammatory drugs (NSAID) which are known to block PG synthesis [1-3]. Data indicating that PG are the mediators of normal thermoregulation are conflicting [4-6]. The present investigation studies the possible relationship between PG, pyrogen and environmental hyperthermia.

METHODS: Male Sprague-Dawley rats 12-15 weeks old weighing 350-500 g were maintained in a room kept at 22 ± 1°C and relative humidity of 35-55% for at least 2 weeks before commencing the experiments. The rats were housed on wood shavings, 2 per cage with water and food *ad libitum*. The experiments started at 10-12:00 h and were carried out at ambient temperatures of 22 ± 1°C (control) and 34 ± 1°C (heat). Rats were injected either with lipopolysaccharide (LPS, E. coli, 350 μg ip) or saline (0.5 ml ip). In the first experiment 22 h postinjection, groups of animals were injected either with indomethacin (INDO 50 mg/kg ip) in $NaHCO_3$ 2% (0.5 ml/100 g ip) or $NaHCO_3$ 2% alone. In the second experiment 20 h after LPS administration rats were transferred to a room kept at 34 ± 1°C. INDO and $NaHCO_3$ were injected 2 h later. Rectal temperatures (RT) were measured before each treatment with a thermistor probe inserted 5 cm into the rectum using a telethermometer. Following the final RT measurement 2 h after the INDO or vehicle administration the rats were decapitated and the anterior hypothalami (HT), weighing about 40 mg, were excised. Two HT were incubated per vial (5-6 vials per group) for 3 h in Krebs-Henselite buffer containing glucose (0.2%) under an environment of O_2 (95%) and CO_2 (5%) at 37°C. The buffer was replaced every 30 min. The buffer samples that were recovered were frozen and were assayed at a later time for PGE_2 by radioimmunoassay [7]. Statistical evaluation was carried out using student's t-test (two tailed) and factorial analysis.

RESULTS: In rats treated with LPS the RT was significantly higher compared to control (p < 0.01) and always was above 38°C (Table I). The *ex vivo* experiments show that the enzymatic activity of PGE_2 synthesis in the HT was affected by LPS treatment resulting in increased PGE_2 released into the incubation medium at the times measured (p < 0.01). INDO reversed the LPS induced elevation in RT and decreased PGE_2 synthesis as is shown by the decreased rate of PGE_2 release into the incubation medium by the hypothalami of LPS treated rats. On the other hand INDO did not decrease the normal temperature of control rats but reduced PGE_2 synthesis (Table I). The same trend was shown in the animals exposed for 4 h to 34°C (Table II). Deep body temperature was increased significantly compared to control by more than 2°C. Treating the animals with LPS or INDO did not change the rectal temperature. The PGE_2 synthesis was however elevated by LPS treatment and was decreased by INDO treatment in the heat exposed rats.

DISCUSSION: Most investigators agree that PG are involved in the thermoregulatory mechanism in the brain to produce fever and antipyretics (NSAID) lower pyrogen induced fever by inhibiting the synthesis of PG in the HT [8,9]. Our approach, using *ex vivo* experiments confirms that LPS in animals kept at control temperatures increase RT and PGE_2 synthesis. INDO in these LPS treated rats reverts both effects (Table I). The role of PG in thermoregulation in afebrile humans and animals is not clear. Ad-

ministration of salicylates to nonfebrile humans did not cause changes in core temperature [10]. No evidence was found for PG taking part in thermoregulation in afebrile rabbits [11] or cats [9]. High doses of salicylates resulted in a fall in RT in afebrile rats exposed to low ambient temperatures [4]. On the other hand, we have demonstrated that at high ambient temperatures the RT of rats treated with salicylates increased rather than dropped [6]. Similar results with salicylates were observed in humans exposed to heat [12]. Here we have demonstrated a similar trend. INDO in rats exposed to 22°C or to 34°C did not decrease the RT although the activity of PG cyclooxygenase was significantly reduced (Table I and II). Moreover, elevation of 2°C in the deep body temperature in rats exposed to high ambient temperature did not change PG synthesis (Table II). Our results seem to indicate that acute changes in the core temperature in afebrile rats by high ambient temperature are not related to PG formation in the brain.

TABLE I   Effects of INDO, LPS and LPS + INDO on RT and *in vitro* PGE$_2$ production in HT of rats
             exposed to 22 ± 1°C

Treatment	RT (°C)		PGE$_2$ (pg/mg tissue/min)	
	22 h	24 h	60 min	90 min
CONT + SALINE	37.5±0.1	37.9±0.08	0.79±0.18	0.92±0.19
CONT + INDO	37.6±0.09	37.9±0.06	0.12±0.03[1]	0.25±0.09[1]
LPS + SALINE	38.8±0.1[1]	38.8±0.2[1]	1.76±0.12[1]	1.24±0.11[1]
LPS + INDO	38.5±0.4[1]	37.5±0.1[2]	0.24±0.02[2]	0.57±0.11[2]

[1]Significant difference (p < 0.01) versus control
[2]Significant difference (p < 0.01) versus LPS

TABLE II   Effect of INDO, LPS and LPS + INDO on RT and on *in vitro* PGE$_2$ production in HT of
             rats exposed to 34°C + 1°C

Treatment	RT (°C)		PGE$_2$ (pg/mg tissue/min)	
	22 h (2 h in 34°C)	24 h (4 h in 34°C)	60 min	90 min
CONT + SALINE	39.7±0.07	39.3±0.01	0.64±0.04	0.43±0.09
CONT + INDO	39.7±0.07	39.5±0.08	0.26±0.05[1]	0.25±0.02[1]
LPS + SALINE	39.5±0.08	39.4±0.07	0.74±0.1[1]	0.79±0.08[1]
LPS + INDO	39.5±0.08	39.4±0.06	0.11±0.02[2]	0.27±0.03[2]

[1]Significant difference (p < 0.01) versus control
[2]Significant difference (p < 0.01) versus LPS

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MODIFICATION OF BODY TEMPERATURE BY GABAERGIC DRUGS IN THE RAT AND THEIR INTERACTION WITH NOCICEPTIVE STIMULI

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Dose-dependent hypothermia induced by GABA ip is not antagonized by bicuculline [1]. Neurochemical and electrophysiological studies have suggested a possible interaction between GABAergic, opiates [2] and prostaglandin systems [3]. The present work studies the effect of GABAergic agonists on body temperature in stressed rats and the implication of GABAergic receptors, prostaglandins and opiate peptides in such effects. The results have been compared with those obtained in the same experimental model when a mechanical noxious stimulus was added.

Female albino rats (n, 434) stressed by fasting and restraint were used, core body temperature was measured at 30 min intervals over 3 h. Mechanical painful stimulation was produced by means of a pressure clip applied to the hindleg of the animals according to Hayes et al. [4]. Drugs (mg/kg) were administered ip GABA (250, 500, 1000, 2000) and muscimol (0.01, 0.1, 1, 2) produce a dose-dependent hypothermia which is antagonized by indomethacine. Baclofen (1, 10, 30, 60) did not significantly modify temperature; however at 30 mg/kg it induced hyperthermia in some animals, which was antagonized by indomethacine and naloxone. Bicuculline potentiated the effect of GABA (1000) and baclofen (1). Pain stimulus enhanced hypothermia or reduced hyperthermia induced by the drugs tested.

The results suggest that prostaglandins play a role in the effects of GABA, muscimol and baclofen, while endogenous opiates seem to be implicated only in baclofen induced hypothermia. Hypothermia induced by painful stimuli appears to be due to an increase in GABA, prostaglandins and opiates release.

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NEUROLEPTIC INDUCED HYPOTHERMIA: PERIPHERAL α-ADRENERGIC INVOLVEMENT

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The hypothalamus is generally considered to be the site of action of neuroleptics in producing a decrease in temperature. However, there are conflicting accounts on the thermoregulatory effects of centrally administered neuroleptics. Injection into preoptic nuclei of rats either does not affect rectal temperature [1,2] or induces hyperthermia [3,4]. We have therefore studied a series of neuroleptics, representing each of the major chemical classes, for their ability to induce hypothermia when administered ip or icv to mice. Some neuroleptics possess adrenergic blocking properties [5] which could be responsible for the induction of the hypothermia. Hence, the effects of an α-adrenergic agonist on this hypothermia were investigated.

METHODS: Male OF1 mice, 6 weeks old and weighing 26-28 g, were implanted with a guide cannula into the right lateral ventricle. The mice were housed in groups of 6 and were maintained at 22 ± 1°C for 1 week. Central injections were made in a volume of 0.5 to 1 μl over a period of 50 to 100 sec. Peripheral injections were given in a volume of 0.1 ml/10 g ip. Controls received the same volume of vehicle alone. Rectal temperature was measured with a thermocouple. Statistical analysis was performed using the Mann-Whitney U test for comparison between two independent groups. When more than two means were compared, a one-way analysis of variance (Kruskal-Wallis) and subsequently the Mann-Whitney U test were used.

RESULTS: Five chemical classes of neuroleptics including phenothiazines, butyrophenones, benzamides, thioxanthenes and diphenylbutylpiperidines were examined. The effects of twelve neuroleptics are shown in Fig. 1. The phenothiazines produced the greatest hypothermia; other neuroleptics decreased rectal temperature to a lesser extent. Neither sultopride nor sulpiride modified rectal temperature except that the latter produced hypothermia at a dose (200 mg/kg ip) inducing toxic effects (rigidity, convulsions and sometimes death).

Fig. 2 shows that none of the neuroleptics tested induced hypothermia after icv injection at doses which were devoid of any toxic effects. So, a toxic dose (which produced rigidity) of sulpiride (16 μg) was needed to obtain a hypothermic response. Lack of solubility prevented icv testing of the other neuroleptics.

To study the mechanisms involved in this neuroleptic hypothermia, two neuroleptics from different chemical groups were chosen. The concomitant administration of phenylephrine (6 mg/kg ip) and chlorpromazine (2.5 mg/kg ip) totally suppressed the hypothermia at 30 min and reduced it at 1 and 2 h (Fig. 3A). Similarly, simultaneous injection of phenylephrine (6 mg/kg ip) and haloperidol (4 mg/kg ip) suppressed the hypothermia at 30 min (Fig. 3B).

DISCUSSION: These results demonstrate that none of the neuroleptics was able to elicit hypothermia when administered icv, although systemic administration produced hypothermia. This lack of effect cannot be attributed to inadequate doses of neuroleptics. If these drugs act by a central mechanism icv injection should induce at least a similar decrease in temperature. The doses injected by the icv route were ineffective and higher doses produced only slight hypothermia, with occasionally concomitant toxic effects. A possible explanation is that the hypothermia produced by systemic injection of neuroleptics may be mediated via the peripheral nervous system. Neuroleptics, such as chlorpromazine, pimozide, haloperidol have been shown to block α-adrenoceptors [5]. This antagonistic action of neuroleptics could induce hypothermia by promoting vasodilation. The fact that chlorpromazine and haloperidol hypothermia was abolished by phenylephrine, an α-adrenergic agonist which does not cross the blood brain barrier, is in agreement with the view that peripheral α-adrenoceptors may mediate hypothermia induced by neuroleptics.
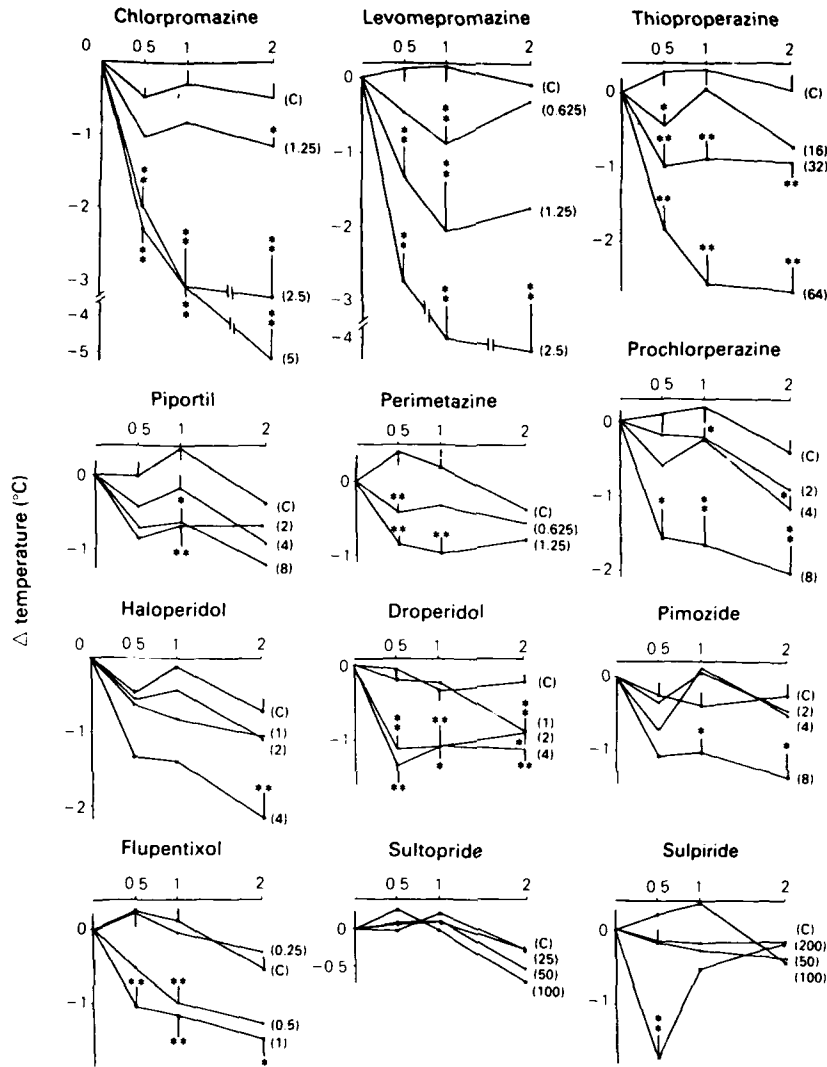
FIGURE 1: Time course of the change in rectal temperature in mice produced by increasing doses of neuroleptics given ip. The effects of neuroleptics are expressed as the difference between pre- and post-injection temperature. Each point represents the mean of 7 to 8 mice. In order to simplify the figure, the vertical lines indicating the standard error of the mean are shown only for the significant values. $^*p < 0.05$; $^{**}p < 0.01$ (Kruskal-Wallis and Mann-Whitney U tests). (C) Control responses. Number in parentheses : doses of neuroleptics in mg/kg.

