HYPOTHERMIA INDUCED BY 5-THIO-D-GLUCOSE: EFFECTS ON TREADMILL P--ETC(U)
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while on the treadmill were significantly (p .001) increased while rates of skin temperature (Tsk) heating were significantly (p .001) reduced when compared to normothermic controls. Administration of 5-TG effected significant (p .001) hyperglycemia which returned to control levels following the exhaustive run in the heat. Prolonged endurance times among the hypothermic rats caused slight increases in the levels of circulating plasma indices of heat/exercise injury. We concluded from these studies that hypothermia induced by 5-TG administration and cold exposure is effective in increasing the endurance capacity of rats exercising in the heat. However, homeostatic mechanisms supercede to increase the heating rate, and thus return Tre to equilibrium levels.
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Hypothermia Induced by 5-Thio-D-Glucose: Effects on Treadmill Performance in the Heat

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Running title: Preinduced hypothermia and treadmill performance
Abstract

In continuing our studies on the effects of preinduced hypothermia on the endurance capacities, thermoregulatory responses, and clinical chemical indices of heat injury, 10 mg of 5-thio-β-glucose (5-TG) were administered intravenously to restrained rats kept at 4°C. When rectal temperatures ($T_{re}$) fell to 29.3°C, the rats were removed to a hot environment (35°C) where they exercised on a level treadmill (9.14 m/min) to hyperthermic exhaustion ($T_{re} = 41.5-43°C$). Pre-induced hypothermia was effective in significantly ($p < .001$) prolonging the time to hyperthermic exhaustion. In these hypothermic rats increments in $T_{re}$ (°C/min) while on the treadmill were significantly ($p < .001$) increased while rates of skin temperature ($T_{sk}$) heating were significantly ($p < .001$) reduced when compared to normothermic controls. Administration of 5-TG effected significant ($p < .001$) hyperglycemia which returned to control levels following the exhaustive run in the heat. Prolonged endurance times among the hypothermic rats caused slight increases in the levels of circulating plasma indices of heat/exercise injury. We concluded from these studies that hypothermia induced by 5-TG administration and cold exposure is effective in increasing the endurance capacity of rats exercising in the heat. However, homeostatic mechanisms supercede to increase the heating rate, and thus return $T_{re}$ to equilibrium levels.

Key words: hyperthermic exhaustion, physical performance, hyperglycemia
INTRODUCTION

Reports in the literature have described a variety of experimental techniques designed to maximize physical performance. Investigators have utilized pharmacological agents (7), nutritional regimens (1,24), as well as vitamin supplements (9) to either increase physical performance or reduce the physiological cost of exercise. While Strydom et al. (22) have demonstrated the efficacy of vitamin C supplements in reducing the time required for heat acclimatization, very few investigations have addressed the possibility of reducing the adverse effects of exercise during acute exposure to high ambient temperatures. Tal and Sulman (23) demonstrated the benefits of exogenously administered dehydroepiandrosterone in increasing the survivability of rats when passively exposed to an environmental temperature of 37°C, while Hubbard et al. (11) have shown the critical importance of ambient temperature on the endurance capacity of exercising rats.

Since it had been previously demonstrated that when rats exercise on a treadmill under a moderate (26°C) (11) or hot (35°C) (3) environmental temperature, hyperthermic exhaustion usually occurs at a rectal temperature ($T_{re}$) of 42.5-43°C. The consistent rate of body heating as well as the reproducibility of final $T_{re}$ suggested that experimental manipulation of $T_{re}$ might delay the onset of hyperthermic exhaustion. Thus, we examined the effects of preinduced hypothermia ($T_{re}=33°C$) on the endurance capacity of rats exercising in the heat (4,12). The results of these earlier experiments demonstrated that the hypothermic animals had an endurance capacity which was increased 62% over that of controls.

More recently, we completed a series of experiments in mice which
demonstrated marked hypothermic effects of 5-thio-D-glucose (5-TG) when this agent was administered while the animals were exposed to cold (4°C) (5,13). Since the experimental animals demonstrated extreme sensitivity to relatively low doses of 5-TG, we hypothesized that the hypothermia induced by this agent and cold exposure would be even more efficacious in increasing the endurance capacity of rats exercising in the heat.

