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# Depletion of brown fat norepinephrine content by acute cold exposure and adrenoceptor blockade

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**Abstract**

Experiments were conducted to characterize the effects of acute cold exposure, with and without adrenoceptor blockade, on intrascapular brown adipose tissue (IBAT) and adrenal catecholamine content in male Sprague–Dawley rats. Groups of animals with indwelling temperature transmitters were tested following treatment with saline, the alpha-adrenoceptor blocker phentolamine, the beta-adrenoceptor blocker propranolol, combined blockade with phentolamine plus propranolol, and the ganglionic blocker chlorisondamine. IBAT norepinephrine (NE) content was not affected in animals tested at 22°C, but was reduced in 4°C-exposed animals treated with phentolamine (−57%), phentolamine plus propranolol (−97%), and chlorisondamine (−42%). Adrenal NE and epinephrine (EPI) content were not altered by the treatments at 4°C or 22°C. None of the treatments affected the temperature of animals at 22°C, but significant hypothermia occurred at 4°C after chlorisondamine (−2.3±0.3°C) and the combination of phentolamine and propranolol (−1.5±0.4°C). These results suggest that cold exposure alone did not affect IBAT NE content, but when cold exposure was combined with adrenoceptor blockade, the sympathetic activation was sufficient to cause a reduction in IBAT NE content. In addition, alpha- and beta-adrenoceptor-mediated mechanisms contribute to the maintenance of core temperature. However, both alpha- and beta-receptor mechanisms had to be interrupted before a deficit in body temperature was detected. 

*Keywords:* Adrenal medulla; Alpha-receptors; Beta-receptors; Epinephrine; Thermoregulation

## 1. Introduction

The sympathetic nervous system is activated as a part of the homeostatic adjustments that are initiated in response to acute cold exposure (Landsberg et al., 1984; Maickel et al., 1967). Sympathetic neurons, via the release of norepinephrine (NE) and the consequent stimulation of alpha-adrenoceptors, promote heat conservation through constriction of the cutaneous vasculature and piloerection (Kent and Satinoff, 1990; Kent et al., 1991). NE stimulation of beta-adrenoceptors results in heat generation in brown adipose tissue (BAT) (Arch, 1989; Bukoweicki et al., 1980; Tsukazaki et al., 1995) and stimulation of the heart (Barney et al., 1980; Fregly et al., 1989; Sun et al., 1997). These actions of NE released from sympathetic neurons are augmented by the adrenal medullary catecholamines (Himms-Hagan, 1975). Adrenal NE and epinephrine (EPI), activate alpha- and beta-receptors accessible from the circulation, thereby contributing to both heat conservation and heat generation.

Despite the general acceptance that the sympathetic nervous system-mediated responses are important to thermoregulation during cold exposure (Hsieh and Carlson, 1957; Johnson, 1963; Leduc, 1961; Thomas and Palmiter, 1999), there is surprisingly little information that quantitates the extent to which body temperature may fall if they are blocked. Thus, there were two major objectives to the present study. The first objective was to determine if catecholamine content of the adrenal gland and intrascapular BAT (IBAT) could be used as indicators of the magnitude of the sympathetic activation during acute cold exposure. This objective was based on the previous observation in our laboratory that cold exposure depleted adrenal medullary NE stores (Vollmer et al., 1992) and the observation by others that IBAT NE content was attenuated during acute cold exposure (Brito et al., 1998; King et al., 1999). The second objective was to determine if pharmacologic blockade of the thermoregulatory actions of the catecholamines resulted in deficits in body temperature. The experi-

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ments were designed to utilize conscious, unrestrained animals with implanted temperature telemetry probes to eliminate temperature artifacts due to anesthetic agents or handling of animals.

## 2. Methods

### 2.1. General

Male Sprague–Dawley rats (Hilltop Labs, Scottsdale, PA) weighing 250–275g on delivery were housed in a room maintained at 22±1°C with a 12:12-h light–dark cycle with food and water available ad libitum. A 1-wk acclimatization period was allowed before surgery was conducted to implant temperature transmitters (Minimitter, Sunriver, OR).

