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Paradoxical Effects of Exogenous Norepinephrine on Cold-Induced Thermogenesis in the Rat[1]

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ZYLAN, K. D. AND H. J. CARLISLE. *Paradoxical effects of exogenous norepinephrine on cold-induced thermogenesis in the rat.* PHARMACOL BIOCHEM BEHAV **39**(1) 21–24, 1991.—The effect of norepinephrine (NE, 250 μg/kg IP) on thermoregulatory behavior of rats in a cold environment was tested. Norepinephrine produced an increase in operant responding for heat reward but a decrease in core temperature. Since animals compensate behaviorally for alterations in autonomic thermoregulation, this suggested that NE might inhibit cold-induced thermogenesis, an effect contrary to the expected action of this agent. A second experiment showed that NE increased oxygen consumption when rats were tested at 25°C as expected, but decreased oxygen consumption when tested at 5°C. The beta-adrenoceptor antagonist propranolol decreased oxygen consumption both at 25 and at 5°C as expected. These results suggest that the thermogenic effect of NE is highly dependent on ambient temperature.

Norepinephrine Behavioral thermoregulation Oxygen consumption Thermogenesis Rat

NOREPINEPHRINE (NE) was first implicated in the initiation and maintenance of cold-induced nonshivering thermogenesis by Hsieh and colleagues who found that injection of exogenous NE increased oxygen consumption in curarized cold-acclimated rats (15) and was more effective than epinephrine in preventing decreases in oxygen consumption caused by ganglionic blockade (16). Since these early findings, much work has shown that NE is the primary stimulatory agent in cold-induced thermogenesis (4, 8, 31). This relationship is well established and peripheral injection of exogenous NE is often used as a test of nonshivering thermogenesis (8, 9, 14, 21–23, 25, 30, 31).

Although NE-mediated nonshivering thermogenesis is a major autonomic means of temperature regulation in the rat, behavioral thermoregulatory responses also contribute to the maintenance of body temperature in the cold (7,33). A complementary relationship exists between autonomic and behavioral thermoregulatory systems such that impairment of one typically results in increased utilization of the other (1, 29, 32, 33).

To examine the effects of pharmacological augmentation of nonshivering thermogenesis on behavioral thermoregulation, we tested the effects of NE on an operantly conditioned thermoregulatory response (Experiment 1). It was expected that animals injected with NE would leverpress less than controls due to enhanced autonomic heat production. The results of this first experiment were contrary to expectations since NE increased leverpressing for radiant heat reward in a cold environment. This suggested that NE was not stimulating, but perhaps inhibiting, metabolic heat production.

Previous research characterizing the thermogenic response to NE has been conducted at a neutral ambient temperature [e.g., (15, 16, 26)], whereas the present experiment was conducted in the cold. Since ambient temperature is known to significantly alter the effect of certain drugs (3,11) we tested the effects of NE on metabolism in both a neutral and a cold ambient temperature (Experiment 2).

EXPERIMENT 1

To examine the effects of altering nonshivering thermogenesis on behavioral thermoregulation, NE was injected into rats trained to leverpress for radiant heat reinforcement in a cold environment.

METHOD

Subjects

Twelve male Sprague-Dawley rats (290–350 g) were used as subjects. All animals were housed individually in wire mesh cages at 25°C, exposed to a 12:12 LD cycle and given free access to Purina laboratory chow and water.

Apparatus

The experimental chamber consisted of a cylindrical wire-mesh cage (23 × 25 cm) equipped with a Plexiglas lever (4 × 5

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TABLE 1

EFFECT OF NOREPINEPHRINE ON OPERANT RESPONDING FOR HEAT

	Saline	NE	*t*-Value*
Leverpresses	298.9 ± 32.1	475.8 ± 39.1	3.94 ($p<0.01$)
Heat Intake†	6.6 ± 0.5	9.6 ± 0.6	4.25 ($p<0.01$)
Change T_c‡	0.4 ± 0.2	−1.2 ± 0.4	3.62 ($p<0.01$)

Note: All values are means ± standard error.

