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# THERMOREGULATORY EFFECTS OF ATROPINE IN THE COLD USING A HYPOTRICHOTIC RAT MODEL

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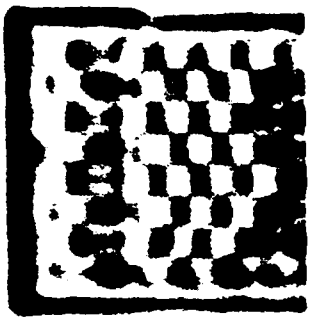

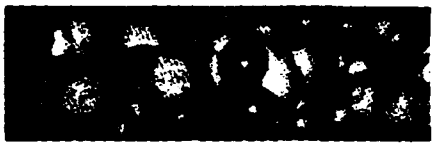

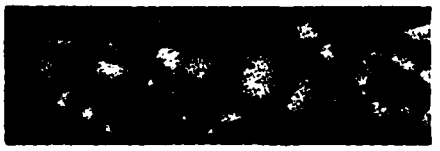

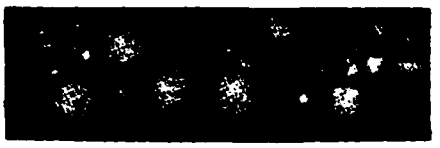

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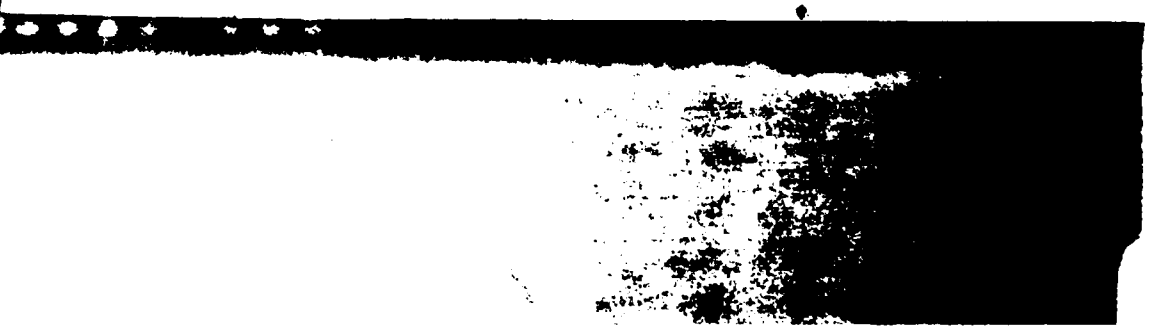

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The objectives of this research were to determine the effects of atropine on thermoregulation and peripheral vasodilation in the cold. A conscious, confined but unrestrained, hypotrichotic (Wistar-Furth, fuzzy) rat model was used. Electromyography (EMG) was utilized to assess the shivering response of the trapezius muscle. An EMG frequency band between 3 Hz and 1 kHz was rectified, then integrated for determination of a shivering index. Infrared thermography was used to monitor dorsal body skin temperature as an indirect assessment of cutaneous blood flow. Rats were injected in the lumbar musculature with either 1 mg/kg atropine (A) or an equivalent volume (0.15 ml) of saline (S) 30 minutes after exposure to either 25°C, 18°C or 12°C. Data were then collected for an additional 90 minutes. There were no significant between group (A vs. S, $p<0.05$) differences in shivering, rectal temperature ($T_{re}$), skin temperature or tail temperature at 25°C (n=6/group), 18°C (n=7/group), or 12°C (n=12/group). Modest within group decrements in $T_{re}$, occurred between 10 and 90 min following A injection at 18°C and 12°C. A transient decline in shivering occurred following atropine administration at 12°C, but baseline levels were reached by 20 min post-injection. We concluded that intramuscular injection of A caused a small decrease in $T_{re}$ in a cold-stressed hypotrichotic rat model. However, this decrease could not be entirely explained by shivering inhibition nor by cutaneous vasodilation.