Animals were anesthetized with pentobarbital sodium, 60 mg/kg, i.p., and temperature transmitters were implanted into the abdominal cavity via a midline incision. Following surgery, the animals were housed individually and a minimum of 5 days was allowed for recovery before experiments were performed. On the day before the experiment, animals were transferred to plastic observation boxes, which were placed on temperature receivers. Food and water were available at all times.

Ten groups of animals ($n=6–8$/group) were studied. Five of the groups were studied at the laboratory room temperature of 22°C, and five groups were exposed to a cold environment by transferring them to a walk-in cold room maintained at 4°C. The cold room is within the same laboratory and immediately adjacent to the 22°C observation area. Entry to the cold room is via a glass door that permits the lighting conditions to be equivalent for all animals. Five treatments were investigated, at 22°C and 4°C. The treatments were: saline (vehicle); phentolamine, 2 mg/kg, i.p.; propranolol, 3 mg/kg, i.p.; phentolamine, 2 mg/kg, i.p., plus propranolol, 3 mg/kg i.p.; and chlorisondamine, 3 mg/kg, i.p. Doses of propranolol and chlorisondamine were selected based on previous studies conducted in this laboratory and by other investigators in which it was demonstrated that sympathetic neuronal responses were more than 90% abolished for the time frame in which the current studies were conducted (Bush and Vollmer, 1983; Deitchman et al., 1980; Vollmer et al., 1988; Young et al., 1992). The dose of phentolamine, 2mg/kg, i.p., was shown, by other investigators, to cause cutaneous vasodilation in rats (Lin et al., 1979). In all groups, baseline temperatures were recorded for 2 h prior to treatment. After treatment, the animals were monitored for 4 h. During the 6-hr experimental period, temperature and locomotor activity data were recorded at 2-s intervals and averaged every 15 min via a computerized data acquisition system. At the completion of the experiment, IBAT and adrenal glands were removed and frozen (−70°C) for later analysis of catecholamines (Keller et al., 1976).

### 2.2. Analysis of IBAT and adrenal glands for determination of catecholamines

Adrenal glands and IBAT were homogenized in a solution of 0.1 N perchloric acid containing EDTA and sodium metabisulfite. The samples were centrifuged and the supernatant was stored at −70°C until assayed. Catecholamines were assayed using high performance liquid chromatography (HPLC) with electrochemical detection (Waters, Marlborough, MA) (Weicker et al., 1984). After a 1:100 dilution with perchloric acid, adrenal supernatants were directly injected into the HPLC system. Dihydroxybenzylamine (DHBA) was added to IBAT supernatants as an internal standard and the catecholamines were eluted from alumina. The recovery of catecholamines after extraction was between 65% and 70%. Catecholamines were separated on a 5 μm, 3.9×150 mm C-18 reverse-phase column (Waters). The mobile phase consisted of sodium acetate (50 mM), citric acid monohydrate (20 mM), sodium-1-octane-sulfonate (2 mM), di-*n*-butylamine (1.0 mM), disodium EDTA (0.1 mM), and methanol (4%). Catecholamines were oxidized during exposure to a glassy carbon electrode set at a potential of 0.6 V vs. Ag/AgCl. Data were acquired using ChromPerfect software (Justice Innovations, Mountain View, CA), and calculations were based on peak areas. The limit of quantitation for NE and EPI was 50 pg/ml.

