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Effects of Epinephrine on Thermoregulatory Behavior in Lean and Obese Zucker Rats in the Cold

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CARLISLE, H. J., P. U. DUBUC AND M. J. STOCK. *Effects of epinephrine on thermoregulatory behavior in lean and obese Zucker rats in the cold.* PHARMACOL BIOCHEM BEHAV 51(2/3) 255-261, 1995.—This series of experiments examined whether epinephrine (EPI) produces the same thermoregulatory effects in the cold that have been reported for norepinephrine and isoproterenol. Lean and obese Zucker rats were trained to press a lever to activate infrared heat lamps in a cold (−8°C) environment. Operant thermoregulatory behavior increased dose-dependently following EPI (0-100 μg/kg), but posttest colonic temperature (Tc) fell. Thermal balance calculations showed a substantial increase in net heat loss, more so in obese than lean animals. EPI is therefore thermolytic—i.e., disrupts thermal balance. A low dose (100 μg/kg) of the α-antagonist phentolamine produced a marked improvement in operant behavior, Tc, and thermal balance, whereas a comparable dose of the β-antagonist propranolol had no beneficial effect. Increasing the dose of phentolamine worsened the responses with respect to the 100-μg/kg dose. The selective α_1-antagonist prazosin ameliorated the decrease in Tc induced by EPI but had little effect on operant behavior or thermal balance; the selective α_2-antagonist yohimbine had no effect on any parameter compared to EPI alone. These results suggest that the paradoxical effects of EPI in the cold are mediated by α-adrenoceptors, but definitive identification of the subclass of receptor involved cannot be determined.

Epinephrine Propranolol Phentolamine Prazosin Yohimbine Temperature regulation Thermal balance Zucker rat

THE HYPOTHALAMIC-PITUITARY-ADRENAL axis has been reported to be defective at all levels in the obese (fa/fa) Zucker rat (1,16). Although not all work confirms the global nature of these defects (29,35), there is little doubt that adrenocortical function is impaired in the Zucker rat (36). Adrenomedullary function has received relatively little attention although decreased epinephrine (EPI) excretion has been noted in the obese (ob/ob) mouse (25). The sympathetic innervation of the adrenal appears to be intact in the obese rat because electrical stimulation of the hypothalamus elicits a comparable release of EPI in lean and obese animals (27). Resting plasma levels of EPI are reported to be increased (27) or not different (33) in obese compared to lean animals, whereas exaggerated postsynaptic responses have been noted in the obese rat (33). The catecholamines norepinephrine (NE) and EPI together mediate a variety of functions of the sympathetic nervous system (SNS) by interacting with α and β adrenoceptors in many tissues. Temperature regulation is one of these functions to which many tissues contribute in the control of heat production and heat loss. An impairment in energy balance contributes significantly to the development of obesity in genetically prone animals, such as the Zucker rat [see (18,21) for reviews]. This defect is generally believed to be due to a thermogenic defect involving the SNS activation of brown adipose tissue (BAT) (2,18,28,32). BAT is of unique importance in heat production, and is activated by the SNS via NE acting on β-adrenoceptors with a small contribution from α_1-adrenoceptors [see (18,32) for reviews]. More recent evidence indicates that BAT α_1-adrenoceptors increase with cold acclimation, and thus their density correlates with increased thermogenic capacity (30). NE is clearly implicated in mediating SNS thermogenic responses, but the contribution of EPI

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is less clear. EPI, a mixed α/β agonist, is a less potent thermogenic agonist than NE, but is still capable of activating thermogenic responses (19,34) and brown adipocyte respiration (3).

The overall importance of the catecholamines in mediating multiple aspects of thermogenesis in a cold environment is clear, yet paradoxical responses have been noted for both NE and isoproterenol. Both are potently thermogenic when tested at a neutral ambient temperature (Ta), but thermolytic (i.e., disrupt thermal balance) in the cold (5,6,8,37). A reduced or impaired metabolic rate has been reported for NE (38) and isoproterenol (7), whereas operant heat-seeking behavior increases for both, but is not sufficient to prevent a decrease in core temperature (5,37). The basis for the paradoxical effects of isoproterenol have been ascribed to β_2-adrenoceptors because these thermolytic effects are blocked by the selective β_2-antagonist ICI 118551 (8). Conversely, the decrease in metabolic rate produced by NE in the cold is inhibited by yohimbine (38), which suggests an α_2-adrenoceptor mechanism.