FIGURE 2: Time course of the change in rectal temperature produced by increasing doses of neuroleptics given icv. The effects of the neuroleptics are expressed as the difference between pre- and post-injection temperature less the effect of vehicle alone (controls). Each point represents the mean of 7 to 11 mice. *p < 0.05; **p < 0.01 (Mann-Whitney U test). Numbers in parentheses : doses of neuroleptics in μg.

FIGURE 3: Time course of the change in rectal temperature produced by a combination of phenylephrine and chlorpromazine (A): (1) water (ip) and phenylephrine (6 mg/kg ip); (2) chlorpromazine (2.5 mg/kg ip) and phenylephrine (6 mg/kg ip); (3) chlorpromazine (2.5 mg/kg ip) and water (ip) and a combination of phenylephrine and haloperidol (B): 1) water (ip) and phenylephrine (6 mg/kg ip); (2) haloperidol (4 mg/kg ip) and phenylephrine (6 mg/kg ip); (3) haloperidol (4 mg/kg ip) and water (ip). The drugs were given simultaneously but separately. Each point represents the mean of 9 to 10 mice. compared with the group treated by phenylephrine + water, † compared with the group treated by the neuroleptic + water; *p < 0.05; **p < 0.01 (Kruskal-Wallis and Mann-Whitney U tests).

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EFFECTS OF CENTRAL ADMINISTRATION OF GABA ON THERMOREGULATION

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Gamma-aminobutyric acid (GABA) has been reported to induce a rise or fall in body temperature when it is administered by the intracerebroventricular (icv), intrahypothalamic (ih) or intraperitoneal (ip) route in different species including the rat [1,2]. However, the precise nature of the variable responses to GABA seems to depend on factors such as ambient temperature, the dose of GABA, and the species being tested. Thus, at a high dose, GABA administered icv, ih, as well as ip, evokes a hypothermia in the rat [3,4]. Given in a low dose, either icv or ih, GABA produces a hyperthermia in the rat [3].

The purpose of the present experiments was: (1) to determine the effects of GABA when administered icv and into the rostral and caudal hypothalamus in the unrestrained rat maintained at an ambient temperature of $22 \pm 1°C$; and (2) to evaluate the action of GABA in the rat treated with a lipopolysaccharide (LPS) bacterial endotoxin, *Salmonella typhosa*.

METHODS: Male albino rats of the Wistar strain, weighing 250-300 g, were housed at an ambient temperature of $22 \pm 1°C$ on a 12 h light-dark cycle. Using standard stereotaxic procedures, stainless steel guide tubes were implanted to rest just 1 mm above the lateral cerebral ventricle and the preoptic/anterior hypothalamic nuclei (PO/AH) or the posterior hypothalamus (PH) according to the stereotaxic atlas of Paxinos and Watson [5]. Experiments were begun after at least 1 week after surgery and conducted between 10:00 and 15:00 h. Body temperature was recorded every 30 min by a thermistor probe inserted 6 cm into the colon with the rat restrained only momentarily during temperature recording. An injection was made only after the body temperature had stabilized for a period of 1-2 h. Each solution was prepared daily in sterile saline. The volume of an injection was 5.0 µl for the administration of a solution into the lateral ventricle and 0.5 µl into the two hypothalamic areas. The injector cannula which was 1.0 mm longer than the implanted guide cannula was always kept in place for an additional 45 sec following the injection.

To test the effect of GABA on fever induced by icv injection of *S. typhosa* (0.1 µg), GABA (1.0, 10 or 100 µg) was administered 30 min after the pyrogen. Analysis of variance in conjunction with a Newman-Keuls test was used to test for significant differences between different groups.

RESULTS: GABA (1.0-100 µg icv) caused variable effects on body temperature. As illustrated in Table I, 1.0 µg produced a significant hypothermia, 10 µg increased body temperature, but 100 µg had no effect on body temperature, when compared with saline injections. When GABA was microinjected in the same doses into the PO/AH, a dose-dependent rise in body temperature occurred, when compared with the response to saline (Table I).

GABA (1.0-100 µg) injected into the PH caused a slight hyperthermia, although only that evoked by the 1.0 µg dose was significantly greater than that of saline (Table I). When injected either icv or into the PO/AH or posterior area *S. typhosa* (0.1 µg) produced characteristic febrile responses (Table I). In addition, the hyperthermic response to GABA (1.0 and 100 µg) microinjected into the PO/AH, augmented further the febrile response to *S. typhosa* (0.1 µg icv), injected 30 min before GABA when compared with the response of the febrile animal (Table II). GABA (1.0 and 100 µg) injected into the PH also caused a significant increase in body temperature in *S. typhosa* (0.1 µg icv) treated animals (Table II).

TABLE I   Effects of GABA on body temperature in conscious unrestrained rats

Treatment (dose)	Route	n	Mean Change Temp (°C + S.E.)
Saline (0.9%)	icv	7	-0.06 ± 0.04
GABA (1 μg)	icv	6	-0.23 ± 0.05[1]
GABA (10 μg)	icv	5	+0.23 ± 0.02[2]
GABA (100 μg)	icv	5	+0.03 ± 0.05
Pyrogen (0.1 μg)	icv	5	+0.46 ± 0.05[2]
Saline (0.9%)	PO/AH	7	-0.03 ± 0.01
GABA (1 μg)	PO/AH	5	+0.18 ± 0.03[2]
GABA (10 μg)	PO/AH	6	+0.53 ± 0.07[2]
GABA (100 μg)	PO/AH	6	+0.59 ± 0.07[2]
Pyrogen (0.1 μg)	PO/AH	6	+0.65 ± 0.09[2]
Saline (0.9%)	PH	7	+0.19 ± 0.03
GABA (1 μg)	PH	6	+0.35 ± 0.05[2]
GABA (10 μg)	PH	6	+0.28 ± 0.06
GABA (100 μg)	PH	6	+0.16 ± 0.05
Pyrogen (0.1 μg)	PH	6	+0.63 ± 0.03[2]

[1]P < 0.05 and [2]P < 0.01 compared to respective saline-treated rats.
n, Number of animals.

TABLE II   Effects of GABA on body temperature in rats treated with pyrogen (*S. typhosa*, 0.1 μg icv)

Treatment (dose)	Route	n	Mean Change Temp (°C + S.E.)
Pyrogen (0.1 μg)	icv	7	+0.52 ± 0.05
Pyrogen + GABA (1 μg)	PO/AH	6	+0.95 ± 0.09[2]
Pyrogen + GABA (100 μg)	PO/AH	6	+0.95 ± 0.10[2]
Pyrogen + GABA (1 μg)	PH	6	+0.80 ± 0.09[1]
Pyrogen + GABA (100 μg)	PH	6	+1.24 ± 0.07[2]

[1]P < 0.05 and [2]P < 0.01 compared to pyrogen-treated animals.
n, Number of animals.

DISCUSSION: These experiments suggest that GABA in the CNS of the freely moving rat subserves the heat gain/conservation mechanism. The microinjection results revealed that a principal site of action of GABA in the animal's brain is the PO/AH. In terms of the functional system underlying GABA thermogenesis in the rat, GABA could act either by stimulating an endogenous substance implicated in the control of body temperature or by an interaction with GABA receptors on nerve cells at the level of the diencephalon. GABA may also activate the release of 5-HT from PO/AH neurons which modulate the serotonin sensitive heat gain mechanism in this area [6]. In this connection, the intracerebral injection of GABA elevates the level of serotonin in the brain of the rat [7]. Since GABA infused in a low dose in PH produced only a hyperthermic response, it would appear that GABA may act by an alternative mechanism than that in the PO/AH. Thus, GABA could facilitate the release of ACh in the hypothalamus [8] which in turn could mediate the thermogenic effect of GABA at this level.

Bacterial endotoxin (LPS) when injected centrally induces fever in a variety of animals including the rat [6]. When microinjected into the PO/AH, both GABA and *S. typhosa* elicited a thermogenesis of approximately equal magnitude. However, when GABA was injected into the PO/AH in conjunction with the pyrogen, the hyperthermic response produced by *S. typhosa* was significantly greater than that caused by GABA alone. This summation of the hyperthermic effects, when GABA and pyrogen are given concurrently into PO/AH and icv, respectively, suggests that different functional processes are involved at this level.

On the other hand, since the hyperthermia due to pyrogen was accentuated by GABA injected into the PO/AH, it is conceivable that a febrile response is mediated, at least in part, by GABA containing neurons in this region of the hypothalamus. Hence, GABA in the rat's PO/AH could comprise a terminal link in endotoxin fever.

Further, the ability of GABA in the PH to potentiate the pyrexic response raises the possibility that (1) GABA in PH operates via a non-pyrogen mechanism as well as amplifying the fever of bacterial endotoxin and (2) GABA inhibits substances that are activating compensatory heat loss mechanisms in febrile rats at this level. Finally, because GABA can interact in the hypothalamus on numerous transmitters, including dopaminergic, noradrenergic, cholinergic and serotonergic systems, all of which apparently participate in the control of body temperature [6], it is likely that one or more of these substances acts together with GABA in the final common pathway for heat production.

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THERMOREGULATION AND α₂-ADRENERGIC AGONISTS: EFFECTS OF ENVIRONMENTAL TEMPERATURE AND SEX

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Clonidine (CLO), an α_2-adrenergic agonist (A-2-AA), is used therapeutically as a central antihypertensive drug. Several studies have documented the hypothermic effect of CLO, following central or peripheral administration [1-3]. This pharmacologic activity is shared by many A-2-AA, such as xylazine and naphazoline [2-5]. Structure activity relationship studies have revealed that drugs with very diversified structure may possess α_2-adrenergic activity [6]. While CLO is an imidazoline derivative, guanabenz (GUA), a central A-2-AA, has a guanidine moiety and B-HT 920, a potent A-2-AA, contains a thiazolazepine nucleus.

In the present study we investigated the thermoregulatory effect of 3 centrally active A-2-AA (CLO, GUA and B-HT 920) and 2 A-2-AA that do not cross the blood brain barrier to an appreciable amount, namely paraaminoclonidine (pAC) and ST-91.

METHODS: Male and female Charles River derived rats, 10-12 weeks old were maintained at 23°C with 12 h light/12 h dark cycle, switching at 06:00 h and 18:00 h. Food and water were given *ad libitum*. Experiments were conducted at 23°C or 31°C. In the experiments at 31°C, rats were exposed at this temperature for 3 h prior to administration of saline or drug. Rectal temperature was measured in unrestrained rats at 30 min intervals for 3 h after drug administration with a thermistor probe.

Statistical evaluation was determiend by analysis of variance followed by Duncan's test.

RESULTS: As can be seen from Fig. 1 both peripheral (pAC and ST-91) and central A-2-AA (CLO, GUA and B-HT 920) had potent hypothermic activity in male and female rats, at an environmental temperature of 23°C. When rats were maintained for 3 h at 31°C, the A-2-AA caused hyperthermia in male rats, while in female rats the effect was variable: the two centrally acting A-2-AA, GUA and B-HT 920 maintained their hypothermic activity. CLO at low dose caused hypothermia while higher doses led to hyperthermia. The two peripherally acting A-2-AA, pAC and ST-91, had only hyperthermic activity in female rats at 31°C, as in male rats.

The thermoregulatory effects of CLO were reversed by YOH at both environmental temperatures (Table I). It should be noted that at 23°C a higher dose of YOH was needed for reversal than at 31°C.

DISCUSSION: These results show that A-2-AA reduce rectal temperature at an environmental temperature of 23°C and they are in agreement with previously published studies [2,3]. The hypothermic effect of CLO in females is much more pronounced compared to males.