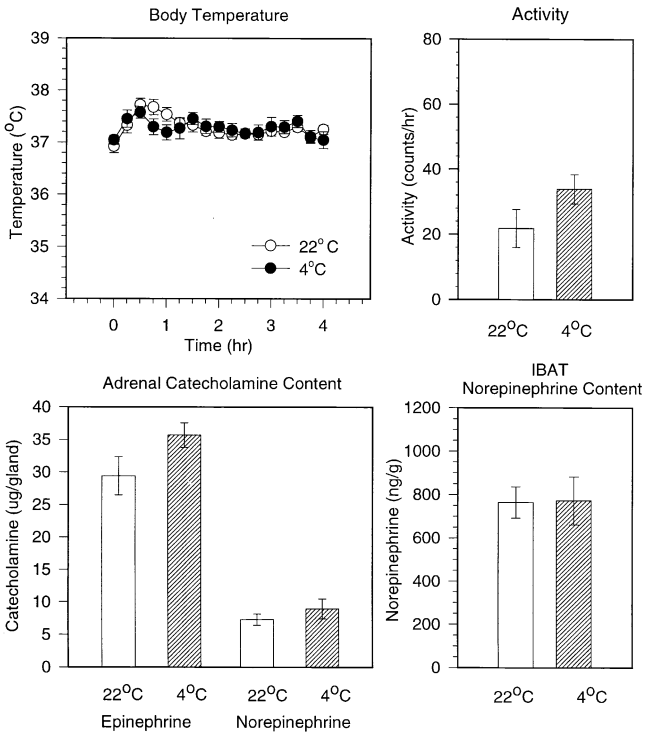


Fig. 1. Effect of saline, 1 ml/kg, i.p., on body temperature (top left panel), locomotor activity (top right panel), adrenal EPI and NE content (bottom left panel), and IBAT NE content (bottom right panel) in rats kept at 22°C ($n=7$) and 4°C ($n=7$).

### 2.3. Statistical analysis

Results are presented as mean ± S.E. The differences between multiple groups for individual parameters were assessed using one-way analysis of variance (ANOVA). If ANOVA revealed a statistically significant difference, post-hoc, pairwise comparisons of groups were done using Bonferoni $t$ test (SigmaStat, Jandel, San Rafael, CA). The differences in multiple measurements taken over a period of time were assessed using one-way ANOVA for repeated measures. Post-hoc contrasts between measurements taken at different time points were done using Bonferoni $t$ test. A statistically significant effect was accepted when $P < .05$.

## 3. Results

### 3.1. Baseline measurements

At the commencement of the experiments, the mean weight of the animals was 346 ± 4 g. During the first 2 h of the experimental protocol, half of the animals were kept at 22°C and the other half at 4°C. During this 2-h period, there was no difference in the body temperature of groups of animals maintained at 22°C and 4°C. The mean body temperature of the rats before treatment was 37.3 ± 0.1°C.

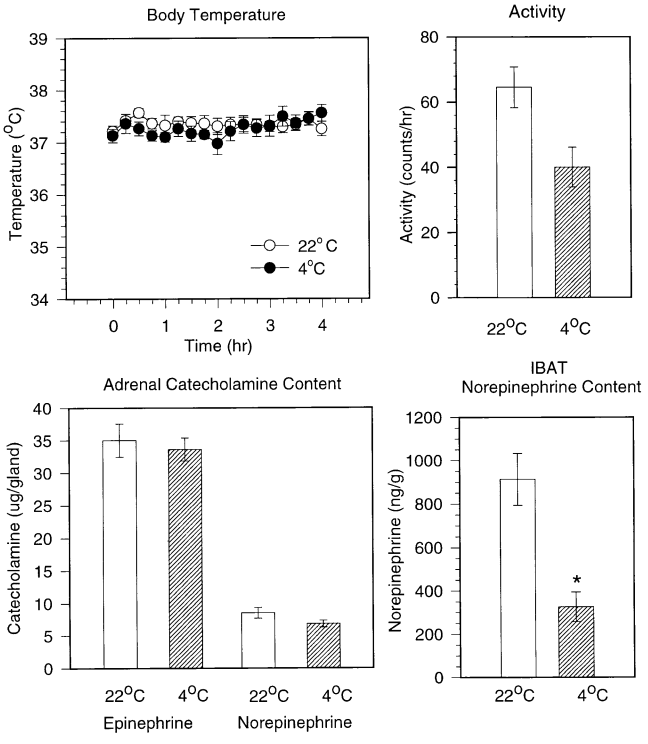


Fig. 2. Effect of phentolamine, 2 mg/kg, i.p., on body temperature (top left panel), locomotor activity (top right panel), adrenal EPI and NE content (bottom left panel), and IBAT NE content (bottom right panel) in rats kept at 22°C ($n = 7$) and 4°C ($n = 7$). An asterisk indicates a significant difference between groups, $P < .001$, $t$ test.