The purpose of the present study was to determine whether EPI has the same paradoxical effects that have been found for NE and isoproterenol. Having confirmed this, the specific receptor responsible for these effects was examined by using selective and nonselective adrenergic antagonists in conjunction with EPI. These studies were complicated by an apparent interaction between environmental temperature and some of the antagonists, and this became the subject of an another report (9). Lean and obese Zucker rats were used to determine whether any phenotype differences exist in the response to EPI and the antagonists. Such differences might be expected because the glycemic response to infusions of EPI in lean and obese rats is quite different (33). In addition, the density of α_1-adrenoceptors in BAT is significantly lower in obese animals (31), which implies a diminished thermogenic capacity (30). However, the density of α_1-adrenoceptors is increased in some brain areas of the obese Zucker rat, whereas the ratio of α_2/α_1 receptors is increased specifically in the ventromedial hypothalamic area (26). Thus, pharmaceutic agents that interact with α or β receptors either peripherally or centrally might be expected to have differential effects in obese compared to lean animals.

METHODS

Animals

Lean (Fa/?) and obese (fa/fa) female Zucker rats were obtained from the colony maintained at the University of California, Santa Barbara, when they were between 3 and 6 mo of age. The Santa Barbara colony has been derived from the Zucker colony of the University of California at Davis. The animals were maintained in pairs in plastic tubs on pine shavings, and fed Purina Chow (5001) and water ad lib. The colony room was maintained at 22°C with a relative humidity of 50% and a 12 L : 12 D cycle (lights on 0700 h); all tests were conducted during the light phase of the cycle.

Drugs

(-)-Epinephrine HCl was obtained from Parke-Davis (Morris Plains, NJ). The α-antagonist phentolamine HCl, β-antagonist (±)-propranolol HCl, α_1-antagonist prazosin HCl, and α_2-antagonist yohimbine HCl were obtained from Sigma (St. Louis, MO). All drugs except prazosin were dissolved in normal saline (0.9% NaCl), which also served as the control vehicle. Prazosin was dissolved in a 25% propylene glycol (PG) solution at a concentration of 1 mg/ml; saline was used for dilutions of this stock solution. The PG vehicle was tested as the control for prazosin. EPI, prazosin, and yohimbine were administered intraperitoneally (IP), whereas phentolamine and propranolol were given subcutaneously (SC). All injections were in a volume of 1 ml/kg. When two drugs were coadministered, the antagonist was given first and the agonist 10 min later to permit receptor occupancy by the antagonist before the agonist. EPI was tested previously for glycemic responses in rats at a dose of 200 μg/kg (20); the maximal dose tested here was 100 μg/kg. Doses for other drugs (specified subsequently) were based on a consideration of values in the literature (12,13,38) with the exception of phentolamine, usually given in milligrams per kilogram (22), but tested here at a dose of 100 μg/kg to correspond to the low dose of propranolol previously found to be effective (6). All drugs were given per kilogram of body mass because adipose tissue sequestration of drug was expected. Because adipose tissue stores constitute a large proportion of the mass of obese animals, this meant that the amount of drug received by obese animals was roughly double that received by lean animals.

Apparatus

The test apparatus allowed animals to obtain unlimited heat in a cold environment by pressing a lever to activate infrared lamps. A circular 22-cm diameter and 22-cm deep wire-mesh cage was equipped with a 3 × 4 cm Plexiglas lever, which protruded 5 cm into the cage 2 cm above the floor. Two 250-W red-bulb infrared lamps were mounted at each side of the cage at a 45° angle to the floor and focused on the rat at the lever. The power dissipated by the lamps was set to 300 W, which produced an irradiance of 180 mW/cm^2 as measured by an Eppley thermopile. The apparatus was placed in a 0.48-m^3 freezer maintained at 8 ± 2°C. A 25-W red incandescent lamp provided low-level background illumination. The heat lamps were activated by pressing the lever and remained on as long as the lever was held down. Equipment in an adjoining room provided a cumulative record of the pattern of responding as well as the number of lever presses and the cumulative duration of heat lamp activation.

Procedure

The animals were shaved closely with an Oster clipper the day before a test. The reason for shaving the animals was to prevent the sporadic performance that occurs as a result of piloerection when the fur is intact. The rats were trained to press the lever to activate the heat lamps and then given at least four additional trials 90 min in duration so that operant responding for heat and body temperature were stable for two consecutive tests. The standard test procedure was to allow 30 min of baseline responding to permit adaptation to the test conditions, and to obtain a measure of colonic temperature (Tc) maintained by the behavior in the absence of drug treatment. The animal was removed from the test apparatus after the 30-min baseline, and Tc was measured with a Physitemp BAT-12 meter (Clifton, NJ) and thermocouple probe inserted 7 cm. The drug(s) for that test was then injected, and the animal returned to the apparatus for an additional 60 min. Tc was again measured on removal from the test. The animals were tested once per week.