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# THERMOREGULATORY EFFECTS OF ATROPINE IN THE COLD USING A HYPOTRICHOTIC RAT MODEL

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## ABSTRACT

The objectives of this research were to determine the effects of atropine on shivering and peripheral vasodilation in the cold, with attendant effects on thermoregulation. A conscious, confined but unrestrained, hypotrichotic (Wistar-Furth, fuzzy) rat model was used. Electromyography (EMG) was utilized to assess the shivering response of the trapezius muscle. An EMG frequency band between 3 Hz and 1 kHz was rectified, then integrated for determination of a shivering index. Infrared thermography was used to monitor dorsal body skin temperature as an indirect assessment of cutaneous blood flow. Rats were injected in the lumbar musculature with either 1 mg/kg atropine (A) or an equivalent volume (0.15 ml) of saline (S) 30 minutes after exposure to one of three ambient temperatures (25°C, 18°C or 12°C). Data were then collected for an additional 90 minutes. There were no significant between group (A vs. S, $p<0.05$) differences in shivering, rectal temperature ($T_{re}$), skin temperature ($T_{sk}$) or tail temperature ($T_{tail}$) at 25°C (n=6/group), 18°C (n=7/group), or 12°C (n=12/group). However, modest within group decrements in $T_{re}$, (0.6°C and 0.8°C, $p<0.05$) occurred between 10 and 90 minutes following A injection at 18°C and 12°C, respectively. A transient decline in shivering occurred immediately following atropine administration at 12°C, but baseline levels were reached by 20 minutes post-injection. We concluded that intramuscular injection of A caused a small decrease in $T_{re}$ in a cold-stressed hypotrichotic rat model. However, this decrease could not be entirely explained by shivering inhibition nor by cutaneous vasodilation, and may have been due to a reduction in non-shivering thermogenesis.

## INTRODUCTION

While the thermoregulatory effects of atropine administration in hot environments have been extensively investigated[1-5], little is known of its effects in the cold. Atropine is fielded as a self-administered antidote for organophosphate poisoning[6], and may have detrimental effects on visual performance[7], cognition[8], comfort ratings[9], and short term memory[10]. If atropine-induced decrements on heat production in the cold result in hypothermia, then the effects of the antidote on performance may be exacerbated. Since many areas of prospective military activity are cold during variable times of the year, it is conceivable that atropine may be used properly, unnecessarily, or accidentally during cold weather operations.

Systemic administration of atropine (1 mg/kg) in rats at an ambient temperature ($T_a$) of 20°C, causes $T_{re}$ to fall[11]; however the mechanism for this decrease is unknown. Atropine administration in man exercising at cool temperatures elicits peripheral vasodilation[12,13]. Body temperature is thus decreased due to increased convective and radiative heat dissipation from the skin to the cool ambient air[13]. Atropine may attenuate the ability to increase heat production by inhibiting shivering[14]. Thus, both decreased cutaneous blood flow and increased shivering thermogenesis may be significantly compromised by atropine resulting in hypothermia during cold exposure.

This study is designed to evaluate the effects of atropine on thermoregulation in a conscious, cold-stressed hypotrichotic rat model. We hypothesize that atropine inhibits shivering and/or elicits cutaneous vasodilation in the cold, thereby impairing the ability to maintain normothermia. Thermographic techniques are applied to indirectly assess skin blood flow, and the intensity of the shivering response is quantified using integrated electromyography.

## METHODS

Adult, male Wistar-Furth "fuzzy" rats (366 $\pm$ 19 g) were used. The rats were caged individually and housed in animal care facilities ($T_a$ = 23-25 °C) until the time of use. Two days prior to an experiment, two 0.20 mm x 10 cm, stainless steel teflon coated EMG electrodes were aseptically inserted 2-3 mm apart into the trapezius musculature, under methoxyflurane anesthesia. A 26 gauge needle was used for intramuscular penetration of each electrode. A preformed barb anchored each electrode in the muscle as the needle was withdrawn. One electrode was similarly implanted subcutaneously on each side of the chest to monitor heart rate.