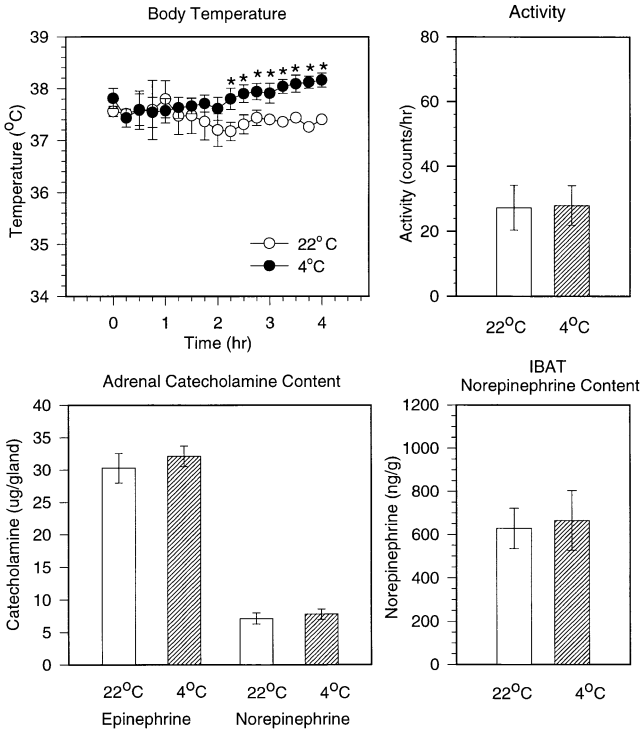


Fig. 3. Effect of propranolol, 3 mg/kg, i.p., on body temperature (top left panel), locomotor activity (top right panel), adrenal EPI and NE content (bottom left panel), and IBAT NE content (bottom right panel) in rats kept at 22°C ($n = 7$) and 4°C ($n = 7$). Top left panel — one-way ANOVA for repeated measures indicated that the groups are significantly different, $P < .001$. Asterisks indicate that there is a difference between the groups at a specific time point, Bonferoni $t$ test.

### 3.2. Effects of treatment with adrenergic-blocking drugs on body temperature, IBAT and adrenal NE content, and locomotor activity

The differences in IBAT and adrenal NE content and locomotor activity between groups of animals treated with adrenoceptor blockers and saline and kept at 22°C, were analyzed using a one-way ANOVA, and no significant differences were found among the treatment groups.

There was no difference in the temperature records for rats treated with saline and maintained either at 22°C or 4°C, Fig. 1. Also, there were no differences in IBAT NE content, adrenal gland NE and EPI, or locomotor activity.

The temperature records for animals treated with phentolamine under ambient and cold conditions were not different, Fig. 2. However, IBAT NE content was significantly decreased (−57%) in the cold-exposed animals. Phentolamine treatment did not influence adrenal NE and EPI or locomotor activity.

Treatment of animals with propranolol caused body temperature to rise in the group kept at 4°C compared to

the group at 22°C, and was significantly elevated after 2.5 h, Fig. 3. Propranolol treatment did not affect IBAT NE content, adrenal NE and EPI, and locomotor activity.

Cold-exposed animals that received combined phentolamine and propranolol treatment showed a significant decline in body temperature that was maximal approximately 45 min after administration, Fig. 4. Temperature recovered to pretreatment levels at the end of the 4-h observation period. Cold-exposed animals that received the combined treatment also showed a very substantial reduction in IBAT NE content ( − 97%). The phentolamine plus propranolol treatment did not affect IBAT NE content or core temperature in animals kept at 22°C. No differences were observed for adrenal NE and EPI and locomotor activity in the 4°C and 22°C groups.