MATERIALS AND METHODS

The techniques utilized in the current experiments were essentially identical to those of our earlier report (4). Adult, male rats (Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. An indwelling jugular catheter was implanted on the day prior to experimentation for rapid withdrawal of 1 ml blood immediately prior to initiation of the exercise in the heat ($T_{re} = 29.4^\circ C$) and immediately subsequent to attaining hyperthermic exhaustion ($T_{re} = 41.7^\circ C$). To induce hypothermia in the experimental rats, 10 mg 5-TG/0.1 ml sterile, non-pyrogenic saline was administered intravenously followed immediately by exposure under restraint to 4°C ambient temperature. Control rats were treated identically, but injected only with physiological saline. The animals weighed from 280-340 g at the time of the experiment. Runs were made on a level treadmill at a speed of 9.14 m/min at an ambient temperature of 35°C and a relative humidity of 20-25%.

In several of these experiments the rats were food-deprived for 18h prior to the start of experimentation since we had earlier demonstrated the augmented thermoregulatory sensitivity of food-deprived animals to the 5-TG (5,13). Two groups of control animals, fed and 18h food-deprived, were used for proper comparisons. Ordinarily, sufficient hypothermia was achieved between 35-50 min after administration of the drug. Blood samples were rapidly centrifuged
Plasma creatine phosphokinase (CPK) was determined by methods based on the technique of Oliver (17) as modified by Rosalki (18). Lactic acid was quantitated by the method described by Henry (8) while plasma potassium (K⁺) levels were assayed on an IL flame photometer (Model 143, Instrumentation Laboratory, Lexington, MA) by methods described in their technical bulletin. Plasma glucose was measured after the procedures described by Slein (21). Statistical analyses were performed by the paired and non-paired t-test, and the null hypothesis was rejected at p < .05.

RESULTS

Fig. 1 illustrates the effects of hypothermia induced by 5-TG administration and cold exposure on the endurance capacity of animals exercising in the heat. The arrows denote the mean endurance time of the initially normothermic control animals as well as the originally hypothermic 5-TG treated rats. Since thermoregulatory characteristics between a group of normothermic, fed controls (n=7) and normothermic, food-deprived controls (n=6) were not statistically different, the results were combined for statistical purposes. The data tabulated in Fig. 1 show clearly the intensity of the preinduced hypothermia in the 5-TG treated animals, and also the significant increment in run-time for the same group of animals. Fig. 2 demonstrates the mean skin temperature (Tsk) responses for the same groups of animals. It is important to observe the divergence in skin temperatures for the two groups of animals particularly after 20 min of exercise in the hot environment (e.g. at 25 min, p < .01; at 30 min, p < .001) despite the fact that both groups had similar starting Tsk due to the exposure to the 4°C environment.

Thermoregulatory data are summarized and quantitated in Table 1. Of the
five parameters compared between control and experimental animals, four
demonstrated highly significant differences. Of particular interest is the
difference noted in increments in $T_{sk}$ which is indicative of reduced vasodilation
and heat loss in the 5-TG treated rats. This is commensurate with the increased
rate of heating ($\Delta T_{re}/\text{min}$, $p < .001$) among these animals.

Fig. 3 illustrates the plasma glucose levels in the three groups of animals. Initially, it can be observed that treatment with 5-TG effects a highly significant
($p < .001$) hyperglycemia when compared with either fed or food-deprived control
animals. Further, the often reported significant ($p < .001$) difference in plasma
glucose levels between fed and fasted control rats can be noted. Following the
exercise in the heat to hyperthermic exhaustion, circulating glucose levels are
significantly reduced ($p < .01$) in the 5-TG-treated rats; in the fed, saline-treated
animals as well as the fasted, saline-treated group trends toward reduction are
not significantly different.

Fig. 4 depicts the effects of 5-TG administration and initial hypothermia
on the response of plasma lactate levels to exercise in the heat. In the blood
samples taken immediately prior to exercise in the heat to hyperthermic
exhaustion, resting plasma lactate levels in the 5-TG, hypothermic animals are
significantly ($p < .02$) elevated when compared to both groups of control animals.
Final lactates (post-run) displayed rather large variability, but in each case were
significantly ($p < .05$, minimal) higher at the conclusion of the exercise to
hyperthermic exhaustion. Analogous results for circulating levels of $K^+$ are
presented in Fig. 5. Parallel significant ($p < .05$, minimal) increments in plasma
concentrations of $K^+$ occurred in both groups of initially normothermic animals,
while 5-TG-treated, hypothermic animals displayed sharper increments. The
steeper rise in circulating lactate and $K^+$ in the hypothermic rats may be
associated with their increased run times. The results depicted in Fig. 6 for CPK are consistent with some of our earlier findings (3). Because the high ambient temperature used in these experiments greatly reduces the expected run times to hyperthermic exhaustion, plasma CPK levels are quite variable and range from large increases in some rats to actual decreases in others when the pre- and post- blood samples are compared. What appears to be a significant increase in mean CPK levels of the initially hypothermic rats is actually mostly accounted for by a single value of 17,750 IU/L in the plasma of one experimental animal. Ordinarily, exercise bouts of longer duration are required before consistent responses of plasma CPK are observed.