The activity of the two peripherally active A-2-AA is comparable to that of the centrally acting A-2-AA. pAC does not penetrate the blood brain barrier and its activity is essentially peripheral [7]. There is, however, some controversy as to the extent of the central activity of ST-91. While Hoefke *et al.* [8] showed that the cardiovascular effects of ST-91 are peripheral, McLennan [5] claims that this drug penetrates to some extent, which may explain its cardiovascular activity by a central mechanism. Tsucaris-Kupfer and Schmitt [2] showed that peripherally acting A-2-AA (naphazoline and tetrazoline) caused hypothermia. Reid *et al.* [4] showed that the activity of CLO following central depletion of catecholamines is peripheral.
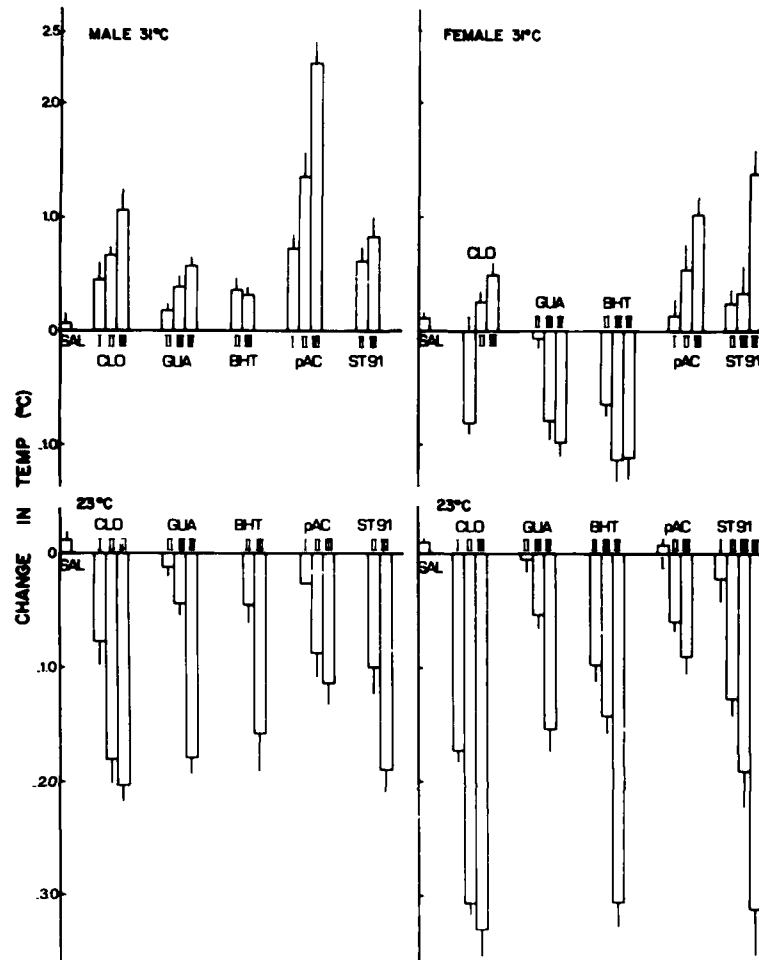
FIGURE 1: Effects of A-2-AA on rectal temperature in male and female rats. n, 6-10 rats in each group. Doses of A-2-AA used: I, 0.05 mg/kg; II, 0.1 mg/kg; III, 0.2 mg/kg; IV, 0.4 mg/kg.

In other species CLO elevated rectal temperature at high ambient temperatures [9]. The reason for this differential effect is unknown. It might be that other biogenic amines, e.g., serotonin [10] and dopamine [4,9], are activated at elevated temperatures leading to hyperthermia.

The effect of clonidine is reversed by YOH both at 23°C and at 31°C. These results are in agreement with results published previously [2], but disagree with those of Livingstone et al. [11] who found potentiation of CLO activity by YOH [11].

TABLE I   Reversal of the effect of clonidine on rectal temperature in male rats by yohimbine

| Drug | ΔT | |
	23°C	31°C
Saline	+0.06 ± 0.01 (15)	+0.06 ± 0.01 (15)
CLO	-2.03 ± 0.13 (17)[1]	+1.05 ± 0.16 (12)[1]
YOH	-0.16 ± 0.06 (7)	+0.16 ± 0.06 (10)
YOH/CLO	-0.32 ± 0.10 (14)	+0.10 ± 0.06 (10)

Results are presented as Mean ± SEM (n).
[1]Significantly different from saline treated rats, p < 0.05. Dose of CLO was 0.2 mg/kg ip and of YOH 2 mg/kg ip.

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ADRENERGICALLY INDUCED THERMOGENESIS IN COLD AND NON-COLD ACCLIMATED RATS: THE PARTICIPATION OF β_1 AND α_1-ADRENOCEPTORS

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Temperature regulation in endotherms is comprised of several factors, one of which involves metabolism [1]. Metabolic adjustments concerned with thermoregulation may be induced by exposing an animal to cold ambient temperatures, and maintaining an endotherm in a cold temperature for several weeks is regarded as cold acclimation. Cold acclimation is accompanied by a variety of physiological effects including increased resting metabolic rate [2], enhanced thermogenic responsivity to catecholaminergic stimulation of β_1-receptors located on brown adipose tissue [3], and the possible emergence of a role for α_1-receptors in the brown adipose tissue mediated thermogenic response [4]. Although β_1 stimulation has been shown to evoke brown fat thermogenesis in non-cold acclimated animals [5], it remains unclear as to whether α-adrenoceptors participate in this response in the non-cold acclimated endotherm. Accordingly, this study assessed the thermophysiological consequence of catecholaminergic adrenoceptor stimulation in cold and non-cold acclimated rats.

METHODS: Thirty-two male, Sprague Dawley rats weighing approximately 120 g were either cold acclimated (6 ± 1°C; n, 16) or non-cold acclimated (23 ± 1°C; n, 16) for 21 days. During the last 6 days of acclimation, thᵉ animals were gradually adapted to mild physical restraint for no more than 4 h/day. Under aseptic conditions and while anesthetized with sodium pentobarbital (60 mg/kg ip), the animals had their descending aorta and right jugular vein chronically catheterized as described elsewhere [6]. Forty-eight hours following recovery, metabolism (oxygen consumption, $\dot{V}O_2$, and carbon dioxide production, $\dot{V}CO_2$), rectal temperature, mean artrial blood pressure, and heart rate were assessed every 10 min over two test sessions, separated by a 46 h rest period. For purposes of testing, the animals were restrained and placed in a metabolic chamber maintained at 23°C. Following a 40 min equilibration and 50 min baseline period, isoproterenol, a β_1-agonist (ISO, 0.5 µg/kg/min iv) and phenylephrine, an α_1-agonist (PE, 10 µg/kg/min iv) were infused over 50 min either individually or simultaneously. Statistical analyses consisted of apriori linear contrasts and three-way mixed effects ANOVAs.

RESULTS: Although PE alone had no thermogenic effect, it did increase mean arterial blood pressure from baseline levels ($\underline{M}$ = 169 vs. 149 mm Hg; p<.0001). When compared with baseline values, ISO resulted in an increase in metabolism ($\underline{M}$ = 3.85 vs. 3.29 ml O_2/g/h and 2.72 vs. 2.43 ml CO_2/g/h; p<.0001), rectal temperature ($\underline{M}$ = 37.2 vs. 36.5°C; p<.0001), and heart rate ($\underline{M}$ = 532 vs. 415 bpm; p<.0001), and in a decrease in mean arterial blood pressure ($\underline{M}$ = 135 vs. 150 mm Hg; p<.0001). As illustrated in Fig. 1, simultaneous administration of ISO and PE augmented the increase in metabolism seen during the infusion of ISO alone (p<.004). This potentiation of metabolism, however, was not paralleled by a similar enhancement of rectal temperature, nor did the cardiovascular variables differ between the ISO and the ISO with PE drug conditions (p>.05). It was also observed that during ISO administration the rise in metabolic rate from baseline was greater for the cold than for the non-cold acclimated rats (e.g., from 2.36 to 2.80 ml CO_2/g/h for the cold acclimated compared with 2.50 to 2.64 ml CO_2/g/h for the non-cold acclimated rats) (p<.05). No other variable, however, exhibited a similar differentiation between the cold and non-cold acclimated animals. Moreover, during the co-administration of ISO and PE, neither the absolute response nor the percentage change from baseline for any metabolic, thermal, or cardiovascular variable differed as a function of the acclimation status of the animal (p>.05).
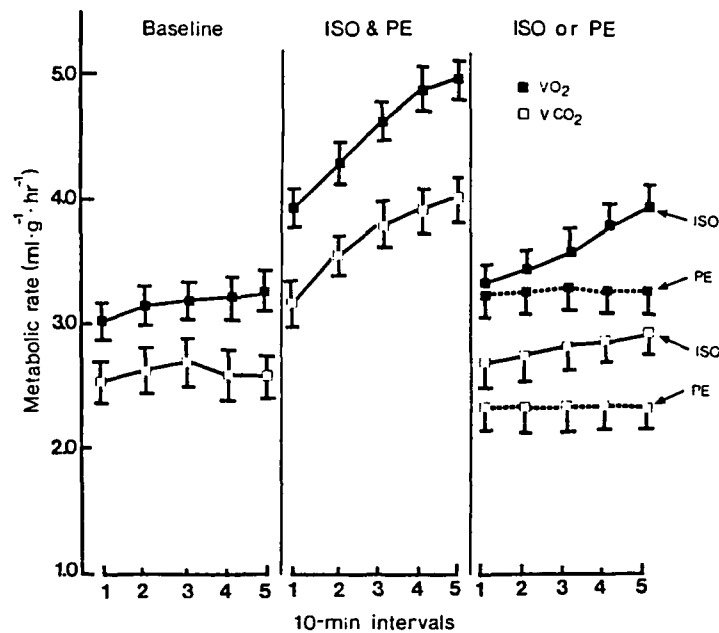
FIGURE 1: Mean (± SEM) oxygen consumption (ml O_2/g/h) and carbon dioxide production (ml CO_2/g/h) collapsed across the cold and non-cold acclimated groups as a function of time for baseline and for the administration of isoproterenol, phenylephrine, and combined isoproterenol and phenylephrine.

DISCUSSION: The finding that the combined ISO and PE administration increased metabolic rate above that seen during the individual infusion of either agent is consistent with several reports [4,7,8]. The general consensus from these findings is that, although β_1-receptors may play an activating role in brown adipose tissue-mediated thermogenesis, the concurrent stimulation of α_1-receptors may potentiate this effect. Although it has been well documented that cold acclimation increases thermogenic responsivity to β_1-stimulation [e.g., 9], the results of the present experiment suggest that *in vivo*, cold acclimation does not potentiate the thermogenic contribution of α_1-receptors following pharmacologic stimulation. In their *in vitro* study using isolated hamster brown fat adipocytes, Mohell *et al.* [7] found that whereas β_1-receptors accounted for 80% of brown fat respiration, α_1-receptors contributed 20% to the thermogenic response. Similarly, other investigators [10,11] demonstrated that when brown fat is activated, either through cold acclimation or hyperphagia, the thermogenic process could be divided according to two receptor populations; 80% due to β_1-receptor activation and 20% to α_1-receptor stimulation. In the present study, stimulation of α_1-receptors accounted for approximately 20% of the thermogenic response to combined ISO and PE infusions in both cold and non-cold acclimated animals. Thus, α_1-receptors appear to play a fairly consistent role in the activation of brown fat thermogenesis, perhaps regardless of the acclimation status of the endotherm. The precise cellular events which are activated by α_1-stimulation remain to be elucidated.

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THERMOGENIC RESPONSES TO ADRENALINE IN ACUTE UNDERNUTRITION

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It is now reasonably established that sympathetically mediated non-shivering thermogenesis does occur in man as cold exposure leads to a rise in metabolic rate in curarized patients [1] and to a rise in urinary noradrenaline excretion in normal subjects [2]. Diminished thermogenesis on cold exposure has been demonstrated in chronically malnourished infants [3], in very thin elderly females [4] and also in normal subjects following 48 h starvation [5]. It is therefore possible that there is some reduction in non-shivering thermogenesis in undernutrition.

The purpose of the present study was to determine whether the thermogenic effect of catecholamines is altered in undernutrition. This possibility is suggested by the observation that the chronotropic response to subcutaneous adrenaline was markedly diminished in malnourished subjects in the Warsaw ghetto in World War II, although thermogenesis was not measured in that study [6].

METHODS: Healthy, normal-weight, young female subjects were recruited for the following studies which were approved by the University of Nottingham Medical School Ethical Committee. In the first study on underfeeding, 7 subjects reduced their daily energy intake to approximately 60 kJ/kg ideal body weight for 7 days under the supervision of our research dietitian. In the second study, 6 different subjects starved for 48 h whilst being allowed non-calorific drinks and being provided with 80 mmol of sodium supplementation per day to prevent salt and volume depletion. The physical characteristics of the subjects and the weight loss during underfeeding and starvation are given in Table I.

TABLE I   Physical characteristics (ranges) of subjects and weight loss (mean ± SEM) during underfeeding or starvation

	Underfeeding Study	Starvation Study
Age (years)	20-28	21-26
Initial weight (kg)	43.5-69.0	52.7-66.5
Body mass index (kg/m^2)	17.2-23.0	19.0-22.5
Weight loss (kg)	1.8±0.4 (P<0.01)	1.3±0.1 (P<0.001)

Studies were performed both in the normally fed state and in the underfed or starved state. In each experiment, subjects were studied in the post-absorptive state, wearing shorts and a T-shirt only and resting supine in a thermoneutral room at 30°C. In each study, measurements were made for 3 consecutive 30 min periods before, during and after infusion of adrenaline (25 ng/kg/min). Metabolic rate was measured continuously by indirect calorimetry [7]. Skin temperatures were recorded at 5 min intervals from thermocouples located on the left upper quadrant of the abdomen and the anterior aspect of the left thigh.

Blood was sampled for catecholamine levels [8] from a cannula inserted retrogradely in a vein on the dorsum of the left hand which rested in a hot-air box maintained at 50°C.

Data were analysed by analysis of variance with repeated measures.

RESULTS: The principal results are given in Table II.