Protocols

Experiment 1 was a dose-response study of the effect of EPI on thermoregulatory behavior and thermal balance in the

cold in five lean and five obese female rats. Saline and EPI doses of 25, 50, 75, and 100 μg/kg were given IP. The order of drug treatments was counterbalanced across doses, but each lean-obese pair received the same dose on the same day. Body weight (±SEM) averaged 300 ± 9.1 g for the lean animals during these tests and 615 ± 26.7 g for the obese ones.

Experiment 2 examined whether the thermolytic effects of EPI noted in Experiment 1 were due to α- or β-adrenoceptors. The nonselective α-antagonist phentolamine and the nonselective β-antagonist propranolol were tested using eight lean body weight (BW = 301 ± 10.3 g) and eight obese (BW = 570 ± 22.4 g) female Zucker rats. The strategy was first to determine whether a relatively low dose of propranolol (100 μg/kg SC) was an effective antagonist to a standard EPI dose of 100 μg/kg (IP). Following this, the effect of a similar dose of phentolamine (SC) was tested against the standard dose of EPI. Additional doses of phentolamine of 0.5 and 1.0 mg/kg were tested against EPI, followed by phentolamine alone at a dose of 1 mg/kg. Finally, the effect of both propranolol and phentolamine (100 μg/kg each, given SC on contralateral sides) was tested against EPI (100 μg/kg IP).

Experiment 3 examined whether the selective α_1-antagonist prazosin and the selective α_2-antagonist yohimbine were effective antagonists to the standard EPI dose of 100 μg/kg. Prazosin doses of 0.1 and 0.5 mg/kg were tested, whereas the yohimbine dose was 0.5 mg/kg. The animals and procedures were the same as in Experiment 2.

Data Analysis

The primary data were the duration of heat lamp activation and the change in Tc resulting from the treatments. To evaluate how these variables interact using common units, several derived measures of thermal balance were calculated, as described previously (5). The change in heat storage (dS, kJ) is the product of the change in Tc (posttest − preinjection), body mass, and the specific heat of the body [assumed to be 3.47 J/g for lean animals and 3.1 J/g for the obese ones based on body composition data provided in (10)]. Heat influx (HI, kJ) is the amount of energy absorbed from the heat lamps. HI considers primarily the surface area of the animal exposed to the radiant energy, and the irradiance and duration of activation of the lamps. Net heat loss (NHL, kJ) is the amount of energy absorbed less the change in heat stored (HI − dS). Because the amount of heat obtained (s/heat per min) could be influenced by either the duration of a response (s heat/R) or the frequency of responding (R/min), these parameters were examined for consistent trends.

A two-way repeated-measures analysis of variance (24) was used to test the overall significance of the main variables (duration of heat lamp activation and posttest Tc) in Experiment 1, and analyses of variance were used in Experiments 2 and 3. Paired *t*-tests were used for all within-group specific comparisons to saline or agonist alone for the main variables and the derived measures of thermal balance; unpaired *t*-tests were used to compare lean and obese groups. All probabilities quoted are two-tailed.

RESULTS

Experiment 1

Figure 1 shows that both lean and obese groups worked to obtain more heat in a dose-dependent manner following EPI, but the increase in operant responding was not sufficient to balance heat loss and posttest Tc decreased. The lean and obese groups did not differ from each other with respect to operant responding for heat. Two-way repeated-measures analysis of variance showed a significant effect of drug treatment on operant responding [$F(4, 32) = 26.1, p < 0.01$], but insignificant group and interaction effects. For posttest Tc, the groups were different [$F(1, 8) = 22.7, p < 0.01$], as was the drug treatment [$F(4, 32) = 23.5, p < 0.01$] and the interaction [$F(4, 32) = 8.0, p < 0.01$]. Although posttest Tc decreased more in the obese than in the lean rats, the preinjection Tc was the same (39.4°C) in both groups.