*t-value obtained from paired t-tests ($df=11$).

†Heat Intake = s heat/min.

‡Change T_c = core temperature after 60-min session minus core temperature before session.

cm). Two red-bulb infrared lamps were positioned laterally at 45° to the lever and adjusted to provide an irradiance of 180 mW/cm^2. Each leverpress response turned the lamps on for 2 s. Responses made during the time the lamps were on did not prolong the reward duration. The entire experimental chamber was housed in a Kelvinator freezer set at -8 ± 2°C.

Procedure

The trunk and limbs of each rat were shaved the day before each session to facilitate learning and performance (5). All rats were given at least five training sessions prior to the first experimental session. On the test day rats were injected intraperitoneally (IP) with either 250 μg/kg norepinephrine bitartrate (Levophed, Winthrop-Breon; calculated as the base) or an equal volume (1 ml/kg) of isotonic saline and placed in the chamber for 60 min. Number of leverpresses and reinforcements were recorded on an electronic counter located in an adjacent room. Colonic temperature was measured with a Sensortek (Model BAT-12) thermocouple inserted 6 cm into the rectum before and after the session. Each animal received two test sessions in a counterbalanced order, one with NE and one with saline. All training and test sessions were conducted during the light phase of the LD cycle. Test sessions were separated by one week. Data were analyzed by paired t-tests.

RESULTS

Contrary to expectation, NE did not decrease leverpressing for radiant heat reinforcement, but rather significantly increased behavioral responding as shown in Table 1. Consequently, test animals received more heat during the NE tests than during the saline tests ($p<0.01$). Despite increased behavioral heat intake, animals injected with NE showed a significant decrease in core temperature over the 60-min session ($p<0.01$).

EXPERIMENT 2

The results of the first experiment suggest that NE did not increase metabolic heat production, but perhaps impaired it since peripheral administration of NE resulted in increased exogenous heat intake and a fall in core temperature. Although the animals in Experiment 1 were exposed to an ambient temperature of −8°C, leverpressing activated the infrared lamps which warmed the animal and surrounding area. Thus the prevailing ambient temperature fluctuated depending on the rate of leverpressing. An ambient temperature of 5°C was selected for Experiment 2 because it is cold and not as a replication of the conditions of Experiment 1. This experiment was conducted to examine the effect of a cold ambient temperature on NE-induced calorigenesis. Propranolol, an inhibitor of cold-induced thermogenesis (2,18), was also tested for comparison.

TABLE 2

BASELINE OXYGEN CONSUMPTION (ml/min)

	25°C	5°C
Saline	7.47 (±0.46)	21.18 (±0.71)
NE	6.89 (±0.39)	20.69 (±0.66)
Propranolol	8.30 (±0.48)	21.68 (±0.69)

METHOD

Subjects

Thirty-six male Sprague-Dawley rats (285–450 g) were used as subjects. The rats were maintained in the same environment as those in Experiment 1.

Apparatus

The experimental chamber was a 4-liter spherical plastic container through which air was drawn at a flow rate of 3–4 liters per minute. Oxygen consumption was measured by the open circuit method with a Beckman Oxygen Analyzer (Model OM-11) located in an adjacent room. Measurements were recorded on a strip-chart recorder (Microscribe 4500, Houston Instruments) and were corrected to standard temperature and pressure (0°C, 760 mmHg, dry). The experimental chamber was housed in a thermostatically controlled room set at 25 ± 2°C for the neutral condition and 5 ± 2°C for the cold condition.

Procedure

Rats were randomly assigned to one of three groups: NE, propranolol or saline. All animals were shaved prior to each test session. All animals were adapted to the chamber prior to the first test session. Each animal was given two 90-min test sessions, one at 25°C (neutral) and one at 5°C (cold). Order of exposure to the test temperatures was counterbalanced.