TABLE II   Catecholamine, metabolic rate and skin temperature responses to underfeeding and starvation before and during infusions of adrenaline. Values are means with standard errors. Differences between fed and underfed or starved states are shown

| | Underfeeding Study | | | Starvation Study | | |
	Fed	Underfed		Fed	Starved	
Plasma adrenaline (nmol/l)						
basal	0.29±0.06	0.28±0.06	NS	0.34±0.07	0.27±0.04	NS
increment	1.62±0.14	1.30±0.23	NS	1.72±0.14	1.47±0.10	P<0.05
Plasma noradrenaline (nmol/l)						
basal	1.01±0.17	0.78±0.22	NS	0.93±0.09	0.97±0.11	NS
increment	0.06±0.12	0.01±0.06	NS	0.03±0.07	0.16±0.11	NS
Metabolic rate (kJ/min)						
resting	3.94±0.22	3.83±0.19	NS	4.44±0.08	4.58±0.10	P<0.02
increment	0.37±0.08	0.43±0.08	NS	0.42±0.05	0.56±0.07	P<0.001
Abdominal skin temperature (C)						
basal	34.50±0.18	34.54±0.13	NS	34.62±0.17	35.04±0.14	NS
increment	0.76±0.09	0.94±0.09	P<0.05	0.75±0.11	0.63±0.26	NS
Thigh skin temperature (C)						
basal	34.26±0.32	33.57±0.17	NS	34.67±0.24	35.03±0.25	NS
increment	0.77±0.17	1.19±0.13	P<0.01	0.68±0.08	0.50±0.18	NS

The period of underfeeding led to no significant changes in basal plasma catecholamine concentrations although there was a trend towards a fall in plasma noradrenaline following 7 days underfeeding. There were no changes in plasma noradrenaline concentrations with the adrenaline infusions in either study. The increment in plasma adrenaline concentration during the adrenaline infusions was significantly diminished in the starved state compared with the normally fed state and there was a similar, albeit non-significant, trend following underfeeding.

Resting metbolic rate increased by approximately 3% (P<0.02) following 48 h starvation but was not significantly altered by underfeeding. The thermogenic effect of the adrenaline averaged over the 30 min of the infusion period was enhanced in the starved state, increasing from 0.42 to 0.56 kJ/min (P<0.001). A similar, but non-significant, trend was noted following underfeeding.

Basal abdominal and thigh skin temperatures were not affected by underfeeding or starvation. There were considerable increases in skin temperatures (0.5 to 1.2°C) at both of these sites during the adrenaline infusions in each of the normally fed, underfed and starved states, with significantly greater responses after underfeeding.

DISCUSSION: Basal plasma noradrenaline concentrations were unaffected by either underfeeding or 48 h starvation, although there was a trend towards a fall with the former intervention. A difficulty concerning the use of plasma noradrenaline concentrations as a measure of SNS activity is that steady state catecholamine levels reflect only the balance between spillover and clearance from the plasma. With the use of radiolabelled tracer techniques, release of noradrenaline into the circulation has been reported to be

diminished following 11 day energy restriction in normal subjects. However clearance of noradrenaline was also reduced [9], so any decline in plasma noradrenaline concentration with underfeeding was thereby attenuated.

As a corollary of the study by O'Dea *et al.* [9], if plasma clearance of noradrenaline is diminished by underfeeding, an infusion of adrenaline might be expected to give rise to higher plasma levels following nutritional depletion as adrenaline and noradrenaline are cleared by similar mechanisms [10]. In the present study, the increment in plasma adrenaline levels during the adrenaline infusions was, however, lower in the starved state than in the normally fed state and a similar trend was observed following underfeeding. The explanation for this is obscure. However, the enhancement in the thermogenic response to infused adrenaline in the starved state was certainly not due to a difference in the plasma concentration of adrenaline achieved, as this was actually lower than in the normally fed state.

Resting metabolic rate was significantly enhanced by 48 h starvation as observed previously in normal male subjects [5]. This is perhaps due to incomplete adaptation to starvation, with energy requiring metabolic processes such as gluconeogenesis proceeding at a high rate. The enhanced thermogenic response to infused adrenaline following starvation (and the similar trend following underfeeding) observed in the present study may be due to increased adrenoceptor responsiveness or sensitivity induced by undernutrition. Recently, and in contrast to earlier reports [6], an enhanced chronotropic response to isoprenaline has been shown in undernourished human subjects [11] and in isolated heart preparations from starved rats [12]. In the latter case there was increased cardiac adrenoceptor sensitivity but no change in receptor number. Whatever the mechanism for the enhancement of the thermogenic effect of adrenaline in starvation, this may represent increased mobilisation and recycling of energy resources.

Skin temperatures over the abdomen and thigh increased during the adrenaline infusions. This may simply represent cutaneous vasodilatation induced by the adrenaline. An alternative explanation is that this increase in skin temperature is due to heat conducted from increased thermogenesis in underlying tissues. Oxygen consumption in both the leg [13] and the splanchnic region [14] is known to increase in response to sympathomimetic, or direct adrenergic, stimulation.

A decrease in SNS activity has been proposed as a contributory factor in the physiological adaptation to undernutriton [15], although the possibility of any concomitant change in responsiveness to catecholamines has been less extensively investigated. The present study demonstrates that the thermogenic effect of adrenaline is enhanced by 48 h starvation and that a similar trend occurs following underfeeding. This factor needs to be considered when assessing the physiological significance of any apparent change in SNS activity with undernutrition. In particular, any diminution in non-shivering thermogenesis which occurs in undernutrition is more probably due to changes in SNS outflow than to a loss of sensitivity to catecholamines.

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DOES CHLORPROMAZINE ENHANCE THE HYPOTHERMIC RESPONSE
TO Δ9-TETRAHYDROCANNABINOL BY BLOCKING PERIPHERAL α-
ADRENOCEPTORS?

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Previous studies have shown that the hypothermic response of mice to Δ^9-tetrahydrocannabinol (THC) can be enhanced by pretreatment with subhypothermic doses of chlorpromazine [1]. No enhancement of THC induced hypothermia has been detected after pretreatment with lignocaine, desipramine, pimozide, methysergide, atropine or mepyramine suggesting that the interaction between chlorpromazine and THC does not depend on the ability of the phenothiazine to act as a local anaesthetic, as an inhibitor of the neuronal uptake of noradrenaline or as an antagonist at dopamine (D_2), 5-hydroxytryptamine ($5HT_2$), acetylcholine (muscarinic) or histamine (H_1) receptors [1]. In contrast, THC induced hypothermia can be enhanced by pretreatment with phentolamine [1,2]. Since there is also evidence that chlorpromazine potentiates the hypothermic response to THC by acting outside the brain [1], it is possible that the phenothiazine interacts with THC by blocking α-adrenoceptors located on cutaneous blood vessels so as to reduce the ability of mice to retain heat by vasoconstriction. To investigate this possibility, experiments were carried out to determine whether the ability of chlorpromazine and phentolamine to enhance the hypothermic response to THC could be mimicked on the one hand by two other α-adrenoceptor blocking drugs, prazosin [3] and indoramin [4] and on the other hand by the ganglion blocking drug, pentolinium [5]. A more detailed account of the work presented in this paper is published elsewhere [6].

METHODS: Experiments were performed with unrestrained adult male albino MF1 mice at an ambient temperature of 22°C. Chlorpromazine hydrochloride, phentolamine mesylate and pentolinium tartrate were dissolved in saline and indoramin hydrochloride in distilled water. The dose level of each of these drugs was expressed in terms of the salt. Prazosin was dissolved in a minimum volume of lactic acid, this solution then being diluted with distilled water. THC was mixed with 2 parts of Tween 80 by weight and dispersed in saline. The cannabinoid was injected intraperitoneally at time zero using a sub-maximal hypothermic dose (5 mg/kg). The other drugs were administered subcutaneously 10 sec before THC. Body temperature (T_r) was measured with a rectal thermistor probe at -30 min, again immediately before drug administration, then at 20 min intervals until +2 h and finally at +3 h. Temperature responses have been expressed both as T_r and as a temperature response index (TRI), defined as the area in °C h between the curve for T_r against time and a horizontal line passing through the T_r value measured at time zero. Negative values for TRI denote falls in T_r. Differences between means have been evaluated by the Mann-Whitney U-test or by the Student t-test for paired data (P > or < 0.05).

RESULTS: As shown in Table I, the hypothermic response to THC, expressed as the TRI, was significantly enhanced by pretreatment with chlorpromazine (0.375 mg/kg), phentolamine (15 mg/kg), prazosin (0.1875 mg/kg) and indoramin (6 mg/kg). For each of these pretreatment drugs, halving the dose abolished the interaction with THC. Pretreatment with pentolinium (5 mg/kg) also significantly enhanced THC hypothermia. Chlorpromazine, phentolamine and prazosin interacted with THC at doses which, in the absence of this cannabinoid, were followed by falls in T_r of the same order as those following administration of the appropriate vehicle (Table I). Slightly larger falls in T_r were detected in the absence of THC, after injection of indoramin or pentolinium.

DISCUSSION: The results confirmed that the hypothermic response to THC is enhanced by pretreatment with either chlorpromazine or phentolamine. Similar enhancement was produced by pretreatment with prazosin or indoramin, strengthening the view that the only pharmacological property which chlorpromazine has in common with other drugs capable of enhancing THC hypothermia is the ability to antagonize α-adrenoceptors. The hypothermic response to THC was also enhanced by the ganglion blocking drug, pentolinium, a finding consistent with an earlier report that THC induced hypothermia can be

enhanced by hexamethonium and mecamylamine [2]. Like hexamethonium, pentolinium is a bisquaternary ammonium compound and would be expected to penetrate the blood-brain barrier only very slowly, so it is likely to have interacted with THC by acting peripherally. Presumably, its main action was to decrease sympathet: 'one and hence to reduce the ability of mice to retain heat by vasoconstriction and to generate heat by non-shivering processes under sympathetic control [8]. The finding that pentolinium interacted synergistically with THC therefore supports the idea that enhancement of the hypothermic response to THC can be induced by drugs acting outside the brain. This idea in turn supports the hypothesis that the other drugs used in the present study could also have interacted with THC at peripheral sites.

TABLE I The effect of treatment with chlorpromazine (CPZ), phentolamine (PHE), prazosin (PRZ), indoramin (IND) and pentolinium (PEN) on the hypothermic response of mice to THC (5 mg/kg ip)

Treatment at Time Zero			TRI	Time Zero T_r	Minimum T_r	T
s.c. Drug	s.c. Dose (mg/kg)	i.p. Drug	(°C h ± S.E.M.)	(°C ± S.E.M.)		(min)
CPZ (6)	0.375	THC	-10.5±2.2[2]	37.7±0.2	32.7±1.0	+60
CPZ (6)	0.375	Tween	-1.2±0.6	38.0±0.1	36.9±0.2	+180
VEH (6)	-	THC	-3.4±0.5	37.7±0.1	35.6±0.3	+20
VEH (6)	-	Tween	-1.6±0.5	38.1±0.3	36.9±0.2	+180
PHE (5)	15	THC	-11.8±1.8[2]	37.6±0.2	32.1±0.6	+60
PHE (5)	15	Tween	-2.7±0.7	38.1±0.1	37.1±0.2	+20
VEH (5)	-	THC	-4.1±1.1	37.9±0.2	35.1±0.6	+20
VEH (5)	-	Tween	-1.7±0.3	38.0±0.1	37.0±0.2	+180
PRZ (5)	0.1875	THC	-9.6±2.2[1]	38.2±0.1	33.5±1.0	+40
PRZ (5)	0.1875	Tween	-2.7±0.4	38.1±0.3	36.9±0.2	+40
VEH (5)	-	THC	-3.9±1.4	38.0±0.2	36.1±0.7	+40
VEH (5)	-	Tween	-1.5±1.4	37.9±0.3	36.9±0.4	+180
IND (6)	6.0	THC	-14.5±2.8[2]	37.9±0.1	30.9±0.7	+60
IND (6)	6.0	Tween	-5.4±1.2	37.9±0.1	34.2±0.7	+40
VEH (6)	-	THC	-3.9±0.5	37.9±0.1	35.8±0.1	+40
VEH (6)	-	Tween	-1.5±0.4	37.9±0.1	37.0±0.3	+180
PEN (6)	5	THC	-7.4±1.2[1]	38.2±0.2	33.2±0.5	+40
PEN (6)	5	Tween	-2.2±0.4	37.7±0.2	35.8±0.2	+20
VEH (6)	-	THC	-3.2±0.5	38.0±0.2	36.3±0.4	+40
VEH (6)	-	Tween	-1.3±0.5	37.9±0.2	37.2±0.2	+120

Vehicle = VEH (see Methods for the vehicles used).
Temperature response indices (TRI) were determined for the period between time zero and +3 h. The superscripts indicate that the interaction between pretreatment drug and THC was greater than additive [[1]$P<0.05$; [2]$P<0.01$; Mann-Whitney U-test applied to the procedure for multi-way analysis described by Bradley (7)]. Digits listed under T denote the times at which mean rectal temperatures reached their minimum values. Minimum T_r values were significantly less than the corresponding time zero T_r values in all groups of mice apart from those receiving VEH-Tween in the PRZ experiment ($P<0.05$ to $P<0.001$; Student's t-test for paired data). No statistical analyses were carried out to determine significance levels for differences between time zero T_r values or between minimum T_r values. The number of mice in each group is given in parentheses. The data in this Table are reproduced by permission of Neuropharmacology.

It is concluded that chlorpromazine and other α-adrenoceptor antagonists used in this study augmented THC induced hypothermia in mice by blocking α-adrenoceptors, most probably those located on vascular smooth muscle. At ambient temperatures at which mice thermoregulate by decreasing cutaneous blood flow (below 34°C [9]), this action would be expected to increase heat loss from the body surface by reducing the ability of the animals to maintain vasomotor tone. In the absence of THC, an increase in heat loss induced in this way would be expected to be followed immediately by an increase in heat production, triggered reflexly by the thermoregulatory centres. If such an increase in heat production exactly balanced an increase in heat loss, deep body temperature would remain unaltered and it is therefore noteworthy that chlorpromazine, phentolamine and prazosin each interacted with THC at a dose which had no significant effect on the rectal temperatures of animals subsequently receiving Tween 80 instead of THC. It follows that THC could have interacted with chlorpromazine, phentolamine, prazosin and indoramin by suppressing heat production and indeed there is evidence that THC can act on thermoregulatory centres located in mouse brain to reduce thermogenesis triggered by these centres [10]. This mechanism would also account for the interaction between THC and pentolinium which, as discussed above, is also expected to augment heat loss from the body surface. In addition, there is evidence that THC can act centrally to reduce sympathetic outflow from the brain [11] and such a mechanism could also have contributed towards the interactions described in this paper.