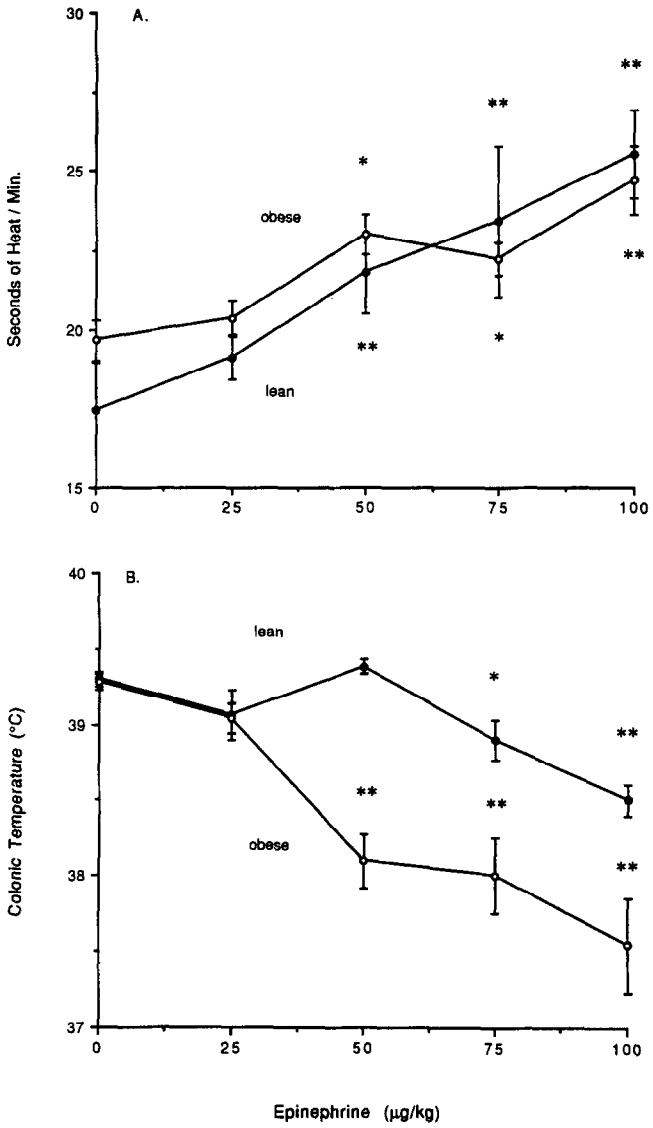

FIG. 1. The effect of epinephrine doses on (A) operant responding for heat and (B) posttest colonic temperature in lean and obese rats. Values are means ± SEM. *$p < 0.05$; **$p < 0.01$ compared to respective saline doses (paired *t*-test).

The increase in heat lamp activation noted in Fig. 1 was due primarily to an increase in the frequency of responding and not to an increase in response duration. The lean animals increased the frequency from 1.7 R/min (saline) to 2.6 R/min (at 100 μg/kg), an increase of 50% ($p < 0.05$). The obese increased frequency from 2.0 to 2.8 R/min, an increase of

37% ($p < 0.01$). Response duration averaged 10.8 s/R for the four EPI doses compared to 10.0 for saline for the lean animals, an increase of 8% [not significant (NS)]. Similarly, the obese averaged 9.0 s/R for the EPI doses compared to 9.7 for saline, a decrease of 7% (NS). Thus, the EPI-induced increase in the demand for heat was accomplished by more frequent responses.

Table 1 presents the effects of EPI on thermal balance for lean and obese groups. The change in Tc from preinjection to posttest influenced dS, and this was significant for the lean animals only at the 100-μg/kg dose, whereas the obese showed significant effects at doses of 50 and 100 μg/kg. The increase in the duration of heat lamp activation influenced HI, and both phenotypes showed significant increases compared to saline at doses ≥50 μg/kg. The effects of EPI on dS and HI were additive such that NHL became progressively greater as the EPI dose increased. Comparing obese to lean animals, the obese showed substantially greater values of HI at all doses including saline. The decrease in dS was also greater in obese than in lean animals at doses of ≥50 μg/kg. Taken together, these changes resulted in a substantial increase in NHL for the obese compared to the lean animals as the EPI dose increased. The net outcome was that the increase in operant responding for heat failed to prevent the loss of heat, and thus the overall effect of EPI was a dose-dependent thermolytic effect that was greater in obese than in lean animals.

Experiment 2

Figure 2 presents the effects of propranolol and phentolamine on EPI-induced changes in operant responding for heat and posttest Tc. The specific statistical comparisons shown are for EPI (100 μg/kg) alone. The obese animals obtained significantly more heat after propranolol + EPI than after EPI alone, whereas there was no difference in the lean animals. The decrease in posttest Tc after EPI was not affected by pretreatment with propranolol. Conversely, pretreatment with a 100-μg/kg dose of phentolamine substantially reduced the demand for heat in both lean and obese animals, and also resulted in a marked increase in posttest Tc with respect to EPI alone. The thermal balance data in Table 2 show that this dose of phentolamine produced a significant improvement in all parameters with respect to EPI, but the values were also different from saline except for dS in the lean group. Increasing the dose of phentolamine to 0.5 and 1 mg/kg worsened the responses with respect to the beneficial effect of the 100-μg/kg dose. Posttest Tc was not improved by the higher doses, especially in the obese group. The data in Table 2 indicate that increasing the dose of phentolamine tended to worsen the thermal balance. Pretreatment with both phentolamine (100 μg/kg) and propranolol (100 μg/kg) before EPI resulted in an increase in operant responding in the lean animals with respect to saline, and posttest Tc was no different from saline. Thus, HI and NHL were significantly elevated, whereas dS was not different from saline for the lean group. For the obese group,