The group of cold-exposed animals treated with chlorisondamine showed a significant decline in body temperature in contrast to animals treated with chlorisondamine and kept at 22°C, Fig. 5. The peak decline occurred approximately 1 h after administration and temperature recovered to pretreatment levels by the end of the experimental observation period. IBAT NE content was significantly less ( − 42%) in the cold-exposed, chlorisondamine-treated animals compared to the ambient chlorisondamine-treated animals. Also, we observed that the animals exposed to cold and treated with chlorisondamine shivered.

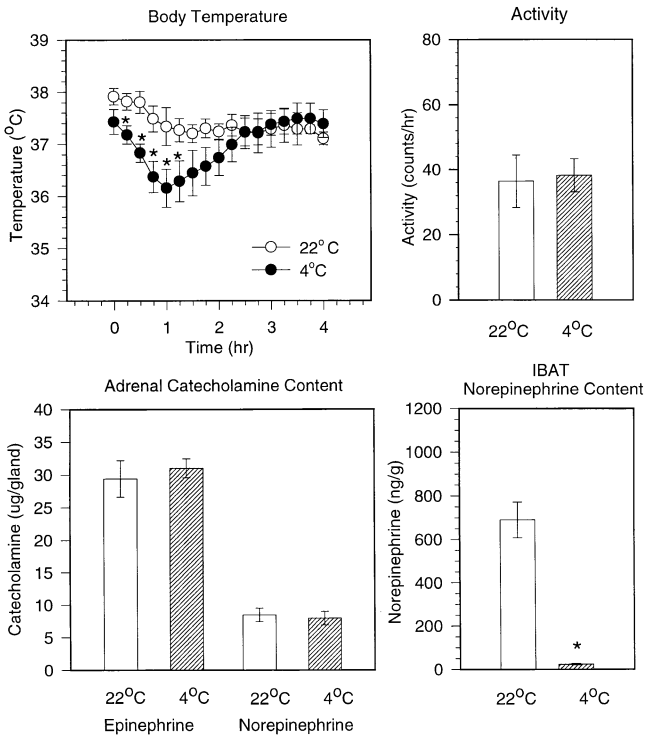


Fig. 4. Effect of phentolamine, 2 mg/kg, i.p., and propranolol, 3 mg/kg, i.p., on body temperature (top left panel), locomotor activity (top right panel), adrenal EPI and NE content (bottom left panel), and IBAT NE content (bottom right panel) in rats kept at 22°C ($n=7$) and 4°C ($n=7$). Top left panel – one-way ANOVA for repeated measures indicated that the groups are significantly different, $P < .001$. Asterisks indicate that there is a difference between the groups at a specific time point, Bonferoni $t$ test. Bottom right panel — an asterisk indicates a significant difference between groups, $P < .001$, $t$ test.