All animals were tested individually. Each rat was given 30 min of baseline testing. Following this baseline, the animal was removed from the chamber and injected with either 250 μg/kg of NE, 10 mg/kg of propranolol hydrochloride (Sigma; calculated as the salt) or an equal volume of saline (1 ml/kg), all given IP. After the injection, the rat was replaced in the chamber and oxygen consumption was measured for an additional 60 min. Rectal temperature was measured before (prior to baseline) and after the session. All tests were conducted during the light phase of the LD cycle. Oxygen consumption data were analyzed as difference scores (test minus the last 15 min of baseline) to correct for differences in basal metabolic rate. The first 15 min of baseline testing were not used in the analysis to avoid inclusion of effects due to high activity levels. Two-way analyses of variance were conducted on the metabolic differences scores of each 15-min interval to assess time-course effects. When warranted, post hoc tests were conducted using Fischer's LSD test (20).

RESULTS

Baseline oxygen consumption values prior to drug injection

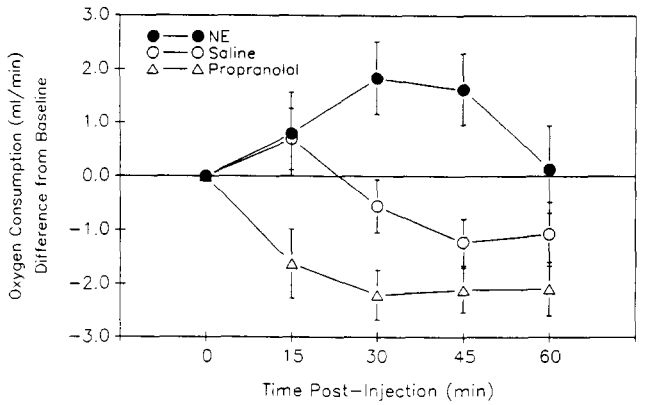

FIG. 1. Effects of norepinephrine and propranolol on oxygen consumption at 25°C.

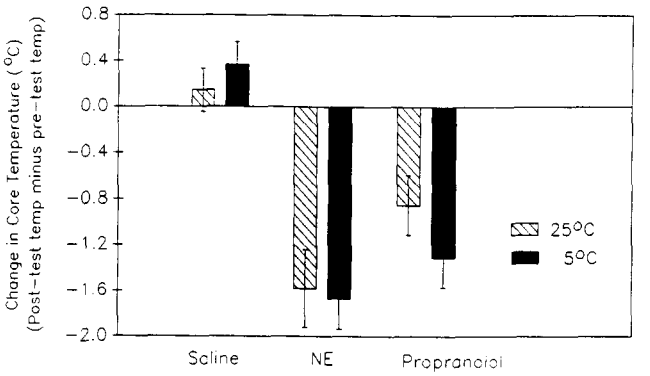

FIG. 3. Effects of norepinephrine and propranolol on core temperature at 25°C and 5°C.

are shown in Table 2. Rats consumed significantly more oxygen at 5°C than at 25°C ($p<0.001$) during the baseline condition, irrespective of assignment to a drug group. Thus exposure to the cold environment was sufficient to demonstrate cold-induced thermogenesis. Figures 1 and 2 illustrate the effects of the drugs at 25°C and 5°C, respectively. A two-way analysis of variance revealed a significant drug effect, $F(35,2)=20.10$, $p<0.001$, and ambient temperature effect, $F(1,33)=66.63$, $p<0.001$, as well as an interaction between these two variables, $F(2,33)=16.39$, $p<0.001$, at 15 min postinjection. During peak effects, NE significantly increased oxygen consumption at 25°C (Fig. 1) ($p<0.05$), but decreased oxygen consumption at 5°C (Fig. 2) ($p<0.01$). Propranolol decreased oxygen consumption at both ambient temperatures, although to a significantly greater degree at 5°C than at 25°C ($p<0.01$). There was no change in oxygen consumption from baseline for saline-treated animals at either ambient temperature.