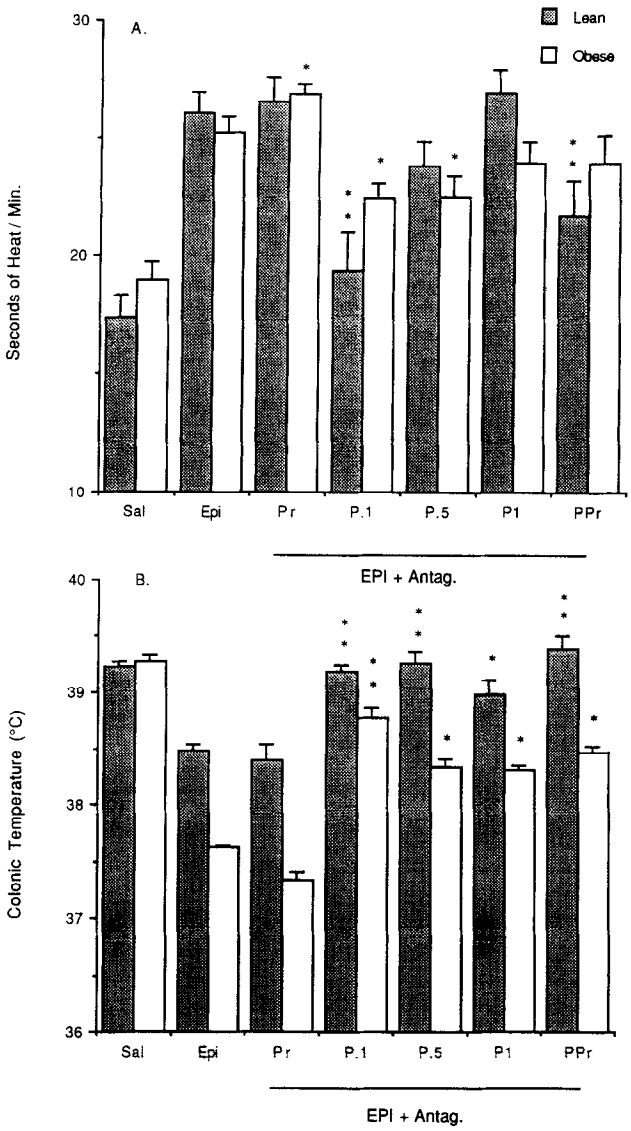

FIG. 2. The effect of epinephrine (Epi) alone (100 μg/kg) or with propranolol (Pr) 100 μg/kg; phentolamine (P) 0.1, 0.5, and 1 mg/kg; and epinephrine + propranolol + phentolamine (PPr), 100 μg/kg each, on (A) operant responding for heat and (B) posttest colonic temperature. *$p < 0.05$; **$p < 0.01$ compared to epinephrine alone (paired t-test).

TABLE 1

EFFECT OF EPINEPHRINE ON THERMAL BALANCE

Dose (μg/kg)	dS (kJ)	HI (kJ)	NHL (kJ)
Lean			
0	−0.1 ± 0.04	20.9 ± 1.76	21.0 ± 1.74
25	−0.4 ± 0.17	23.3 ± 1.12	23.7 ± 1.23
50	−0.2 ± 0.11	26.2 ± 1.65*	26.4 ± 1.56*
75	−0.4 ± 0.13	28.5 ± 3.05*	28.8 ± 3.11*
100	−0.9 ± 0.51*	30.5 ± 2.14*	31.5 ± 2.12*
Obese			
0	−0.1 ± 0.12	36.3 ± 1.13‡	36.4 ± 1.20‡
25	−0.5 ± 0.26	37.9 ± 0.62‡	38.4 ± 0.79‡
50	−2.6 ± 0.53†‡	43.0 ± 0.83†‡	45.6 ± 1.11†‡
75	−3.0 ± 0.45*‡	41.3 ± 0.66*‡	44.3 ± 0.61*‡
100	−3.7 ± 0.60*‡	44.7 ± 1.62*‡	48.4 ± 1.87*‡

Values are means ± SEM ($n = 5$). dS, Change in heat storage; HI, heat influx; NHL, net heat loss.

*$p < 0.01$ and †$p < 0.05$ compared to respective salines (paired t-test).