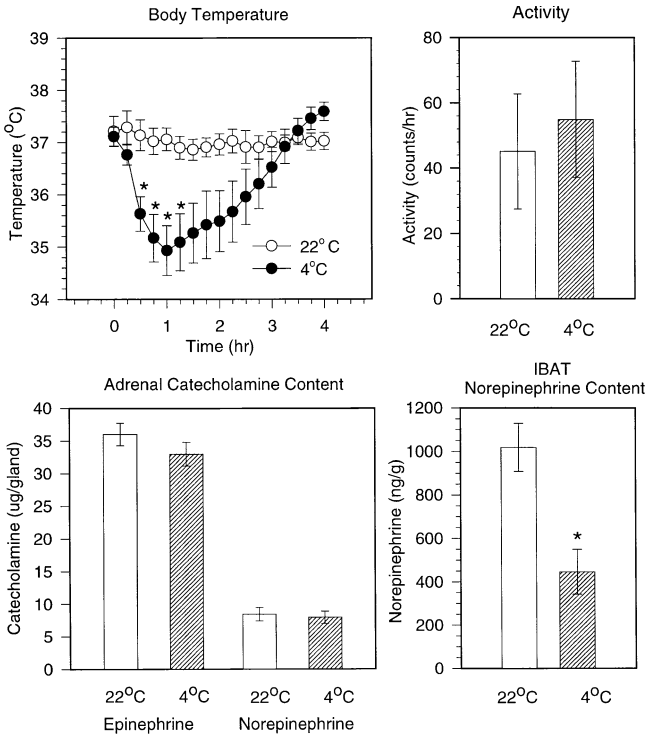


Fig. 5. Effect of chlorisondamine, 3 mg/kg, i.p., on body temperature (top left panel), locomotor activity (top right panel), adrenal EPI and NE content (bottom left panel), and IBAT NE content (bottom right panel) in rats kept at 22°C ($n=7$) and 4°C ($n=7$). Top left panel — one-way ANOVA for repeated measures indicated that the groups are significantly different, $P < .001$. Asterisks indicate that there is a difference between the groups at a specific time point, Bonferoni $t$ test. Bottom right panel — an asterisk indicates a significant difference between groups, $P < .001$, $t$ test.

## 4. Discussion

There are two major new findings of this study. First, alpha- and beta-adrenoceptor-mediated mechanisms contribute quantitatively to the maintenance of core temperature during acute cold exposure. However, due to the overlapping compensations, both alpha- and beta-receptor mechanisms had to be interrupted before a significant deficit in body temperature was detected. The second major finding of the study was that cold exposure alone did not affect IBAT NE content but when cold exposure was combined with adrenoceptor blockade, the sympathetic activation was sufficient to cause a reduction in IBAT NE content.

The experiments of this investigation assessed the impact of pharmacological blockade of adrenoceptors on the thermoregulatory function of conscious animals in response to acute cold exposure. Catecholamine content of IBAT and the adrenal gland was measured to determine if the neurotransmitter stores would be reduced proportionately to the

intensity of sympathetic nervous system activation. The major finding was that IBAT NE content but not adrenal content was decreased during acute cold exposure combined with adrenoceptor blockade. The relationship was consistent with the conclusion that IBAT NE content declined when sympathetic neural activity increased to compensate for blockade of adrenoceptor-mediated heat conservation or heat generation mechanisms.

Untreated animals exposed to cold (4°C) maintained body temperature at the same level as animals kept at the normal laboratory temperature of 22°C, and no change in IBAT NE content was measured. While it has been shown by other investigators that IBAT is neurally activated during acute cold exposure, as evidenced by increased blood flow to IBAT (Foster and Frydman, 1978), increased GDP binding to mitochondria of IBAT (Trayhurn et al., 1987), and increased IBAT NE turnover rate (McDonald et al., 1993; Young et al., 1982), we found that an increase in neural activity to IBAT was not sufficient to change its NE content. However, there are reports that cold exposure alone can cause depletion of IBAT NE (Brito et al., 1998; King et al., 1999). The disparity in experimental results is probably best explained by differences in the experimental design of each study, which include variability in the duration of cold exposure and the handling of animals during temperature measurements.

However, the ability to maintain NE content in the presence of increased sympathetic drive to IBAT appears to be limited because blockade of alpha-receptors with phentolamine in animals exposed to cold resulted in a 57% decline in IBAT NE. We interpret this finding to indicate that phentolamine interfered with alpha-receptor-mediated heat conserving mechanisms, piloerection and cutaneous vasoconstriction, resulting in an augmented activation of sympathetic outflow to IBAT. It appears that the activation of IBAT offset the effects of phentolamine because core temperature was maintained at normal levels. The prevention of piloerection by phentolamine was visually verified in these studies and the dose of phentolamine used was shown to produce cutaneous vasodilation by others (Lin et al., 1979). Phentolamine did not have an effect on IBAT innervation independent of cold exposure because IBAT NE content was not diminished in animals kept at 22°C.