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POSSIBLE INVOLVEMENT OF SEPTUM IN SEASONAL CHANGES IN THERMOREGULATORY RESPONSES TO MET-ENKEPHALINAMIDE IN GROUND SQUIRRELS

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The similarity in the depressed state of physiological systems during hibernation and after high doses of opioids suggests that endogenous opioids may be involved in the process of hibernation. This notion is supported by recent findings that a) administration of opioid antagonists to hibernating animals either reduces the duration of the hibernation bout [1] or induces premature arousal from hibernation [2,3]; b) increased overall brain levels of met- and leu-enkephalin have been observed during hibernation [4]; c) the hibernating ground squirrel is resistant to the development of physical dependence to morphine [5]. Further, we have observed an attenuation in efficacy of thermoregulatory response to intracerebroventricularly (ICV) injected opiates in the Columbian ground squirrel during its hibernating phase supporting the suggestion that there is an increase in endogenous opioid activity commensurate with hibernation [6]. However, the actual loci within the CNS regulating the sensitivity change to exogenous opioids remain unknown. The present study attempts to identify some of the possible neuroanatomical locations within the CNS which may regulate the seasonal changes in thermoregulatory responses to opiates, and perhaps also in hibernation processes.

METHODS: Mature Columbian ground squirrels (*Spermophilus columbianus*) of either sex were trapped live in the foothills of the Rocky Mountains in Alberta, and kept individually at an ambient temperature of 22°C under 12 h light: 12 h dark with *ad lib.* food and water. The hibernation phase was characterized by a rapid weight gain followed by a weight plateau and anorexia. The completion of transition to the hibernation from the non-hibernation phase was further verified by the exhibition of hibernation when the animal was placed in the cold (5°C) and dark without food in a walk in environmental chamber. Animals were used after having completed at least two hibernation bouts and then tested in euthermia following disturbed or spontaneous arousal from hibernation. The non-hibernating phase was evident when the animals showed no weekly weight increase and did not hibernate when placed in the cold and dark without food for up to 7 days.

Prior to being transferred to the cold room, guide cannulae (23-gauge stainless steel tubing) were implanted bilaterally into the lateral septum of each ground squirrel under halothane anesthesia. After completion of the experiments, the precise anatomical location of the injection site was verified histologically.

On the day of experiment, the animal was transferred to a circular, plexiglas water jacketed metabolic chamber in which the ambient temperature could be controlled accurately at 22°C. The core temperature (T_b) was recorded continuously with a brain thermocouple or a pre-calibrated temperature sensitive radiotransmitter, which was implanted in the peritoneal cavity under halothane anesthesia at the same time as the guide cannula implantation. Using a microcomputer interfaced with a data acquisition system, oxygen consumption and CO_2 production were recorded and integrated simultaneously with transmitter signal. Heat production was calculated from oxygen consumption and respiratory quotient using Kleiber's equation [7] and the T_b was calculated using pre-established calibration curves for each individual transmitter. Behavioral observations were noted throughout the experimental period.

The following compounds were used: [D-Ala^2]-Met-enkephalinamide (met-EK) acetate and naloxone hydrochloride. Each drug was prepared immediately before an experiment in a pyrogen free artificial CSF. Each test solution, except EK, was always passed through a 0.22 μm filter. The doses in the Results are expressed in terms of μg of the salt and the injection volume was 1 μl. In order to minimize the possibility of tolerance development, animals were tested with at least a 7 day rest between experiments.

Animals were anaesthetized with sodium pentobarbital (50 mg/kg) and perfused via the heart with NaCl solution (0.9%) containing heparin (15000 IU/l). After removing from the skull, the brain was immersion fixed with Bouin's solution, embedded in paraffin and cut in serial sections of 5 to 7 μm thick. Sections were processed for the immunocytochemical reaction with met-EK according to the original method of Sternberger [8]. The activity states of the enkephalinergic system was determined (a) by means of cytometrical measurements and (b) by the semiquantitative analysis of the number of immunostained fibers. Standard specificity and cross reactivity tests were applied. None of the animals used was pretreated with colchicin.

RESULTS: Table I shows the effect of met-EK on thermoregulatory changes during non-hibernating and hibernating phases. Bilateral intraseptal injection of CSF had no significant thermoregulatory effect in the non-hibernating ground squirrel maintained at room temperature. Low doses of met-EK (5 and 10 μg/site) elicited a dose-related rise in T_b which was accompanied by an increase in heat production. In most cases, an increase in activity, such as grooming and exploratory behaviour, was also observed after administration of low doses of met-EK. In contrast, the highest dose of met-EK (20 μg/site) caused a marked fall in T_b. The met-EK induced hypothermia may be due to a suppression of metabolic rate as a decrease in heat production was also recorded. However, in most cases the animal lay spread out on the floor of the metabolic chamber after injection, the hypothermia may partly have resulted from an increase in heat loss. Both met-EK induced hyper- and hypothermic responses were attenuated after intraseptal pretreatment with naloxone (7.5 μg/site).

TABLE I   Thermoregulatory responses of the non-hibernating and hibernating Columbian ground squirrels to bilateral intraseptal injection of met-enkephalinamide (EK)

		Mean Max. Change in T_b (°C)	Mean Max. Change in Heat Production (kcal/10 min/animal)
Non-hibernator			
CSF	1 μl/site (n, 4)	+0.15±0.22	0.05±0.11
EK	5 μg/site (n, 6)	+0.82±0.23[1]	0.12±0.06
EK	10 μg/site (n, 7)	+1.19±0.16[1]	0.48±0.04[1]
EK	20 μg/site (n, 5)	-1.31±0.31[1]	0.22±0.09[1]
Hibernator			
CSF	1 μl/site (n, 3)	+0.31±0.12	0.06±0.05
EK	20 μg/site (n, 3)	+0.66+0.24[2]	0.08+0.04[2]

[1]Significantly different from CSF control, p<0.05.
[2]Significantly different from animals in the non-hibernating phase received met-EK (20 μg/site), p<0.05 (two-tailed t-test).

As shown in Table I, intraseptal injection of CSF had no effect on T_b of squirrels in the hibernating phase. In contrast to the hypothermic responses observed in the non-hibernating phase, a slight increase in both T_b and heat production was seen in the hibernating phase after met-EK (20 μg/site) (Table I). Further, rather than the animal lying on the floor, an increase in motor activity was noted after this dose.

As described in other rodents, met-EK immunoreactive perikarya were found in the striatum, the diagonal band of Broca, the amygdala, and the periventricular, lateral and mammillary hypothalamus of the non-hibernating ground squirrel. Further, met-EK immunoreactive nerve fibers were observed in the lateral septum, the interstitial nuclei of the stria terminalis, the pallidal globe and the ventromedial hypothalamus, as well as in various regions of the brainstem including central griseum, interpeduncular and raphé nuclei. In comparison with the non-hibernating animal, a pronounced increase in immunostaining was observed in the lateral septum and the anterior periventricular hypothalamus of the hibernating animal. The increase in immunoreactivity was not uniform, however. That is, the immunostaining appeared to be decreased in several hypothalamic regions, the nucleus striae terminalis and nucleus accumbens, whereas no change was found in the amygdala and the infundibulum as compared to the non-hibernating brain sections.

DISCUSSION: Even though the role of opioids in thermoregulation has been investigated during past decades [9], little is known about the participation of the septal enkephalinergic system in regulating T_b. Similar to those observed after either ICV or intrahypothalamic administration in other species, intraseptal injection with met-EK caused a biphasic dose-related change in T_b in squirrels in their non-hibernating phase: lower doses elicited hyperthermia whereas higher doses induced hypothermia. Pretreatment with naloxone reduced these changes, suggesting that the thermoregulatory effects of met-EK was mediated in part by mu-receptors.

The most notable finding in our present study is the change in thermoregulatory responsiveness to met-EK in the ground squirrels during the hibernating phase. A slight hyperthermia, rather than a marked hypothermia, was observed after injection of the highest dose of met-EK. This apparent reduction in sensitivity to met-EK does not seem to be due to a general depression of CNS thermoregulatory function; an increase in thermoregulatory response to serotonin [10] and a constant responsiveness to prostaglandins [11] in ground squirrels during the hibernating phase as compared to their non-hibernating phase have been shown previously. Further, the seasonal change in thermoregulatory sensitivity is unlikely to be due to cold acclimation in the hibernating animals since either no change [12] or increased thermoregulatory responsiveness [13] to exogenous opiates has been reported in cold acclimated rats.

Another possible explanation for the differential thermoregulatory response to met-EK during different phases of the hibernation cycle may be an endogenous change in central opioid activity. This is supported by the finding that the immunoreactivity of met-EK is conspicuously increased in the lateral septum and anterior periventricular hypothalamus during hibernation. The increase in met-EK immunoreactivity appears to be site specific as the intensity of immunostaining is either reduced or remains constant in other brain regions examined. One obvious possibility is that the change in endogenous activity is due to the low T_b of the hibernating animal (about 5°C in our experiments). However, in a preliminary study, there was no difference in brain met-EK immunoreactivity between euthermic squirrels in the non-hibernating phase and hypothermic squirrels in which T_b was maintained at 7°C for 72 h. Therefore, the reduced thermoregulatory response to intraseptal injection of met-EK observed during the hibernating phase could be due to an increase in septal opioid activity during this part of the annual cycle, which in turn might induce a down-regulation of opioid receptors. This suggestion is supported by recent findings that the overall opioid binding sites for dihydromorphine decrease in the septum and some other brain areas during hibernation [14]. It has been proposed that a reduction in septal relay to the hippocampus is a prerequisite for the maintenance of hibernation [15]. Since the physiological exemplification of met-EK activity is primarily inhibitory, its enhanced activity in the septum during hibernation may play an important role in regulating hibernation.

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HEAT ACCLIMATION AND HEAT STRESS: CARDIAC OUTPUT DISTRIBUTION, PLASMA VOLUME EXPANSION AND THE INVOLVEMENT OF THE ADRENERGIC PATHWAY

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When subjected to heat stress the thermoregulatory requirements of the body to transfer heat from core to the periphery are met primarily by the cardiovascular system. This is brought into play by a diversion of blood flow to the skin, particularly to arteriovenous anastomoses (AVA), mostly at the expense of blood flow to the splanchnic organs and the kidney [1,2]. Heat acclimation provides an extended period of adequate circulation in non-thermoreguatory areas in the face of increased requirements for skin blood flow [3]. In concert with the cardiovascular responses, heat induced plasma expansion brings about an increase in the capacity of the circulating blood to dissipate heat [4,5]. It was suggested that plasma volume expansion (PVE) is the outcome of a decrease in blood pressure following the diversion of blood flow from the body core to the periphery [4]. However, the exact mechanism leading to this expansion is not yet clear. The purpose of the present investigation was to study the interaction between cardiac output distribution and plasma expansion in non-acclimated and acclimated rats and to elucidate the role of α and β adrenoceptors in this mechanism.

MATERIALS and METHODS: Male R. norvegicus weighing 250-300 g, fed on pellets and water ad lib. were used. Cardiac output distribution and plasma volume changes during exposure to heat stress were studied in non-acclimated and heat acclimated animals. Intact rats, as well as rats with adrenergic receptor blockade were used.

Non-acclimated rats were maintained at $24\pm1°C$ whereas those assigned to heat acclimation were kept in a climatic chamber at $34\pm1°C$ for 2 weeks [6]. Heat stress was attained by subjecting the rat to $37°C$ until hyperthermic plateau was established [6]. Experiments were performed on conscious rats with chronically implanted catheters. Rectal temperature was monitored throughout the entire experiment using a thermistor probe.

For cardiac output and its distribution measurements intraventricular and femoral artery catheters were implanted and ^{155}Gd labeled 15μ microspheres were used, with the reference organ technique [1,3]. Drugs used: phenoxybenzamine (PNZ) (2 mg/kg iv); prazosin (0.1 mg/kg iv).

For plasma volume changes, jugular vein and femoral artery catheters were implanted. Plasma volume responses were followed by monitoring heamatocrit changes whereas absolute plasma volume was measured using T-1824 labeled albumin dilution technique under normothermic and hyperthermic conditions [7]. Drugs used: phenoxybenzamine (2 mg/kg iv); propranolol (0.3 mg/kg iv); metoprolol (5 mg/kg iv); butoxamine (2 mg/kg iv); isoprenaline (0.4 μg/min/kg iv). To validate the effectiveness of the drugs used, arterial pressure was measured before and after administration of the drug, during administration of phenylephrine (α-adrenergic agonist) or isoprenaline (β-adrenergic agonist).

Both paired and unpaired Student's t test with significance level of 0.05 were employed.

RESULTS: Data are presented in Table I. In the laboratory rat subjected to heat stress there was no change in cardiac output. In thermoregulatory peripheral area, patency of AVAs was striking but no change in skin capillary blood flow was observed. In contrast, both skeletal muscle, kidney and splanchnic organ blood flow tended to decrease. The total reduction was sufficient to compensate for the increased AVAs flow. Acclimation to heat resulted in a decrease in cardiac output. As in the non-acclimated rats, heat stress did not result in further changes in cardiac output. Patency of AVAs in the heat stressed acclimated rats was significant. However, in contrast to non-acclimated rats, the decrease in muscle, kidney

and splanchnic blood flow was highly significant. In order to evaluate the role of sympathetic tone in regional vascular resistance α-adrenergic blockade was induced. The data show that both non-specific α- and the $α_1$-adrenergic blockers had similar effects on AVAs blood flow: Under normothermic conditions, α blockade increased AVAs flow by 56% and 242% for PNZ and prazosin respectively. However, despite complete α-adrenergic blockade heat stress resulted in a further increase in AVAs blood flow and values were similar to those observed in the heat stressed intact rat. A similar pattern was observed for tail blood flow. Another interesting observation was the elevation of GI blood flow in the heat stressed rats. The latter finding was pronounced in the PNZ treated animals only.