‡$p < 0.01$ obese compared to lean animals for same dose (unpaired t-test).

TABLE 2

EFFECT OF PROPRANOLOL AND PHENTOLAMINE ON EPINEPHRINE-INDUCED CHANGES IN THERMAL BALANCE

	Lean			Obese		
Treatment	dS (kJ)	HI (kJ)	NHL (kJ)	dS (kJ)	HI (kJ)	NHL (kJ)
Epi alone	−0.9 ± 0.09	30.5 ± 1.36	31.4 ± 1.36	−3.3 ± 0.41	43.6 ± 1.38	46.9 ± 1.58
Epi + Prop 0.1	−1.1 ± 0.11	31.8 ± 1.60	32.9 ± 1.66	−4.0 ± 0.77	47.4 ± 1.30*	51.4 ± 1.85†
Epi + Phen 0.1	−0.3 ± 0.11*	23.5 ± 2.21†	23.8 ± 2.25*	−1.8 ± 0.35*	39.5 ± 1.11†	41.3 ± 1.29†
Epi + Phen 0.5	−0.3 ± 0.10*	28.7 ± 1.47	28.9 ± 1.52	−1.9 ± 0.20*	39.6 ± 1.33†	41.5 ± 1.46*
Epi + Phen 1	−0.6 ± 0.19	32.2 ± 1.17	32.8 ± 1.29	−2.1 ± 0.24†	41.6 ± 1.44	43.6 ± 1.60
Epi + Phen + Prop	−0.2 ± 0.12*	26.7 ± 1.99†	26.9 ± 2.09†	−1.8 ± 0.50†	43.4 ± 2.74	45.2 ± 3.01
Phen 1 alone	−0.2 ± 0.08*	23.8 ± 1.16*	24.0 ± 1.15*	−0.8 ± 0.11*	34.3 ± 1.69*	35.1 ± 1.66*

Epinephrine (Epi) dose is per 100 μg/kg; propranolol (Prop) and phentolamine (Phen) doses are per mg/kg. Epi + Phen + Prop dose is per 100 μg/kg each. Values are means ± SEM (n = 8). See Table 1 for abbreviations.
*$p < 0.01$ and †$p < 0.05$, compared to Epi alone (paired t-test).

operant responding increased with respect to saline, but posttest Tc decreased and the values of thermal balance reflected no significant improvement over EPI alone, except for dS.

Figure 2 does not show phentolamine alone (1 mg/kg); it increased operant responding slightly but significantly ($p < 0.05$) to 19.7 s heat/min for the lean group with no change in posttest Tc compared to saline. Phentolamine alone produced a slight but significant ($p < 0.05$) decrease in posttest Tc (to 39.0°C) in the obese group with no significant effect on operant responding. These modest effects of phentolamine can be seen in Table 2: HI and NHL increased significantly with respect to saline in the lean group, but not in the obese group, whereas dS decreased slightly in the obese but not the lean group.

As in Experiment 1, preinjection Tc was not different between lean and obese groups (39.4°C for the lean and 39.5°C for the obese). The changes in heat influx noted in Fig. 2 and Table 2 could not be ascribed to systematic changes in either response duration or frequency. Both of these variables contributed to the increases and decreases in the demand for heat by both phenotypes with no obvious strategy for either.

Experiment 3

Figure 3 shows the effects of yohimbine and prazosin on EPI-induced operant responding for heat and posttest Tc. Neither antagonist produced a significant change in the duration of heat lamp activation with respect to EPI alone. Pretreatment with prazosin resulted in a significant increase in posttest Tc at the high dose, but not the low dose ($p = 0.08$) in the lean animals, and at both doses for the obese group. Preinjection Tc was 39.5°C for the lean group and 39.4°C for the obese animals. The slight increase in the demand for heat of the lean group after the high dose of prazosin plus EPI resulted in a significant increase in HI (Table 3), and the increase in posttest Tc after both doses of prazosin resulted in an improvement in dS for the obese group. The high demand for heat by both the lean and obese groups resulted in high values of HI, and thus no improvement in overall NHL. Yohimbine had no significant effect on any parameter in either lean or obese groups.