When beta-receptors were blocked in addition to alpha-receptors, body core temperature significantly declined in cold-exposed animals. Thus, combined blockade of adrenoceptors presents a greater challenge to thermoregulation than if the alpha- and beta-receptors are independently blocked. This was evidenced by an intense activation of IBAT as noted by an almost total disappearance of NE from IBAT ( − 97%).

In rats, there is significant stimulation of heart rate and cardiac output in response to cold exposure (Sun et al., 1997), and the dose of propranolol used in our experiment is sufficient to block these cardiovascular responses mediated via $\beta_1$-receptors (Young et al., 1992). The extent to which this dose of propranolol would interrupt sympathetic activation of IBAT is uncertain because there is substantial evidence to suggest that the functional receptor in IBAT is the $\beta_3$-receptor subtype (Zhao et al., 1998). While propranolol is a potent blocker of the $\beta_1$-and $\beta_2$-receptors, higher doses are required to block $\beta_3$-receptor-mediated thermogenesis. In one report (Benzi et al., 1988), the dose of propranolol used in the present investigation was shown to block IBAT thermogenesis, but other reports suggest that higher doses are required (Shimizu and Saito, 1991). Also, it must be considered that the endogenous ligand, NE, has less affinity for the $\beta_3$-receptor than the $\beta_1$ or $\beta_2$ (Chaudhry et al., 1992; Granneman, 1990, 1992). Nevertheless beta-receptor blockade in conjunction with alpha-receptor block produced an almost total disappearance of NE from IBAT.

Interestingly, the body temperature of animals treated with propranolol while in the cold began to rise and became significantly higher than the animals kept at 22°C. One potential explanation for this unexpected finding is that propranolol may interfere with a $\beta_2$-receptor-mediated cutaneous vasodilatation that may have been present prior to propranolol administration (Carlisle and Stock, 1992).

We measured adrenal content of EPI and NE in these experiments as a potential indicator of adrenal activation, but no change in adrenal catecholamines was observed in any of the groups. The absence of any change in content does not rule out the activation of the adrenal since there might be an accelerated synthesis of catecholamines to compensate for the increased secretion. In fact, there is evidence that the adrenals are activated during cold exposure (Himms-Hagan, 1975; Leduc, 1961; Vollmer et al., 1992). In a previous study, we found that inhibition of catecholamine synthesis by prior treatment with $\alpha$-methyl-*p*-tyrosine resulted in a decreased adrenal NE content when animals were exposed to cold for 3 h (Vollmer et al., 1992). In the same study, we found that a very robust stimulation of the adrenal gland is required before content is measurably reduced. Prolonged cold exposure resulted in a selective depletion of adrenal medullary NE, indicating that the adrenal gland became more active as the duration of cold exposure increased (Vollmer et al., 1992). Another factor that differed from the present investigation was that in the previous study, the animals were shaved, thereby increasing the severity of the cold exposure.

The somatic nervous system contributes to thermoregulation through two major actions, shivering induced thermogenesis and through heat generated by increased locomotor activity. The temperature transmitters used in this study provide a record of locomotor activity, and no increase in voluntary motor activity was detected during cold exposure. We did not measure shivering-induced heat generation directly but visually observed shivering only in the chlorisondamine-treated group exposed to cold. This shivering occurred without an increase in locomotor activity. The chlorisondamine group also showed the greatest hypothermic effect during cold exposure. In addition, we found that the dose of chlorisondamine did not produce a complete blockade of ganglionic transmission because there was a significant depletion of IBAT NE content ( − 42%).

The present experiments demonstrate that the integrity of sympathetic input to thermoregulatory effector systems, which include the adrenals, BAT, cutaneous blood vessels, and piloerector smooth muscle, is required to maintain body temperature during cold exposure. Blockade of sympathetic neural influences, pharmacologically through the use of alpha- and beta-receptor blockers, produced a significant hypothermic response. Clearly, central nervous system centers that coordinate thermoregulatory responses attempted to compensate for the hypothermia by provoking more intense activation of sympathetic neurons, a fact that was demonstrated by the reduction in NE content of IBAT.

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