During experimentation the rats were confined in a plastic-coated cage. The EMG signal frequency and intensity were continually recorded using a shielded coaxial cable connected to a Gould® universal preamplifier and pen-writing oscillograph. A frequency band between 3 Hz and 1 kHz was rectified, then integrated on a separate channel to determine a shivering index

(IEMG). A Yellow Springs Instruments (YSI), model 402 rectal thermistor was inserted 6 cm beyond the anal sphincter to measure $T_{re}$. A surface temperature probe (YSI, model 427), was taped to the ventral surface near the base of the tail to monitor $T_{tail}$. The $T_a$ was measured by an air temperature probe (YSI, model 405).

Dorsal body skin temperatures ($T_{sk}$) were evaluated thermographically. The $T_{sk}$ was recorded using an AGEMA® TIC-8000 infrared system with CATSE-1.0 thermographic software. A thermal range of 10°C was used with a sensitivity of ± 0.1°C. The $T_{sk}$ represented an average of approximately 4000 temperature points on the dorsal body surface from the nose to the base of the tail.

Since shivering in the rat begins at $T_a$ = 18.3 ± 1.2 °C and intense shivering at $T_a$ = 13.2 ± 2.5 °C[15], rats were placed in one of three ambient temperatures: $T_a$ = 25 ± 1 °C, $T_a$ = 18.0 ± 1 °C, or $T_a$ = 12.0 ± 1 °C. The EMG and ECG were continually recorded. The $T_{tail}$, $T_{re}$, $T_{sk}$ and $T_a$ were recorded at 2 minute intervals for 30 minutes, then either atropine (1 mg/kg) or an equivalent volume (0.15 ml) of 0.9% NaCl was injected into the lumbar musculature and measurements taken for an additional 90 minutes. Atropine alkaloid was used in this study, since the atropine in the autoinjectors supplied to at-risk armed forces personnel is in the alkaloid form[16]. At the end of each experiment, the rat was euthanized using $CO_2$ followed by severing of the diaphragm.

Statistical comparisons between and within groups were performed using a two-way analysis of variance with repeated measures for time. All values were averaged over ten minute intervals. The null hypothesis was rejected at the $p<0.05$ significance level. Tukey's test was used for post-hoc analysis.

## RESULTS

There were no differences in $T_{re}$, $T_{sk}$, $T_{tail}$ or IEMG between atropine (A) and saline control (S) groups at 25°C, 18°C, or 12°C. However, there were within group differences from baseline which are summarized below. Baseline values included measurements taken during the 30 minutes prior to A or S injection.

In Figure 1a, b, c, the effects of atropine on $T_{re}$ at $T_a$ = 25°C, 18°C, and 12°C respectively are plotted. At $T_a$ = 18° and $T_a$ = 12°C there are small (<1°C), but significant, decreases in $T_{re}$ following atropine administration within 10 minutes of injection compared to baseline (Fig. 1b, c). There is a small (<0.3°C) decrease in baseline $T_{re}$ at $T_a$ = 18°C in the saline-treated group which achieves significance 50 minutes post-injection (Fig. 1b).

The $T_{sk}$'s at the three ambient temperatures are shown in Fig. 2a, b, c. At 12°C $T_{sk}$ decreased nearly 0.4°C from baseline by 20 minutes following atropine injection (Fig. 2c). There were no differences from baseline $T_{sk}$ at 25°C or 18°C (Fig. 2a, b).

Figure 3a, b, c displays the effects of atropine on $T_{tail}$ at the three ambient temperatures. The $T_{tail}$ decreased approximately 2°C ($T_a$ = 18°C) and 3°C ($T_a$ = 12°C) from baseline in both control and atropine-treated groups (Fig. 3b, c).

A transient, but significant decrease in IEMG occurs immediately after atropine injection at $T_a$ = 12°C, but returns to baseline by 20 minutes post-injection (Figs. 4b and 5b).