TABLE I   Cardiac output and cardiac output distribution

| | NON-ACCLIMATED | | ACCLIMATED | |
	Control	Heat Stress	Control	Heat Stress
CO (ml/100 g)	41.05±3.5	38.75±6.32	32.95±8.29	30.28±3.67
AVAs	1.34±0.26	7.45±1.20[2]	1.30±0.57	7.56±2.00[2]
Tail	0.87±0.25	1.58±0.29[1]	0.39±0.10	1.51±0.85[1]
Skin	11.07±1.22	11.23±0:80	10.06±1.30	9.29±1.01
GI	9.86±1.22	7.19±1.92	17.05±1.51	8.74±1.60[2]
Kidney	4.46±0.70	3.02±0.95	8.17±1.58	5.04±1.79[1]
Heart	14.72±1.92	10.37±2.16	10.32±1.07	14.81±3.94
Muscle	44.21±5.19	34.43±8.20	38.73±5.31	17.37±2.08[3]
Phenoxybenzamine				
CO (ml/100 g)	30.27±2.26	31.58±1.42	36.59±5.72	30.10±8.35
AVAs	2.09±0.42[3]	7.17±2.11[2]	4.23±0.96[3]	6.30±1.24[2]
Tail	1.41±0.20	1.61±0.23	0.56±0.28	1.25±0.28
Skin	9.94±0.72	16.63±2.93[1]	8.53±1.06	10.37±1.19[2]
GI	6.22±1.32	10.44±1.85[2]	13.29±2.24	18.86±1.72[3]
Prazosin				
AVAs	4.59±1.13[3]	11.37±2.53[2]	4.50±1.46[3]	11.24±2.92[2]
Tail	0.35±0.07	1.22±0.40[2]	0.42±0.14	0.90±0.11[1]
Skin	8.25±1.02	8.89±1.12	8.42±2.85	6.97±1.69
GI	10.23±1.98	9.41±3.45	9.19±2.13	9.09±2.13

(% Organ, M ± SE, calculated for animal weighing 100 g) in normothermic and heat stressed, intact and α-adrenergic blocked rats, before and after heat acclimation.
[1] $p < 0.05$; [2] $p < 0.001$ - significant difference from the matched control
[3] $p < 0.001$ - significant difference between intact and α blockade

In non-acclimated rats heat stress induced a 28% expansion in plasma volume (Fig. 1). Heat acclimation attenuated this phenomenon and PVE did not exceed 12%. Fluid shift was rather rapid and was measurable within 5 min following initiation of the heat stress. While neither α- and $β_1$-adrenergic blockade inhibited PVE when administered prior to or following heat stress, the use of both propranolol and butoxamine $(β_2)$ resulted in a maximal PVE under normothermic conditions, with no further increase when heat stress was applied. In contrast, the use of isoprenaline, the non-specific β-agonist decreased the magnitude of heat stress induced PVE.

DISCUSSION: The major cardiovascular response of the heat stressed rat is the shift of blood flow to AVAs. An interesting finding in this investigation is the pronounced increase in AVAs blood flow in the α-adrenergic blocked rats in response to heat stress. This may suggest that in the rat heat induced peripheral vasodilation is due not only to decreased sympathetic tone. Possibly, active vasodilation takes place. Such a mechanism has been found in the past in humans and dogs [8,9].
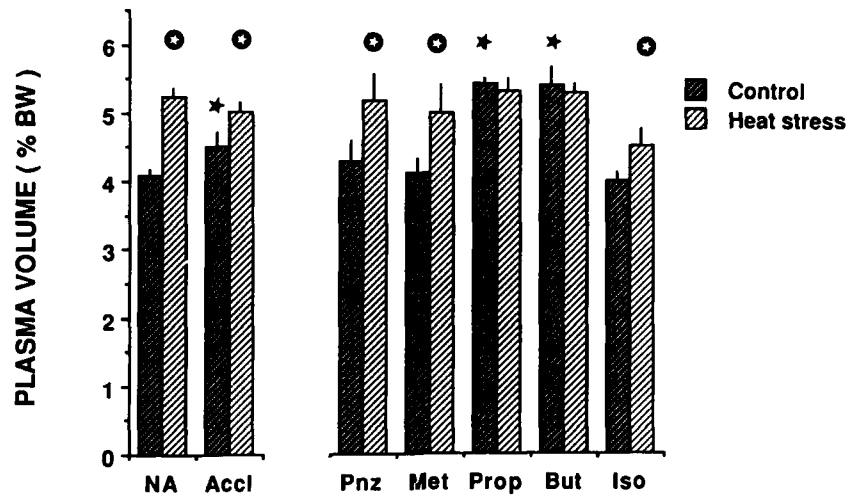
FIGURE 1: Plasma volume (%.b.wt) in control and heat stressed rats subjected to the following treatments: NA - Non-Acclimated; Accl - Heat Acclimated; Pnz - Phenoxybenzamine; Met - Metoprolol; Prop - Propranolol; But - Butoxamine; Iso - Isoprenaline. Vertical lines denote standard error of the mean. n, 6-10.

In the rat peripheral vasodilation is compensated for by a slight decrease in the share of cardiac output distributed to various internal organs particularly to the viscera. Heat stressed α blocked rats exhibit marked splanchnic vasodilation. This may result from the expression of receptors leading to relaxation of the vascular smooth muscle when no masking effect of α receptors takes place.

While cardiac output decreased, in the vasculature, the share of cardiac output distributed to the splanchnic area increased and thus, the capacity to transfer blood flow to the periphery was improved. Indeed, in heat acclimated, heat stressed rats, splanchnic vasoconstriction was more pronounced. In the α-adrenergic blocked rats, under normothermic conditions, AVAs blood flow was much higher than in non-acclimated rats. In contrast to non-acclimated rats, heat stress induced splanchnic vasodilation. This may suggest either an increase in receptors density or increased post-synaptic activity following acclimation. An increased receptors density has been reported by us for cholinergic receptors [10].

Heat induced plasma expansion has been reported in humans [4] and dogs [5] and now, in the rat. Heat acclimation reduced this response. Since fluid shift was very rapid it is likely that a decrease in hydrostatic pressure brought about by decreased tissue perfusion rather than by proteins shifts is responsible for the changes observed. From our results on regional blood flow, one can assume that adrenergic vasoconstriction in splanchnic area and skeletal muscle attribute to PVE. However, the appearance of heat induced PVE in the presence of PNZ rules out simple α-adrenergic mediation. Our data show that inactivation of β_2 receptors produced PVE. Isoprenaline inhibited PVE in the heat stressed rat. Activation of β_2 receptors is known to decrease capillary hydrostatic pressure and increase the capillary surface area available for filtration [11]. It is therefore likely that our results indicate an increase in the ratio between α and β_2 activated receptors, possibly at a precapillary site, leading to transcapillary fluid absorption and heat induced PVE. The mechanism via which this pathway is controlled and where, is not yet clear and hard to reconcile with the increased plasma catecholamine levels during heat stress. However, the pronounced splanchnic vasodilation in the heat stressed α blocked rat may draw attention to this vascular bed as a possible active site for fluid absorption. Heat induced PVE differs from PVE occurring following haemorrage, where activation of β_2 receptors occurs [11].

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A MICROSITE MODEL FOR HEAT GAIN REGULATION IN THE HAMSTER HYPOTHALAMUS: ELECTROPHYSIOLOGICAL CHARACTERISTICS

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Discrete microsites that mediate heat gain have been reported [1-8] in MAHPOA of the hamster hypothalamus. The sites were described in terms of the following experimental parameters: Their location was below bregma between the anterior commissure and the chiasm, within 0.5 mm of the midline and had a dorsal ventral dimension of ≤ 0.5 mm [1]. The sites were initially identified by an increased colonic temperature (T_c) during screening with prostaglandin E_2 (PGE_2) [4,8]. Heat gain was elicited by three additional agonists, serotonin (5-HT), acetylcholine (ACH), and nicotine (Nic). Minimum effective doses of these agonists from 1 ng to 1 ag were found to elicit heat gain [6]. Specificity of the elicited heat gain response was determined with specific antagonists [3,8]. Heat gain was stimulated by the same neurotransmitters in profoundly hypothermic hamsters [4,9,10] and in anesthetized animals at 24°C ambient room temperatures [3,8]. Endogenous release of 5-HT in this site increased with peripheral cold stimulation [2,8]. The data from these studies were in substantial agreement with data in awake and anesthetized rats [Resch and Simpson, unpublished observations] and in the awake monkey [11].

Two kinds of criteria were used to establish the physiologic importance of a thermoregulatory site. One set of criteria used in this laboratory and summarized above were the pharmacologic, dose response, antagonist and endogenous release data. A second set of criteria used by others was the electrophysiologic response of neurons in the PO/AH to peripheral cold stimuli [12]. A more complete description would be obtained by using both sets of criteria in a single site. The present report describes experiments in which electrophysiologic responses were measured at this site following peripheral cold stimulus and micro-injected neurotransmitter stimuli. The relationships between both sets of criteria, i.e., the electrophysiology and the first set of criteria described above, will be discussed.

METHODS: Hamsters *(M. auratus)* between 90 and 120 g under ketamine HCl (50 mg/kg) were implanted stereotaxically with 24 ga stainless steel guide tubes at AP bregma, L 0.0, and Ho -7.5 below the skull. Animals were allowed to recover from surgery for 1 week on food and water *ad lib*. Electrophysiology measurements were made with a recording electrode of a 100 μm diameter wire insulated except for the tip. A reference electrode was placed under the skin, 2 mm lateral to the guide tube. The recording electrode was attached through a preamplifier to a storage oscilloscope. Photographs of neuronal activity were taken of tracings on the oscilloscope screen. In a typical experiment using a ketamine (50 mg/kg) anesthetized hamster, a recording electrode was inserted through the guide tube to the site previously determined by PGE_2 screening. Colonic temperature (T_c) was monitored continuously with a thermistor probe and telethermometer. Control data were recorded before and response data following application of each stimulus. Peripheral cold stimulus was achieved by evaporating alcohol from the surface of the scapular and upper thorax region. Data were monitored for the duration of the response. Micro-injection of neurotransmitter stimuli in a 1 μl volume was achieved, as previously described [1,4]. Antagonists were administered by the same method 20 min prior to the agonist. Site integrity was verified by PGE_2 microinjection following a blockade experiment and the injection site was confirmed histologically.

RESULTS: A peripheral cold stimulus was delivered by alcohol evaporation from the scapular region. Control data taken before peripheral cooling (Fig. 1, panel A) showed a frequency of 2.8 Hz. Fig. 1, Panel B shows a firing frequency after cold stimulus of 50 Hz. The response in Fig. 1 occurred within 2 min and was observed through 20 min.
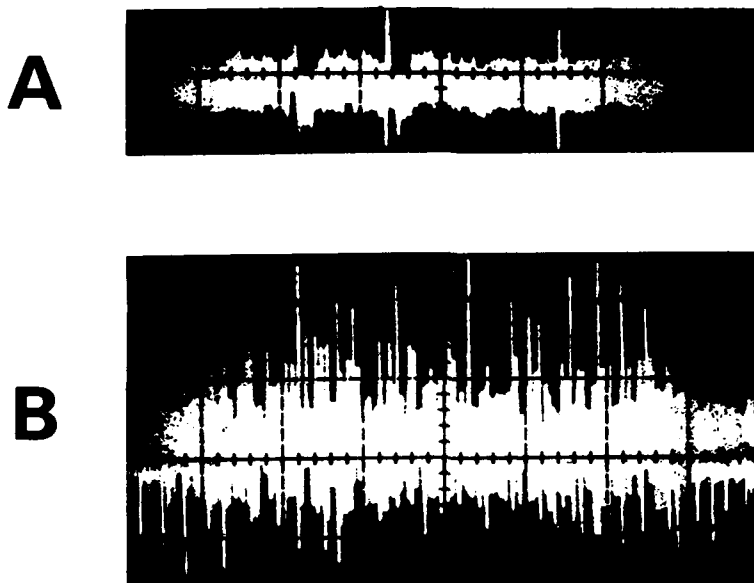
FIGURE 1: Firing frequency in the MAHPOA site before and after peripheral cooling. Panel A shows the recording before the cooling stimulus and panel B shows the increased firing frequency 2 min after application of the stimulus.

A control record was taken 20 min before injection of PGE_2. PGE_2 (100 ng) was then micro-injected directly into the site and the response recorded. Fig. 2 shows a firing frequency of 5.0 Hz before microinjection (Panel A), a rise to 66 Hz at 5 min (Panel B), and 60 Hz at 10 min (Panel C) post injection. The response followed much the same time course as the response to peripheral cold stimulus, i.e., no spikes were recorded within the first 10 to 15 sec after injection and the response was well established within 3 min. Duration of the response was followed for 45 min.

Two kinds of controls for a blocker experiment were recorded. A preinjection control showed a firing frequency of 2.8 Hz (not shown) and an agonist control [5-HT (2 μg)] showed 60 Hz (Fig. 3, Panel A) after microinjection. Twenty minutes later methylergonovine maleate (200 ng) (MTG), a specific 5-HT blocker, was microinjected into the site. Fifteen minutes later a 5-HT (2 μg) challenge was administered and a response frequency of 21.2 Hz recorded (Fig. 3, Panel B). The blocked response was 35% of the unblocked 5-HT response shown in Panel A. In a second experiment in the same animal and at the same recording/injection site, a peripheral cold stimulus elicited a 60 Hz response (Fig. 3, Panel C). This value was the same as recorded following 5-HT microinjection.