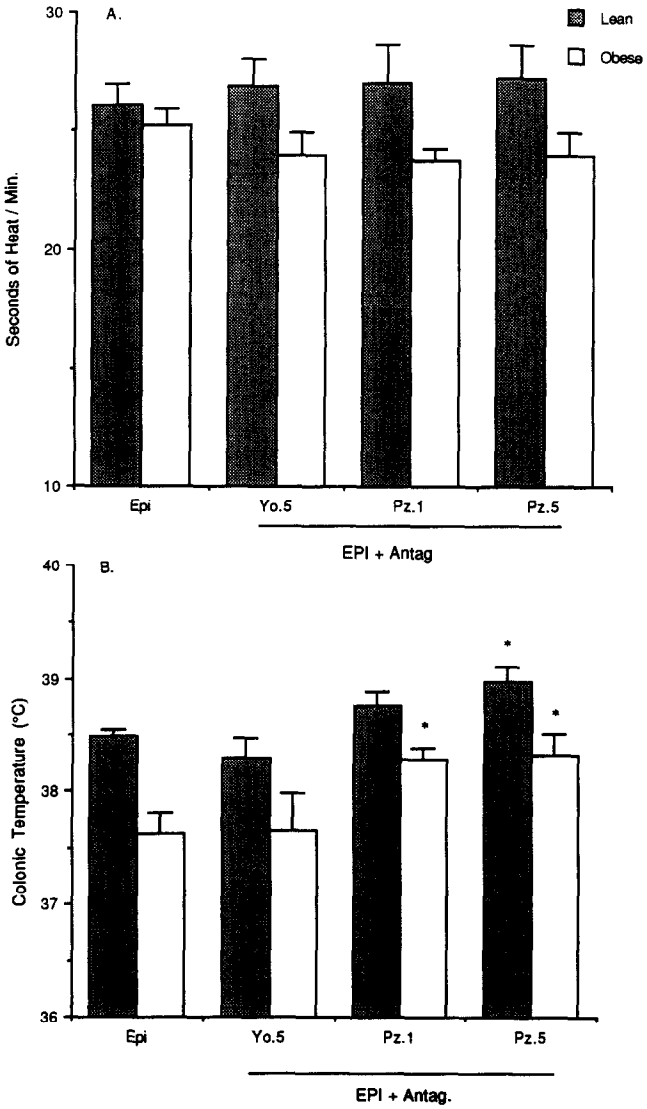

FIG. 3. The effect of epinephrine alone (100 μg/kg) or with yohimbine (Yo) 0.5 mg/kg or prazosin (Pz) 0.1 and 0.5 mg/kg on (A) operant responding for heat and (B) posttest colonic temperature. *$p < 0.05$ compared to epinephrine alone (paired t-test).

DISCUSSION

Epinephrine is similar to NE and isoproterenol in that it is thermogenic at a neutral Ta (19,34) but thermolytic in the cold. Operant responding for heat increased progressively

TABLE 3

EFFECT OF YOHIMBINE AND PRAZOSIN ON EPINEPHRINE-INDUCED CHANGES IN THERMAL BALANCE

Treatment	dS (kJ)	HI (kJ)	NHL (kJ)
Lean			
Epi alone	−0.9 ± 0.09	30.5 ± 1.36	31.4 ± 1.36
Epi + Yo 0.5	−1.3 ± 0.23	32.7 ± 1.43	34.0 ± 1.52
Epi + Praz 0.1	−0.7 ± 0.14	32.9 ± 1.98	33.6 ± 2.03
Epi + Praz 0.5	−0.6 ± 0.17	33.3 ± 1.81*	33.9 ± 1.90
Obese			
Epi alone	−3.3 ± 0.41	43.6 ± 1.38	46.9 ± 1.58
Epi + Yo 0.5	−3.1 ± 0.53	43.3 ± 2.0	46.4 ± 2.39
Epi + Praz 0.1	−1.9 ± 0.29*	42.1 ± 1.59	44.0 ± 1.61
Epi + Praz 0.5	−1.9 ± 0.17†	42.8 ± 2.25	44.7 ± 2.33

Epinephrine (Epi) dose is per 100 μg/kg. Yohimbine (Yo) and Prazosin (Praz) doses are per mg/kg. Values are means ± SEM (n = 8). See Table 1 for abbreviations.

*$p < 0.05$ and †$p < 0.01$, compared to Epi alone (paired t-test).

with an increase in the EPI dose from 25 to 100 μg/kg, yet posttest Tc decreased dose-dependently. A low dose of propranolol either had no effect (lean) or worsened (obese) the thermal balance responses to EPI. This same low dose of propranolol (100 μg/kg) was previously found to block paradoxical responses completely in the cold when the agonist was isoproterenol (5,6). The failure of propranolol to reverse the thermolytic effects of EPI suggests that these effects are not mediated by β-adrenoceptors when the agonist is a mixed α/β agonist. Conversely, pretreatment with a 100-μg/kg dose of phentolamine resulted in a substantial decrease in operant responding with an increase in posttest Tc, and a partial restoration of thermal balance toward control levels. This strongly suggests that the paradoxical effects of EPI are mediated by α-adrenoceptors.