DISCUSSION

The results of the present experiments are generally consistent with those which we had reported earlier for rats in which hypothermia was induced by either L-tryptophan or chlorpromazine administration in combination with cold exposure (4,12). Thus, we have shown clearly that hypothermia induced by 5-TG administration and cold exposure elicits markedly increased endurance time to hyperthermic exhaustion. While the animals were exercising on the treadmill, the non-equilibrium temperatures of the 5-TG-treated, hypothermic animals (<36°C) resulted in greater increments during exercise in the heat. The current results demonstrate, however, that this apparent drive to achieve homeostasis occurred concomitantly with decreased peripheral heat loss as evidenced by reduced rates of tail-skin heating among 5-TG-treated, hypothermic rats (Table 1). It is important to add that it is unlikely that 5-TG, of itself, affects peripheral heat loss since during cold exposure while the 5-TG-treated animals were achieving marked hypothermia, $T_{sk}$ of 5-TG-treated and saline-treated control animals were not different.
Wilson et al. (25) have recently demonstrated that rats exercising at high treadmill speeds (42.6 m/min) can achieve thermal balance when the exercise is carried out under mild temperature conditions (22°C); however, they were unable to demonstrate a proportional increase in $T_{re}$ at four treadmill speeds. Even at a room temperature of 42.5°C Ohara et al. (16) demonstrated that, for varying intervals, equilibrium $T_{re}$ could be achieved in passively heated rats. However, results of the present experiments indicate that the combination of exercise and environmental temperature precluded the achievement of steady-state temperatures. It should be noted in Fig. 1 that for saline-treated animals increments in $T_{re}$ were consistently observed through 25 min, the earliest time at which hyperthermic exhaustion was reached.

The intense hyperglycemic response to 5-TG administration (Fig. 3) which we have observed in even food-deprived animals is analogous to the response elicited by both central and peripheral administration of another analogue of D-glucose, 2-deoxy-d-glucose (6,14,15). It has been hypothesized that the intense circulatory hyperglycemia occurs simultaneously with central and peripheral tissue glucopenia resulting in decrements in heat production. This glucose efflux occurring in the first 30-45 min after 5-TG administration may be the result of glycogenolysis pursuant to sympathicoadrenomedullary discharge. The sharp reduction in plasma glucose levels in 5-TG-treated animals following exercise on the treadmill and the marked increases in heat production in these animals during this interval would be compatible with a rapid re-uptake and hypermetabolism of glucose in central and peripheral tissues. This increased re-uptake and hypermetabolism are commensurate with the increased thermogenic demands as well as the oxidative requirements of the initially hypothermic, exercising animals.
Circulating levels of lactate (2,3,20), potassium, (3,10), and creatine phosphokinase (2,3,10,19) have been repeatedly used as clinical chemical indices of exercise, exercise in the heat, and exercise/heat injury. Results of the present investigation are similar to those which we had demonstrated earlier (3). Thus, lactate and $K^+$ levels are both consistently elevated following exercise to hyperthermic exhaustion in all groups tested. It is noteworthy that the increased endurance capacity of the initially hypothermic animals is reflected in the sharper increments of circulating levels of both lactate and $K^+$. Clearly, the preinduced hypothermia did not reduce the control levels of these indices; in fact pre-run lactates were significantly increased in 5-TG, hypothermic rats. In these and some of our earlier experiments the results indicate that plasma CPK levels are less reliable as an index of heat/exercise induced injury. It should be noted that hyperthermic exhaustion is occurring after relatively brief periods of mild exercise. We believe that both the duration and intensity of the exercise are inadequate to elicit consistent changes in levels of CPK.