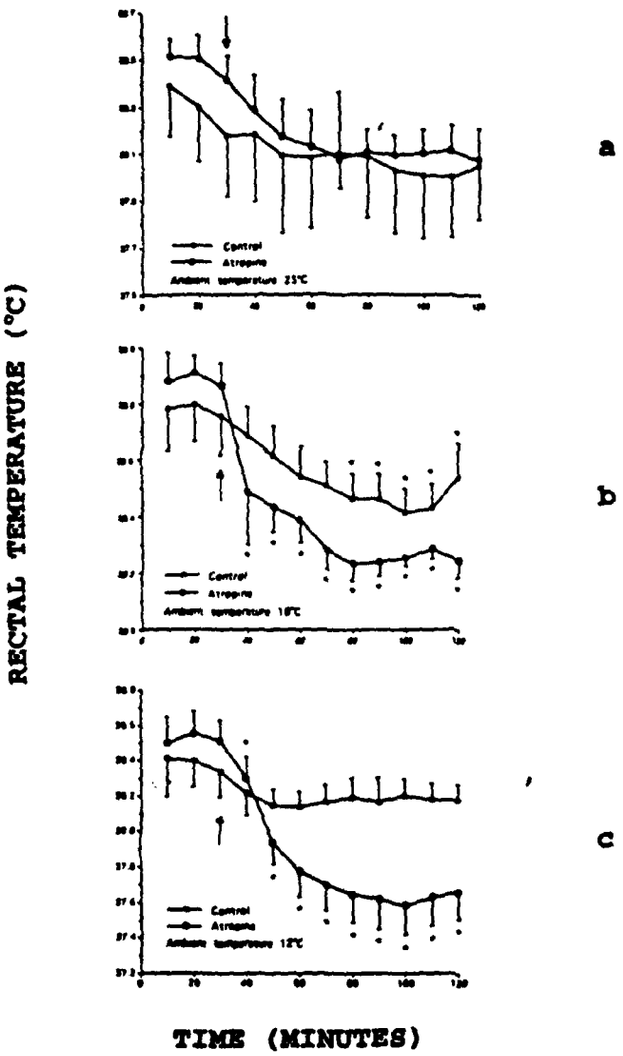


Figure 1a, b, c. The effects of atropine on $T_{re}$ at $T_a$ = 25°C, 18°C and, 12°C. An arrow indicates time of A or S injection. A significant ($p<0.05$) decrease from pre-injection is indicated by an asterisk. Results are reported as mean ± SEM.