Fig. 4 illustrates the firing frequency response at three (3) speeds: 1 sec/div, 100 msec/div, and 10 msec/div. Firing frequency data were obtained with the highest reliability at the 2 slower sweep speeds. The fastest sweep speed, 10 msec/div, shows a typical wave form of approximately 3 msec duration and a 0.75 v amplitude for a single unit response.

In the PGE_2 and 5-HT experiments described above, T_c was simultaneously recorded. This confirmed that the electrophysiologic and temperature responses were elicited from the same site. After microinjection of PGE_2 (100 ng), T_c rose 0.9°C in 10 min and the frequency response rose from 5 Hz to 60 Hz in the same period. Similarly, the T_c response to 5-HT (2 μg) rose 1.2°C in 17 min and frequency rose to 60 Hz in the same time interval. T_c and firing frequency increased together in 100% of the MAHPOA heat gain sites tested and did not change in non-active control sites.
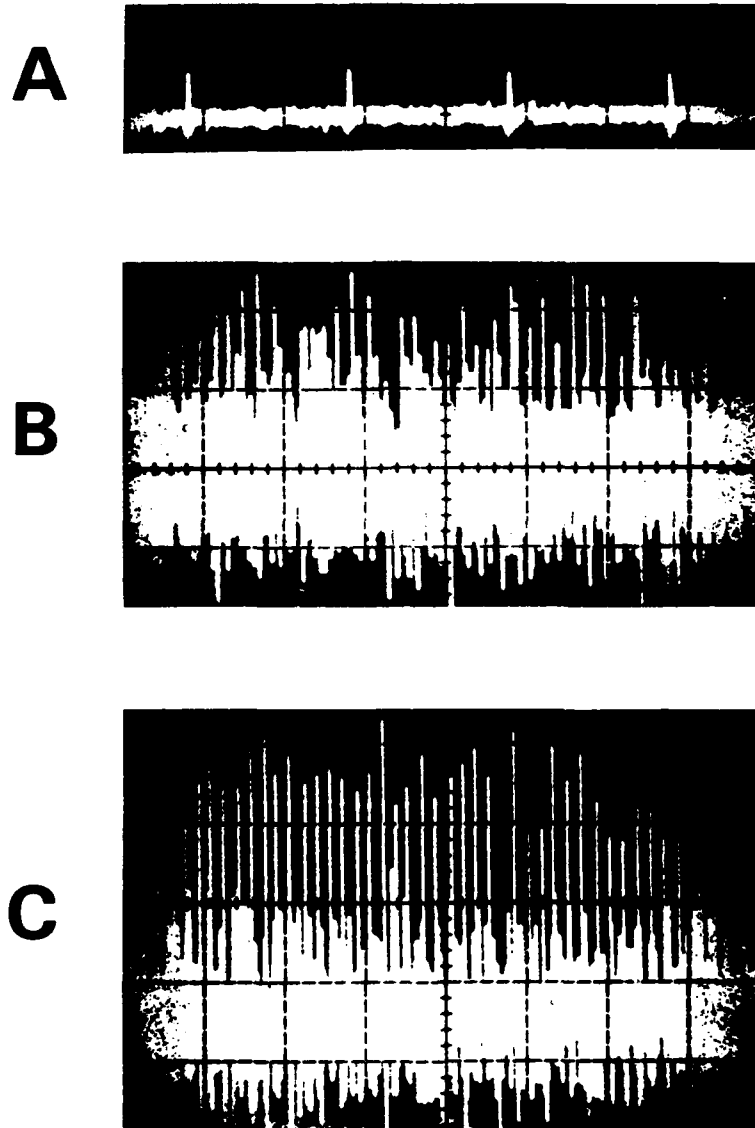
FIGURE 2: Firing frequency in the MAHPOA site before and after microinjection of PGE_2. Panel A shows a control record 20 min before 100 ng of PGE_2 was injected in 1 μl into the MAHPOA site. Panels B and C show the increased frequency at 5 and 10 min after PGE_2 injection, respectively.

During the blockade experiment with 5-HT and MTG, the blocker was administered 20 min before the injection of 5-HT. The T_c response to the MTG was -1.7°C. With the addition of 5-HT, T_c increased 0.3°C in 19 min, which was comparable to vehicle controls. Frequency response to 5-HT in the presence of MTG was only 21.2 Hz. The frequency response and T_c values both increased in response to agonist and both were reduced to antagonist/agonist microinjections.
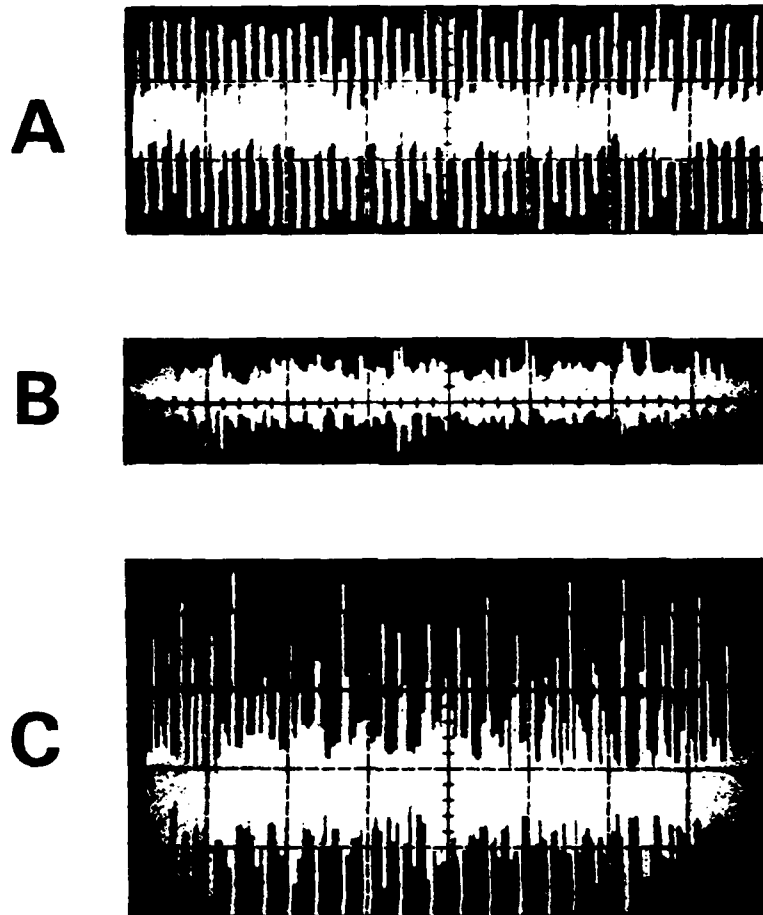
FIGURE 3: Firing frequency in the MAHPOA site after 5-HT alone, after blocker plus 5-HT, and after peripheral cooling in the same animal. Panel A shows the firing frequency after microinjection of 2 μg of 5-HT alone into the MAHPOA site. Panel B shows the firing frequency to 5-HT injected 20 min after 200 ng of methylergonovine. Panel C shows the firing frequency after peripheral cold stimulus.

DISCUSSION: From a neuronal model of a heat gain control system, an increased firing frequency should follow a peripheral cold stimulus. Data from these experiments show that this occurred. Electrophysiologic recordings from the MAHPOA microsite demonstrated that the site receives peripheral input from cooling of the skin. This observation in the ketamine anesthetized hamster is consistent with earlier observations [13-16] in which cold exposure elicited increased firing frequency at hypothalamic sites. The observations are also consistent with thermode and brain slice work which showed changes in firing frequency to cooling of a brain site or lowering of bath temperature [12].

A model of a heat gain site should also predict that increased electrophysiologic activity would result in increased endogenous neurotransmitter release following peripheral cooling. The MAHPOA site from which the electrophysiologic recordings were taken was the same site from which peripheral cooling also elicited increased release of endogenous 5-HT neurotransmitter [2,8]. These obsevations were in agreement with a report of the endogenous release of 5-HT in the conscious monkey after peripheral cooling [11].
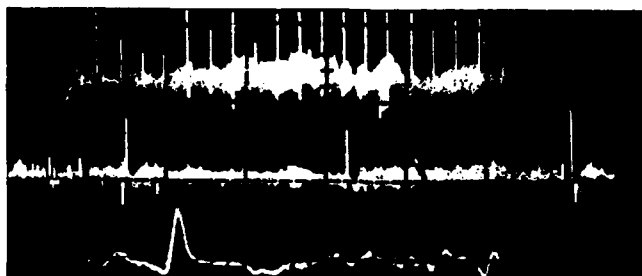
FIGURE 4: Firing frequency recorded at three sweep speeds. The top trace shows a record taken at 1 sec/div, the middle trace at 100 ms/div, and the bottom trace at 10 ms/div. The wave form was 3 msec in duration and 0.75 volts amplitude for the unit response illustrated in the bottom tracing.

TABLE I   Relationships between parameters from the same MAHPOA site in individual hamsters: Changes in colonic temperature, endogenous neurotransmitter release and unit response elicited by microinjection or peripheral cold stimuli

| | PGE$_2$ | | 5-HT | | MTG + 5-HT | | Cold | Released 5-HT |
	(C)[1]	(Hz)	(C)[1]	(Hz)	(C)[1]	(Hz)	(Hz)	(ng)
I	-1.1	5.0	0.1	2.7	1.2	60	2.8	20
II	1.1	66	1.2	60	0.3	21.2	50	80

[1]Values are changes in T_c over a 20 min period before (I) and a 30 min period after (II) injection of PGE$_2$ (cols 1 & 2), 5-HT (cols. 3 & 4), and MTG + 5-HT (cols. 5 & 6). T_c and Hz values in columns 5 & 6 show changes to 5-HT alone (I) and to MTG + 5-HT (II).

Cols. 1 & 2 were measured in the same animal following microinjection of PGE[2]. Cols. 3 & 4 were measured in the same animal following microinjection of 5-HT. Cols. 5 & 6 were measured in the same animal used for cols. 3 & 4 following microinjection of 5-HT 20 min after microinjection of methylergonovine (MTG). Col. 7 shows frequency responses measured in the same animal used for cols. 3-6 following peripheral cold stimuli. Col. 8 shows amounts of 5-HT (before [row I] and after [row II] peripheral cold stimulus) recovered by push-pull perfusion from a MAHPOA site identified by the same criteria as used in all other experiments.

A model of heat gain logically would also indicate that any neurotransmitter that elicites a rise in T_c when injected into the site should do so via an increase in neuronal activity. New observations in the present report show the increased electrophysiologic response to PGE_2 and 5-HT neurotransmitter micro-injected directly into the site (Fig. 2 & 3). PGE_2 and 5-HT were previously shown to elicit increased T_c from this site [2-5,8]. Simultaneous monitoring of T_c during the electrophysiologic recordings in this paper confirm the earlier finding and demonstrate both parameters changed in a coordinated manner from the same MAHPOA site. In the same experiments, specific antagonist to 5-HT reduced increases in both T_c and firing frequency.

The above data satisfy several requirements for an extension of the MAHPOA heat gain model in the hamster proposed in a previous report [1]. An observation made in the context of this model addressed questions of the relationship between heat gain and heat loss control systems. Some theoretical formulations suggest that activation of heat loss inhibits heat gain [14]. In conscious animals it is difficult to separate heat loss and heat gain for independent study. Observations from two experiments reported here suggest that ketamine may functionally separate the heat gain and heat loss systems. Ketamine is known to lower body temperature moderately by increasing heat loss [17,18]. Normally, a lowered core temperature would be expected to provide a strong drive to the heat gain control system. However, in the first experiment reported here in anesthetized animals, no elevation in firing frequencies were observed at T_c values 3 to 5°C below normal in pre-stimulus controls. This observation indicates the core temperature information was blocked from the MAHPOA heat gain site. However, the heat gain system responded to two other stimuli. A second observation from the first experiment indicates skin temperature inputs were not blocked, i.e., cooling of the scapular region with alcohol elicited high rates of discharge measured from the site. A second experiment shows that T_c and frequency increased when neurotransmitters were injected into the site.

Clearly the two experiments show that heat gain is operative. Coupling our observations on heat gain and ketamine's documented effects on heat loss [18] indicates ketamine may provide an experimental tool to separate and study interactions between heat gain and heat loss systems.

The electrophysiologic data in this report, together with data from previous reports [1], directly support the physiologic nature of this MAHPOA heat gain model. The model demonstrates three logical relationships between parameters from three different experimental approaches. Table I shows that three parameters, i.e., 1) T_c responses, 2) endogenous release of neurotransmitter and 3) frequency response, change in a coordinated manner from control values following the stimulus. The following relationships between parameters indicate an endpoint of heat gain was achieved physiologically. First, the relationship between T_c and frequency response was shown to the microinjected agonists. Both parameters increased following microinjection of PGE_2 (Table I, cols. 1 & 2) and also following microinjection of 5-HT (Table I, cols. 3 & 4). Second, the relationship between the endogenous release of 5-HT and frequency response is shown to the stimulus of peripheral cooling (Table I, cols. 7 & 8). Both parameters increased following peripheral cooling. Third, the effect of neurotransmitter antagonist on T_c and frequency response showed reduced responses in both parameters compared to the unblocked state (Table I, cols. 5 & 6). The logical relationships shown here confirm and extend data in previous reports [1,8] and verify a physiologic role for this MAHPOA microsite.