Although the 100-μg/kg dose of phentolamine produced substantial improvement in performance and thermal balance, there was still a residual thermolytic effect of EPI. Increasing the dose of phentolamine might be expected to produce complete restoration to control levels, but all parameters worsened when the dose was increased to 0.5 and 1.0 mg/kg. The explanation for this outcome may be found in studies with phentolamine using doses of 5 and 10 mg/kg, where it produced a dose-dependent decrease in body temperature (23). This decrease was ascribed to an increase in heat loss at a Ta of 2°C as well as 20°C (22). Heat production increased in the cold, but not until body temperature had fallen. Similarly, given the opportunity to obtain warm (40°C) air in a cold (2°C) Ta, the animals worked for warm air, but not until body temperature had decreased (22). These observations suggest that phentolamine is thermolytic at doses of 5 and 10 mg/kg. In the present study, phentolamine alone was given in a dose of 1 mg/kg, which produced a slight but significant increase in operant responding for heat in lean animals, and a slight but significant decrease in Tc in the obese. These effects are modest but in the direction expected for the results based on the higher doses noted previously (22,23). Thus, it seems likely that phentolamine acts as an antagonist to EPI when the dose of phentolamine is 100 μg/kg, but increasing the dose counteracts this antagonist effect because of the thermolytic effects of phentolamine at high doses. This complication does not preclude the possibility that the paradoxical effects of EPI in the cold are due to an interaction with an α-adrenoceptor mechanism, because the 100-μg/kg dose was quite effective.

Yohimbine has been reported to be thermogenic when tested at a neutral Ta (13), but no significant interactions of yohimbine and EPI were noted in the present study. This may have been due to temperature-dependent effects of yohimbine in the cold acting independently of its interaction with EPI (9). In addition, yohimbine can block either pre- or postsynaptic α_2-receptors, but preferential antagonism of the presynaptic autoreceptor results in a significant outflow of NE (11). Administration of yohimbine results in an increase in plasma NE levels (13). This mechanism might account for the lack of interaction of EPI and yohimbine. Prazosin has been reported to block the increase in metabolic rate induced by cold exposure (12), but in the present study prazosin had no significant effect on operant responses to EPI, although it partially ameliorated the decrease in Tc. These effects contributed to an improvement in dS but not NHL because of the high demand for heat. Thus, α_1 antagonism partially rectifies the thermolytic effects of EPI, but temperature-dependent effects of prazosin alone may have complicated its interaction with EPI. This has been investigated in more detail in another series of experiments (9). Although the α_1 antagonist was more effective than the α_2 antagonist, a definitive identification of the α subclass mediating the paradoxical effects of EPI will require tests with more selective and less temperature-dependent antagonists.

Both lean and obese animals responded in the same general way to EPI and the antagonists, but some responses were exaggerated in the obese ones. The fall in posttest Tc after EPI was much greater in obese than in lean animals. Of course, had the EPI dose been calculated in terms of kilograms of body water or lean body mass, the difference between lean and obese animals would have been reduced. Nevertheless, the Tc responses of the obese ones were exaggerated in the presence of antagonists that also were given per kilogram of body mass. Thus, posttest Tc was lower in obese compared to lean animals after all treatments except saline. Propranolol plus EPI compromised thermal balance to a greater extent in obese than in lean animals, and the combination of EPI, propranolol, and phentolamine produced no benefit at all for obese animals compared to EPI alone, whereas the lean group showed significant improvement.

Prazosin reduced the fall in Tc induced by EPI in both obese and lean rats. The mechanism of this α-adrenoceptor effect of EPI might be vascular or an effect on BAT thermogenesis. Because any α inactivation of BAT thermogenesis should reduce metabolic rate, this mechanism seems unlikely. At the same time, however, blocking the α-mediated effects of EPI on peripheral blood flow (i.e., vasoconstriction) should increase heat loss, and this should also reduce thermal balance. Thus, the cause of the thermolytic effects of EPI remains an enigma, albeit an α-mediated one. Perhaps the expected vasoconstrictive effects of EPI may be complicated by exposure to cold (14,17). In addition, EPI and the antagonists have profound effects on cardiovascular and metabolic functioning (15), and these effects may have interacted with exposure to cold to influence some of the results.

Among the many phenotype differences that have been reported for lean and obese animals (21), an exaggerated thermolytic and toxic effect of the mixed β-agonist isoproterenol in the obese ones has been noted (4,5). It now appears that they also are more sensitive to the α-mediated thermolytic effects of EPI. This greater sensitivity may reflect in part the blunted thermogenic capacity of the obese rat which, unlike the lean rat, is less effective in counteracting the thermolytic effects of both isoproterenol and EPI.

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