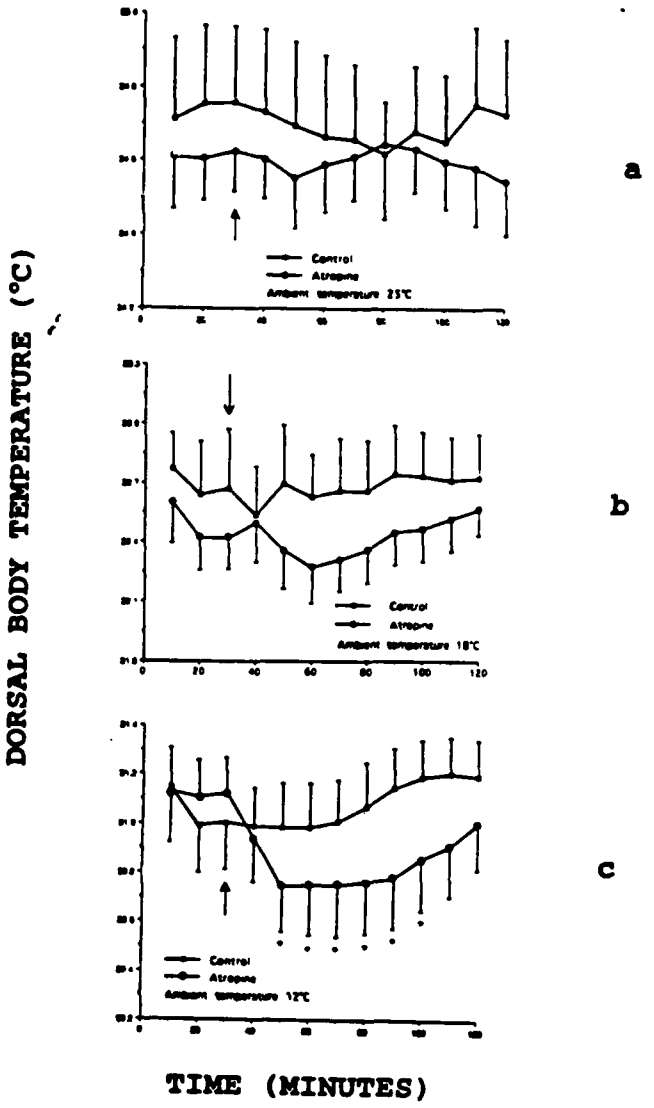


Figure 2a, b, c. The effects of atropine on $T_{sk}$ at $T_a$ = 25°C, 18°C and, 12°C. An arrow indicates time of A or S injection. A significant ($p<0.05$) decrease from baseline is indicated by an asterisk. Results are reported as mean ± SEM.

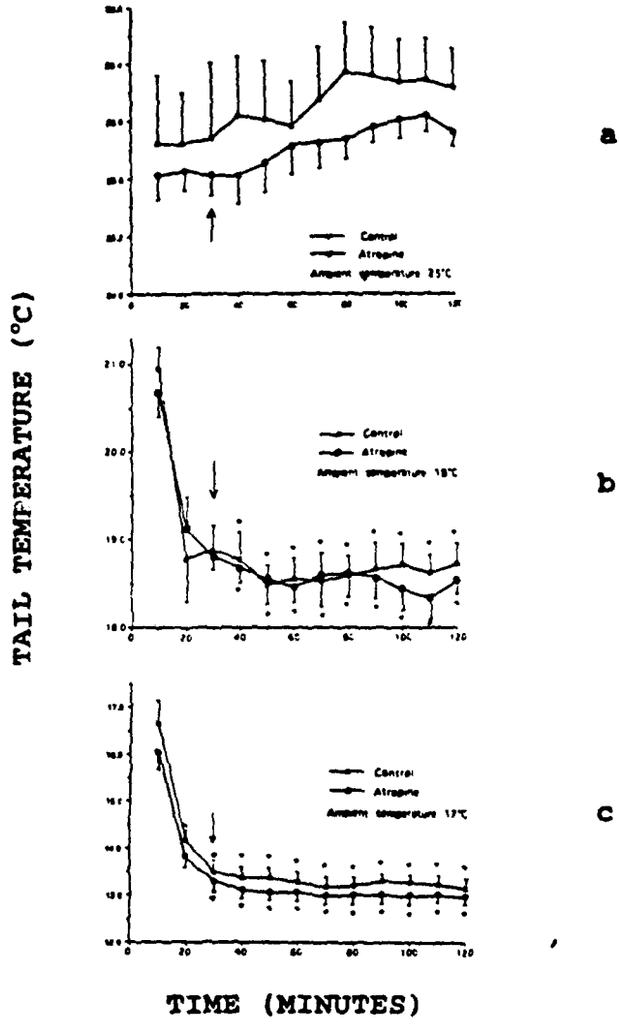


Figure 3a, b, c. The effects of atropine on $T_{tail}$ at $T_a$ = 25°C, 18°C and, 12°C. An arrow indicates time of A or S injection. A significant (p,0.05) decrease from pre-injection 10 and 20 minute time intervals is indicated by an asterisk. Results are reported as mean ± SEM.