In addition to supporting the physiologic nature of the site, the data also suggest this site has an integrative role in thermoregulation. Afferent signals, i.e., skin cooling, stimulate increased firing frequency and 5-HT release in the MAHPOA site. Efferent activity as indicated by increased T_c and increased firing frequency was elicited by microinjection of 5-HT and PGE_2 into the same site. The circuit configuration in this integrative site appears to be complex based on 1) reported responses to 4 agonists and on 2) cross blockade experiments [6]. Taken together, these observations indicate that the site may operate to integrate afferent inputs to determine the nature of efferent events.

The electrophysiologic data reported here contribute to the description of a model for a heat gain microsite in the MAHPOA. The model relates several experimental parameters to electrophysiologic events which must necessarily be part of a neuronal control system. The combined data also support a model that has the necessary characteristics to support its physiologic importance as an integrative site in the regulation of heat gain responses in the animal. The model may therefore be a useful tool to explore other questions in the regulation of body temperature and hypothalamic organization.

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A FUNCTIONALLY IMPORTANT MICROSITE FOR TEMPERATURE REGULATION IN THE HAMSTER HYPOTHALAMUS: PHARMACOLOGICAL CHARACTERIZATION

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Previously we have described midline AHPOA microsites, between the optic chiasm and anterior commissure, which elicit thermogenesis following appropriate neurochemical stimulation [1,2]. Micro-injection of acetylcholine (ACH) or 5-hydroxytryptamine (5-HT) evoked temperature increases from these sites in profoundly hypothermic hamsters [3-5]. At higher ambient temperatures, i.e., 23-24°C, four different hyperthermic agents elicit thermogenesis at these microsites following microinjection [2]. We have also reported that specific antagonists of each of the thermogenic agents will block the heat gain response to that agent [1,2]. In this report we will provide complete dose response curves for the 4 agonists we have investigated: ACH, 5-HT, nicotine (Nic) and prostaglandin E_2 (PGE$_2$). The work was done in ketamine anesthetized hamsters in room air 23-24°C. We will also show blockade experiments between just effective doses of agonists and just effective doses of specific antagonists. The antagonist doses have been specified by a separate analysis which will just block a just effective dose of agonist. Finally we will report the results of cross blockade experiments in these same animals. These data describe the underlying circuit configuration of these functionally important heat gain microsites in the MAHPOA.

METHODS: Female hamsters (*M. auratus*) weighing between 90 and 120 g served as subjects for these experiments. Animals were anesthetized to a surgical plane with ketamine HCl (50 mg/kg ip). A 24 ga stainless steel guide tube was stereotaxically implanted at coordinates AP bregma, L 0.0, and Ho -7.5 in the hamster's brain. Stainless steel anchor screws were placed around the guide tube and both were secured to the skull with dental cement. Hamsters recoverd on *ad lib* food and water for 1 week following cannulation surgery. Animals were anesthetized to a surgical plane by injection of ketamine HCl (50 mg/kg) and maintained at this level of anesthesia throughout the experiment by supplemental injections of ketamine. When anesthetized a thermistor probe, connected to a telethermometer, was inserted 6 cm into the colon for core temperature measurements. Colonic temperatures were recorded every 5 min for all animals throughout the experiments.

Drugs were made fresh daily in 0.9% saline vehicle solution. Vehicle solution was injected alone in control experiments. To identify a thermogenic site, an animal was anesthetized and a 29 ga stainless steel injector inserted into the guide tube. A 1 μl injection of PGE$_2$ (100 ng/μl) was injected over 15 sec beginning 0.5 mm below the tip of the guide tube. Thermogenic sites were described using a criterion of at least a 0.5°C rise in colonic temperature within 30 min following an injection. These same sites were then tested with 3 additional thermogenic agonists: ACH, Nic and 5-HT. Only sites which responded at criterion to all 4 thermogenic agonists were used for these experiments. For specific antagonist or cross blockade experiments, the specific antagonist was injected 20 min before a specific agonist as described. The antagonists used were methylergonovine maleate for 5-HT, atropine for muscarinic ACH receptors, hexamethonium bromide for nicotinic ACH receptors and SC19220 for PGE$_2$ receptors.

At the end of each experiment the data were evaluated with independent t-test. Animals were perfused and sites verified histologically.

RESULTS: Dose response curves (Tables I-IV) established just effective doses for each of the agonists used. The first dose below the just effective dose at which there was no significant difference from saline vehicle is identified as the threshold dose for each agonist. The just effective doses of the agonists were clearly in the physiologic range, i.e., 1 ng to 1 ag. Throughout the entire dose range of all of the agonists only heat gain responses occurred. Specific antagonists were used for each agonist as a test for specificity of response. The data comparing agonist alone with antagonist/agonist pairs, both at just

effective doses, are shown in Tables V-VIII. Cross blockade experiments tested each antagonist against all of the 4 agonists. The data are shown in Table IX.

TABLE I   **Body temperature response to PGE_2 doses (colonic temperature in °C)**

	100 ng	100 pg	1 pg	1 fg	(1×10^{18} g)	(1×10^{21} g)
mean	2.27	1.28	1.54	0.725	0.91	-0.28
SD	(0.66)	(0.08)	(0.68)	(0.35)	(0.63)	(0.43)
n	7	5	5	4	7	10
t	9.99	6.42	6.23	3.32	3.13	-1.43
P	<.001	<.001	<.001	=.004	<0.005	ns

TABLE II   **Body temperature response to ACH doses (colonic temperature in °C)**

	50 µg	12.5 µg	1 ng	100 pg	50 fg	10 fg
mean	1.47	1.60	0.93	1.06	0.91	-0.03
SD	(0.19)	(1.08)	(0.35)	(0.59)	(0.52)	(0.94)
n	7	4	3	5	7	6
t	7.78	4.85	3.37	4.36	5.14	0.97
P	<.001	<.001	=.003	<.001	<0.001	ns

TABLE III   **Body temperature response to nicotine (colonic temperature in °C)**

	10 pg	5 pg	1 pg	500 fg
mean	0.98	1.38	1.44	0.15
SD	(0.69)	(0.49)	(0.39)	(0.79)
n	9	4	5	8
t	4.62	5.22	6.18	1.11
P	<.001	<.001	<.001	=.277

TABLE IV   Body temperature response to serotonin doses (colonic temperature in °C)

	2 μg	10 ng	2.5 ng	1 ng	100 pg
mean	1.40	0.72	1.18	1.31	0.32
SD	(0.61)	(0.29)	(0.48)	(0.77)	(1.17)
n	6	5	4	6	5
t	-5.94	3.47	4.56	4.27	1.06
P	<.001	<.001	<.001	<0.001	ns

TABLE V   Specific antagonism of heat gain (colonic temperature in °C)

	Methy (150 ng) 5-HT (500 pg)	Hex (5 μg) Nic (1 pg)	Atr (2 μg) ACH (50 fg)	SC (5 μg) PGE$_2$ (1x10^{-18} g)
mean	-0.186	0.12	0.22	0.22
SD	(0.79)	(0.98)	(0.58)	(0.75)
n	7	9	5	9
	5-HT alone	Nic alone	ACH alone	PGE$_2$ alone
mean	1.16	1.41	1.05	1.13
SD	(0.60)	(0.53)	(0.42)	(0.59)
n	7	9	6	9
t	3.58	3.46	2.86	2.84
P	<0.01	<0.01	<0.02	<0.02

Methy, methylergonovine; Hex, hexamethonium; Atr, atropine; SC, SC19220.

DISCUSSION: Dose response data (Tables I-IV) demonstrate that the doses of agonists which effect the T_c response are clearly in the physiological range and to the best of our knowledge are the lowest doses reported. The data show that multiple agonists act in the same microsite.

The T_c's in the present report were taken from ketamine anesthetized hamsters. Heat gain increased following the microinjection of agonists in this preparation. Some literature supports the suggestion that ketamine specifically activates heat loss mechanisms, such as tail artery vasodilation in rats [6] and sweating in monkeys [7]. The differences between the ketamine anesthetized hamster in this report and conscious animals suggests three implications for the organization of this heat gain site. First, utilizing ketamine may allow differential study of heat loss and heat gain mechanisms. Second, theoretical notions of heat gain inhibition by heat loss activation need to be rethought. Third, that ketamine may remove endogenous inhibitory influences in the heat gain circuit making it more sensitive to stimulatory influences.

TABLE VI   **Cross blockade experiments with antagonists**

	SC19220 (5 μg) + PGE$_2$ (100x10^{-18} g)	PGE$_2$ (100x10^{-18} g)
mean	0.22[1]	1.13
sem	0.24	0.19
N	9	9
t	2.84	
P	<0.02	

	SC19220 (5 μg) + NIC (1 pg)	Nic (1 pg)
mean	0.67	1.30
sem	0.32	0.19
N	9	9
t	1.68	
P	ns	

	SC19220 (5 μg) + 5-HT (500 pg)	5-HT (500 pg)
mean	0.39	1.01
sem	0.25	0.19
N	10	10
t	1.93	
P	ns	

	SC19220 (5 μg) + ACH (50 fg)	ACH (50 fg)
mean	-0.05[2]	1.19
sem	0.15	0.15
N	8	8
t	5.51	
P	<0.01	

[1]P<0.02
[2]P<0.01
SC19220 for PGE$_2$ receptors

Antagonist blockade of each agonist is shown in Tables VI-VIII. An important feature of the antagonist experiments was that the doses used were just effective doses used against comparable agonist doses as determined in the dose response data (Tables I-IV). The reason these data are important is because in our own work [2] high doses of these same antagonists will block all agonists which elicit heat gain. In cross blockade experiments each antagonist blocked all agonists at doses customarily reported in previous studies [2]. These higher doses of antagonists are those commonly reported in the literature. Cross blockade experiments at just effective doses demonstrated specific blockade against PGE$_2$, 5-HT, ACH, and Nic. However, cross blockade occurred when SC19220 was used against ACH, MTG was used against PGE$_2$ and Nic, and Hex was used against 5-HT. Of the 16 possible combinations of cross blockade, only these 4 occurred. These data suggest that the antagonists may not be entirely specific in all cases. Cross blockade indicates that the neuronal circuitry underlying heat gain is not linear in the site but may involve local neuronal interactions in the microsite.

The data, however, clearly support a model analogous to the sympathetic chain ganglia which shows that multiple receptor populations occur within the same microsite. A model of the microsite [8] consistent with the data is an integrative site that has multiple inputs and outputs.

TABLE VII   Methylergonovine vs all agonists

	MTG (150 ng) + 5-HT (500 pg)	5-HT (500 pg)
mean	0.18[1]	1.16
sem	0.30	0.22
N	7	7
t	3.58	
P	<0.01	
	MTG (150 ng) + PGE$_2$ (100x10^{-18} g)	PGE$_2$ (100x10^{-18} g)
mean	-0.28[1]	0.92
sem	0.15	0.12
N	7	7
t	6.09	
P	<0.01	
	MTG (150 ng) + Nic (1 pg)	Nic (1 pg)
mean	0.57[1]	1.60
sem	0.23	0.15
N	7	7
t	5.39	
P	<0.01	
	MTG (150 ng) + ACH (50 fg)	ACH (50 fg)
mean	0.45	0.91
sem	0.19	0.19
N	7	7
t	1.63	
P	ns	

[1]P<0.01
MTG, methylergonovine maleate for 5-HT receptors

TABLE VIII   **Hexamethonium vs all agonists**

	Hex (5 μg) + Nic (1 pg)	Nic (1 pg)
mean	0.12[1]	1.41
sem	0.32	0.17
N	9	9
t	3.46	
P	<0.01	
	Hex (5 μg) + PGE$_2$ (100x10^{-18} g)	PGE$_2$ (100x10^{-18} g)
mean	1.01	1.01
sem	0.16	0.11
N	9	9
t	0	
P	ns	
	Hex (5 μg) + ACH (50 fg)	ACH (50 fg)
mean	0.67	1.16
sem	0.27	0.14
N	9	9
t	1.55	
P	ns	
	Hex (5 μg) + 5-HT (500 pg)	5-HT (500 pg)
mean	0.33[2]	1.06
sem	0.21	0.21
N	8	8
t	2.39	
P	<0.05	

[1]$P<0.01$
[2]$P<0.05$
Hex, hexamethonium for nicotinic cholinergic receptors

TABLE IX   **Atropine vs all agonists**

	Atr (2 μg) + ACH (50 fg)	ACH (50 fg)
mean	0.21[1]	1.05
sem	0.23	0.17
N	6	6
t	2.86	
P	<0.05	

	Atr (2 μg) + PGE$_2$ (100x10^{-18} g)	PGE$_2$ (100x10^{-18} g)
mean	0.76	0.96
sem	0.24	0.11
N	8	8
t	0.73	
P	ns	

	Atr (2 μg) + 5-HT (500 pg)	5-HT (500 pg)
mean	1.28	1.02
sem	0.30	0.21
N	6	6
t	0.70	
P	ns	

	Atr (2 μg) + Nic (1 pg)	Nic (1 pg)
mean	0.61	1.53
sem	0.43	0.17
N	6	6
t	0.18	
P	ns	

[1]$P < 0.05$
Atr, atropine for muscarinic cholinergic receptors

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THEMATIC LECTURE: The Treatment of Hypothermia

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THEMATIC LECTURE: Temperature Regulation, Sleep and Circadian Rhythm

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Capsaicin Sensitive Vasodilatory Mechanisms in the Brainstem of the Rat

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Successful Resuscitation of a Patient with Severe Hypothermia and Asystole Using Prolonged External Cardiac Massage and Rewarming Using Partial Cardiopulmonary Bypass

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Hypothermic Patients in Odense University Hospital 1975-1988

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Equithesin: A Hibernation-Inducing Drug?

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