Thus, we have confirmed our earlier hypothesis that initial hypothermia can prolong the endurance capacity of rats exercising under hot environmental conditions by delaying the onset of hyperthermic exhaustion. While on the treadmill, the unit-time increments in $T_{re}$ for those animals made hypothermic by 5-TG administration and cold exposure combined with the rapid reductions in levels of plasma glucose indicate a hypermetabolic compensation among these animals to return $T_{re}$ to normal ranges. Similarly, reduced $T_{sk}$ in this group suggests decreased peripheral heat loss, again probably a compensatory mechanism to achieve equilibrium $T_{re}$. Generally, the results indicate that the hypothermia induced by 5-TG and cold exposure do not have marked effects on plasma CPK, $K^+$, and lactate levels, but there are indications that the increased
treadmill time among these animals intensifies these responses after exercise in the heat.
The authors gratefully acknowledge the skilled technical assistance of SP5 Leonard Sakal and Mr. Stephen O'Connor. We express our gratitude to Mrs. A. Debra Caruso for typing the manuscript.

The views, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official department of the Army position, policy, or decision, unless so designated by other official documentation.

In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.
Figure Legend

Fig 1. Effect of IV 5-thio-D-glucose administration (10 mg) and prior cold exposure (4°C) on \( T_{re} \) response to treadmill exercise in the heat (35°C). Mean values are depicted for \( n=6 \) in the 5-TG-treated group and \( n=13 \) in the saline-treated group. The arrows denote the mean times at which hyperthermic exhaustion was reached for both groups. Control animals received 0.1 ml sterile, non-pyrogenic physiological saline.

Fig 2. Effect of IV 5-thio-D-glucose administration (10 mg) and prior cold exposure (4°C) on the \( T_{sk} \) response to treadmill exercise in the heat (35°C). All parameters and conditions are as noted under Fig. 1.

Fig 3. Effect of 5-TG administration on plasma glucose levels in blood samples taken immediately prior to (pre-run) and following (post-run) exercise on a treadmill to hyperthermic exhaustion. Rats which were food-deprived were so for 18h prior to the start of the experiment. Mean values ± SEM are depicted for \( n=6 \) (5-TG, food deprived), \( n=6 \) (saline, food deprived), and \( n=7 \) (saline, fed). Control rats received 0.1 ml sterile, nonpyrogenic saline while 5-TG-treated animals were administered 10 mg 5-TG/0.1 ml saline.

Fig 4. Effect of 5-TG administration on plasma lactate levels in blood samples taken immediately before (pre-run) and after (post-run) exercise on a treadmill to hyperthermic exhaustion. All conditions and parameters are as noted under Fig. 3.

Fig 5. Effect of 5-TG administration on plasma potassium levels in blood samples taken immediately before (pre-run) and after (post-run) exercise to hyperthermic exhaustion. All conditions are identical to those noted under Fig. 3.
Fig 6. Effect of 5-TG administration on plasma levels of creatine phosphokinase in blood samples taken just before (pre-run) and after (post-run) exercise to hyperthermic exhaustion. All conditions are identical to those described under Fig. 3.
REFERENCES


5-Thio-D-glucose treated
Saline treated
### TABLE 1
SUMMARY OF THE RESULTS
DEMONSTRATED IN FIGS. 1 AND 2

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<th>TIME ON TREADMILL (MINUTES)</th>
<th>RECTAL TEMPERATURE MAXIMUM (°C)</th>
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<th>SKIN TEMPERATURE MAXIMUM (°C)</th>
<th>$\Delta T_{sk}$/MIN ON TREADMILL (°C)</th>
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FOOD DEPRIVED, HYPOTHERMIC

SALINE, FOOD DEPRIVED, NORMOTHERMIC

SALINE, FED, NORMOTHERMIC

PLASMA GLUCOSE (MG/DL)

PRE-RUN POST-RUN
5-TG, FOOD DEPRIVED, HYPOTHERMIC

SALINE, FOOD DEPRIVED, NORMOTHERMIC

SALINE, FED, NORMOTHERMIC

PLASMA LACTATE (MG/DL)

PRE-RUN POST-RUN
8
7
6
5
4
3
2
1

PRE-RUN

POST-RUN

\(K^+ (\text{MED/L})\)

5-TG, FOOD DEPRIVED,
HYPOTHERMIC

SALINE, FOOD DEPRIVED,
NORMOTHERMIC

SALINE, FED,
NORMOTHERMIC
5-TG. FOOD DEPRIVED, HYPOTHERMIC

SALINE, FOOD DEPRIVED, NORMOTHERMIC

SALINE, FED, NORMOTHERMIC

CPK (IU/L) $\times 10^3$

PRE-RUN   POST-RUN