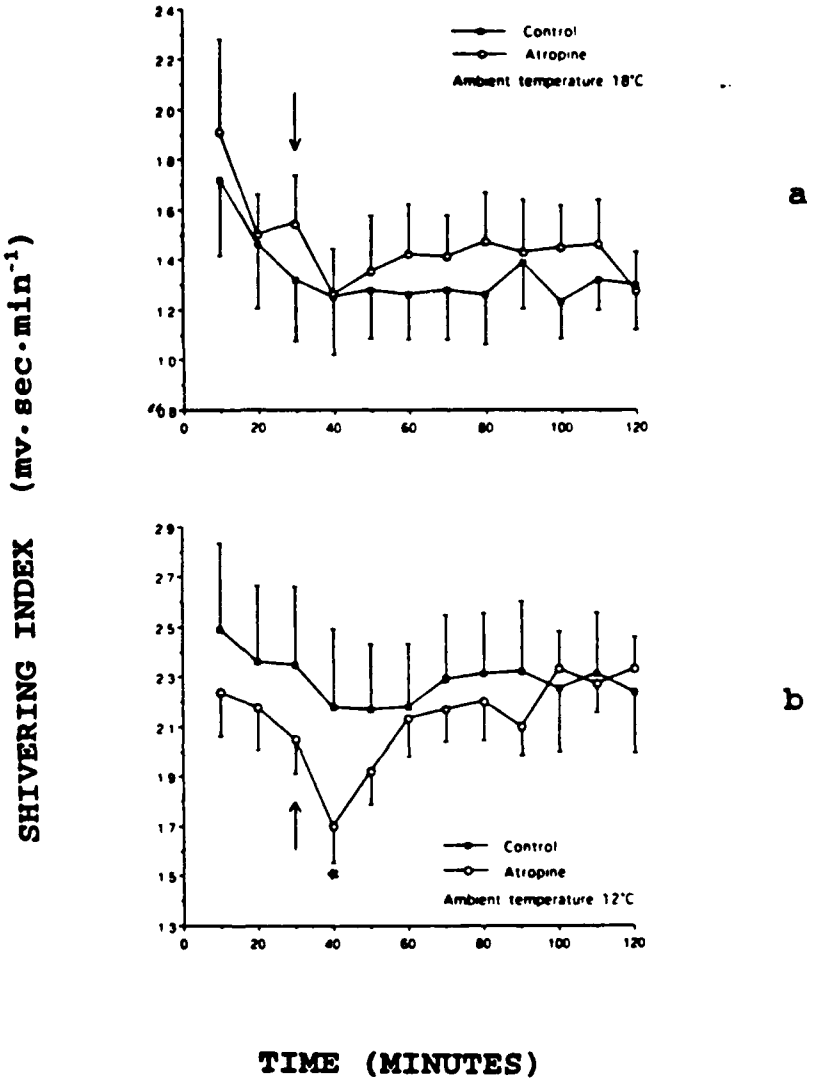


Figure 4a, b. The effects of atropine on shivering at $T_a$ = 18°C and 12°C. An arrow indicates time of A or S injection. A significant ($p<0.05$) decrease from pre-injection and post-injection is indicated by an asterisk. Results are reported as mean ± SEM.