At thirty min postinjection, a significant drug effect, $F(2,33)=6.07$, $p<0.01$, ambient temperature effect, $F(1,33)=30.49$, $p<0.001$, and interaction, $F(2,33)=17.47$, $p<0.001$, persisted. Peak drug effects (for both NE and propranolol) were observed at 15 min postinjection when rats were tested at 25°C. By 45 min postinjection there was no effect of NE or propranolol at 5°C; however, at 25°C the oxygen consumption of NE-injected rats remained higher than propranolol-injected rats ($p<0.05$). At 60 min postinjection there were no significant effects of NE or propranolol on oxygen consumption at either ambient temperature.

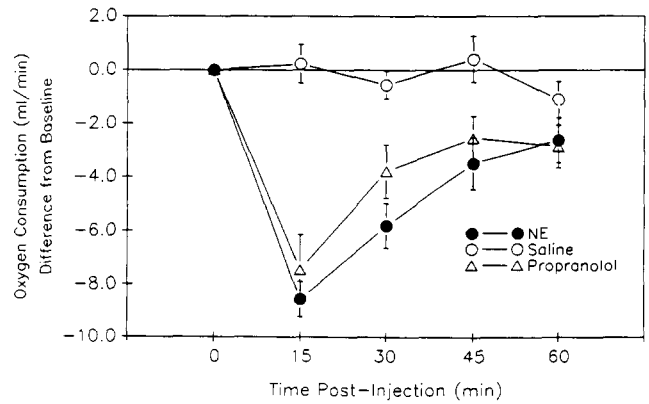

FIG. 2. Effects of norepinephrine and propranolol on oxygen consumption at 5°C.

The effects of NE and propranolol on the change in core temperature over the 60-min session are shown in Fig. 3. Both NE and propranolol decreased core temperature at both ambient temperatures ($p<0.01$). Thus, despite the fact that NE slightly increased oxygen consumption at 25°C, core temperature fell. Saliva spreading and sprawling were observed during this condition. These behaviors increase heat loss (13) and it is possible that NE produced an initial hyperthermic effect which initiated the behavioral adjustments that ultimately resulted in a net decrease in core temperature.

DISCUSSION

The results of the present experiments suggest that NE produces a paradoxical blockade of thermogenesis in a cold ambient temperature. Peripheral administration of NE decreases oxygen consumption at 5°C, a temperature at which cold-induced thermogenesis should be activated (18). It is, therefore, not surprising that animals injected with NE work to obtain more exogenous heat than controls when exposed to a cold temperature. This compensatory response is consistent with previous reports of the complementary relationship between autono mic and behavioral thermoregulation (1, 7, 29, 32, 33).

Since cold-induced thermogenesis is mediated by β-adrenoceptors (4,31), the finding that propranolol, a β-adrenoceptor antagonist (12), inhibited the metabolic response to cold is consistent with previous studies (2). However, since NE, a mixed α- as well as β-adrenoceptor agonist (9) is known to exert a potent calorigenic effect on brown adipose tissue (BAT) (31), the primary site of metabolic heat production in the cold (10), the finding that NE inhibits the metabolic response to cold is difficult to interpret.

One possible explanation is that acute cold exposure involves different receptor mechanisms (α or β) than chronic moderate exposure. A second is that cold exposure stimulates the endogenous release of NE, which may activate a negative feedback mechanism when combined with exogenous NE. This mechanism might involve a presynaptic autoreceptor that decreases production and release of endogenous stores of NE (37).

Alternatively, the inhibitory effects of NE may be due to the nonthermogenic actions of NE (e.g., cardiovascular effects). Since NE exerts widespread effects in many tissues of the body (12), BAT representing just one of these sites, it is possible that one or more effectors may indirectly alter metabolic heat production. Shibata, Nunomura and Nagasaka (35), in a study of

baroreceptor function, noted a similar decrease in oxygen consumption following NE infusion in animals tested at 13°C. These authors suggested that the pressor effects of NE stimulate a baroreflex which inhibits both shivering and nonshivering thermogenesis in the cold (35). Although Shibata et al. found that sinoaortic baroreceptor denervation abolished this effect (34), supporting their hypothesis, it is not apparent why the pressor effect of NE would stimulate the baroreflex only in the cold and not at higher temperatures. Furthermore, since the denervation procedure required the severing of the cervical sympathetic nerves which are known to supply BAT (27), this effect might be due to the decrease of sympathetic outflow to BAT.

Additional non-BAT mechanisms for the paradoxical effects noted here might involve an impairment in cold-induced glucose utilization (36) and perhaps glycogenolysis. Shivering thermogenesis can also be impaired by NE infusion (24). Finally, NE might influence heat loss via its potent effects on peripheral vasculature and blood flow (17). Which of these mechanisms are important in mediating the combined effect of exogenous NE and acute cold exposure await further study. It should also be noted that a 1963 study reported a rapid decrease in oxygen consumption and core temperature following administration of 500 μg/kg of NE at a cold ambient temperature (2°C) (19). Although the work of Hsieh and colleagues (15,16) had been published at the time of this report, the role of NE in cold-induced thermogenesis was not fully appreciated.

In summary, norepinephrine results in a decrease in oxygen consumption in a cold environment and a decreased core temperature resulting in increased behavioral responding for external heat. The mechanisms of these effects are not clear but research currently in progress may elucidate some of the alternatives.

REFERENCES

1. Adair, E. R. Autonomic thermoregulation in the squirrel monkey when behavioral regulation is limited. J. Appl. Physiol. 40:694–700; 1976.
2. Banet, M.; Hensel, H. The control of shivering and nonshivering thermogenesis in the rat. J. Physiol. 269:669–676; 1977.
3. Baumann, I. R.; Bligh, J. The influence of ambient temperature on drug-induced disturbances of body temperature. In: Lomax, P.; Schonbaum, E.; Jacobs, J., eds. Temperature regulation and drug action. Basel: Karger; 1975:241–251.
4. Bukowiecki, L. J. Regulation of energy expenditure in brown adipose tissue. Int. J. Obes. 9(Suppl. 2):31–42; 1985.
5. Carlisle, H. J. Initiation of behavioral responding in a cold environment. Physiol. Behav. 3:827–830; 1968.
6. Carlisle, H. J. Peripheral thermal stimulation and thermoregulatory behavior. J. Comp. Physiol. Psychol. 66:507–510; 1968.
7. Corbit, J. D. Behavioral regulation of body temperature. In: Hardy, J. D.; Gagge, A. P.; Stolwijk, J. A. J., eds. Physiological and behavioral temperature regulation. Springfield, IL: Charles C. Thomas; 1970:777–801.
8. Depocas, F.; Behrens, W. A.; Foster, D. O. Noradrenaline-induced calorigenesis in warm- and cold-acclimated rats: The interrelation of dose of noradrenaline, its concentration in arterial plasma, and calorigenic response. Can. J. Physiol. Pharmacol. 56:168–174; 1978.
9. Foster, D. O. Participation of alpha-adrenoreceptors in brown adipose tissue thermogenesis in vivo. Int. J. Obes. Suppl. 9:25–29; 1985.
10. Foster, D. O.; Frydman, M. L. Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. Can. J. Physiol. Pharmacol. 56:110–122; 1978.
11. Fuhrman, G. J.; Fuhrman, F. A. Effects of temperature on the action of drugs. Annu. Rev. Pharmacol. 1:65–78; 1961.
12. Gilman, A.; Goodman, L. L.; Rall, T. W.; Murad, F. Goodman and Gilman's The pharmacological basis of therapeutics, 7th ed. New York: Macmillan; 1985:145–214.
13. Hainsworth, F. R. Saliva-spreading, activity, and body temperature regulation in the rat. Am. J. Physiol. 212:1288–1292; 1967.
14. Hayashi, M.; Nagasaka, T. Suppression of norepinephrine-induced thermogenesis in brown adipose tissue by fasting. Am. J. Physiol. 245:E582–E586; 1983.