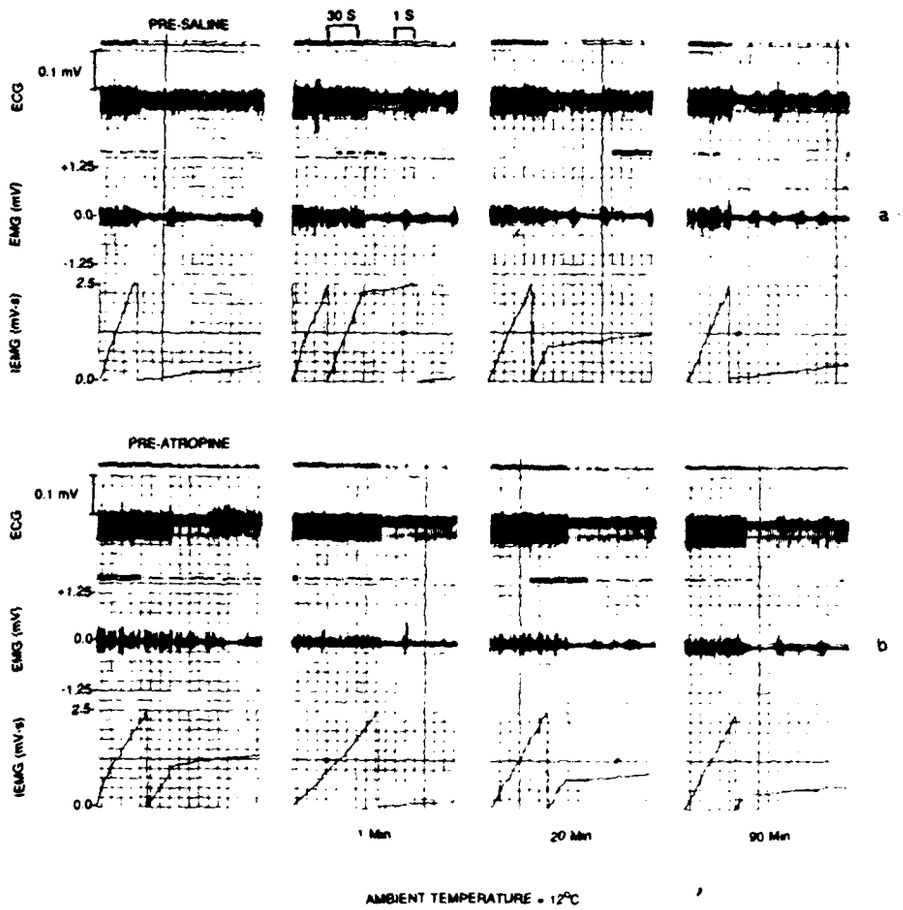


Figure 5a, b. Representative continuous ECG, EMG and integrated EMG (IEMG) tracings fron a saline-treated rat and an atropine-treated rat at $T_a$ = 12°C. Post-injection times are indicated at the bottoms of the tracings. A decrease in the slope of the IEMG immediately following atropine injection indicates a reduction in shivering. The IEMG returns to baseline by 20 min. post-atropine.

## DISCUSSION

Shivering is transiently decreased following atropine administration at $T_a$ = 12°C. This reaction occurs within seconds and shivering returns to baseline by 20 minutes post-injection. In this study, the tachycardiac effects of atropine persist until 90 minutes post-injection (experiment end-time). Others have shown that the length of time for the effects of atropine to subside in rats to be about 3 hours[4]. Because of the immediate onset and transient nature of shivering reduction, it is most likely not a true pharmacologic response. Since the response is brief, its effect on heat production is probably minimal. Furthermore, the transitory reduction in shivering is not seen at $T_a$ = 18°C although $T_{ic}$ decreases at both $T_a$ = 12°C and $T_a$ = 18°C. We were unable to determine why shivering is temporarily reduced at $T_a$ = 12°C and not at 18°C following atropine injection.

In the Hypotrichotic rat, the fall in $T_c$ in the cold following atropine injection is not associated with increases in $T_c$ or $T_{sk}$. Since an increase in skin blood flow would be associated with a rise in either $T_c$ or $T_{sk}$, there is no indication in this model that cutaneous blood flow is augmented by atropine. Indeed, the $T_{sk}$ decreases following atropine injection at $T_a$ = 12°C. This most likely represents a vasoconstrictor response to minimize heat loss.

Thermography has proven to be very effective for measuring surface skin temperatures, and thus, indirectly changes in cutaneous blood flow[5,6]. It is non-invasive and produces no extraneous stress on the animal. In theory, heat energy radiating from the skin will increase as blood flow to that area rises. The "furry" rat is ideally suited for this purpose because of its sparse fur and is potentially a valuable model for studying skin blood flow.

## CONCLUSIONS

Intramuscular injection of the atropine alkaloid results in a modest decrease in core temperature in a cold-stressed hypotrichotic rat model. This decrease cannot be entirely explained by a reduction in shivering nor by cutaneous vasodilation and, may be due to a decrease in non-shivering thermogenesis, and/or increased respiratory heat loss.

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