15. Hsieh, A. C. L.; Carlson, L. D. Role of adrenaline and noradrenaline in chemical regulation of heat production. Am. J. Physiol. 190: 247–251; 1957.
16. Hsieh, A. C. L.; Carlson, L. D.; Gray, G. Role of the sympathetic nervous system in the control of chemical regulation of heat production. Am. J. Physiol. 190:247–251; 1957.
17. Immink, W. F. G. A.; Beijer, H. J. M.; Charbon, G. A. α- and β-receptor blockade of isoproterenol- and norepinephrine-induced effects on regional blood flow and blood flow acceleration. Eur. J. Pharmacol. 50:159–173; 1978.
18. Jansky, L. Non-shivering thermogenesis and its thermoregulatory significance. Biol. Rev. 48:85–132; 1973.
19. Johnson, G. E.; Sellers, E. A.; Schonbaum, E. Interrelationship of temperature on the action of drugs. Fed. Proc. 22:745–747; 1963.
20. Kirk, R. E. Experimental design: Procedures for the behavioral sciences, 2nd ed. Monterey, CA: Brooks/Cole; 1982:114–115.
21. LeBlanc, J.; Pouliot, M. Importance of noradrenaline in cold adaptation. Am. J. Physiol. 207:853–856; 1964.
22. LeBlanc, J.; Villemaire, A. Thyroxine and noradrenaline on noradrenaline sensitivity, cold resistance, and brown fat. Am. J. Physiol. 218:1742–1745; 1970.
23. Levin, B. A.; Sullivan, A. C. Dietary obesity and neonatal sympathectomy. II. Thermoregulation and brown adipose metabolism. Am. J. Physiol. 247:R988–R994; 1984.
24. Marshall, H. W.; Stoner, H. B. Catecholaminergic α-receptors and shivering in the rat. J. Physiol. 282:27–34; 1979.
25. Maxwell, G. M.; Nobbs, S.; Bates, D. J. Diet-induced thermogenesis in cafeteria-fed rats: A myth? Am. J. Physiol. 253:E264–E270; 1987.
26. Moriya, K.; LeBlanc, J.; Arnold, J. Effects of exercise and intermittent cold exposure on shivering and nonshivering thermogenesis in rats. Jpn. J. Physiol. 37:715–727; 1987.
27. Nechad, M. Structure and development of brown adipose tissue. In: Trayhurn, P.; Nicholls, D. G., eds. Brown adipose tissue. London: Edward Arnold; 1986:1–30.
28. Refinetti, K. Z.; Carlisle, H. J. Effects of norepinephrine and propranolol on behavioral and thermoregulatory responses in the rat. FASEB J. 2:A1425; 1988.
29. Refinetti, R.; Carlisle, H. J. Complementary nature of heat production and heat intake during behavioral thermoregulation in the rat. Behav. Neural Biol. 46:64–70; 1986.
30. Rothwell, N. J.; Stock, M. J. Similarities between cold- and diet-induced thermoregulation and brown adipose metabolism. Am. J. Physiol. 247:R988–R994; 1984.
31. Rothwell, N. J.; Stock, M. J. Brown adipose tissue. Recent Adv. Physiol. 10:349–384; 1984.
32. Satinoff, E. Behavioral thermoregulation in response to local cooling of the rat brain. Am. J. Physiol. 206:1389–1394; 1964.
33. Schmidt, I. Interaction of behavioral and autonomic thermoregulation. In: Hales, J. R. S., ed. Thermal physiology. New York: Raven Press; 1984:309–318.
34. Shibata, H. Baroreflex suppression of nonshivering thermogenesis in rats. Jpn. J. Physiol. 32:937–944; 1987.
35. Shibata, H.; Nunomura, T.; Nagasaka, T. Inhibition of shivering as a cause of metabolic suppression with norepinephrine in warm- and cold-acclimated rat. Jpn. J. Physiol. 32:519–527; 1982.
36. Shibata, H.; Perusse, F.; Vallerand, A.; Bukowiecki, J. Cold exposure reverses inhibitory effects of fasting on peripheral glucose uptake in rats. Am. J. Physiol. 257:R96–R101; 1989.
37. Starke, K. Presynaptic autoregulation: Does it play a role? News Physiol. Sci. 4:1–4; 1989.