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Effects of α_1-Adrenoceptor and Ca^{2+} Channel Inhibition on Norepinephrine-Induced Thermoregulatory Behavior in the Cold

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CARLISLE, H. J., AND M. J. STOCK. *Effects of α_1-adrenoceptor and Ca^{2+}-channel inhibition on norepinephrine-induced thermoregulatory behavior in the cold.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 185–189, 1997.—This experiment examined whether paradoxical temperature-dependent effects of norepinephrine (NE) can be blocked by the α_1-adrenoceptor antagonist WB 4101 (WB) and the Ca^{2+}-channel blocker nifedipine. An operant lever-pressing task was used to measure the demand for heat in a cold environment. As noted previously, NE alone (250 μg/kg) produced a substantial and significant increase in the demand for heat, and yet post-test colonic temperature (Tc) fell. When tested alone, WB and nifedipine also increased the demand for heat, but this was sufficient to maintain Tc. When combined with NE, WB and nifedipine reduced the demand for heat and the fall in Tc such that there were no differences between the effects of the blockers given alone or with NE. These results indicate that paradoxical thermoregulatory effects of NE in the cold can be antagonized effectively by either an α_1-adrenoceptor antagonist or a Ca^{2+}-channel blocker. © 1997 Elsevier Science Inc.

WB 4101 Nifedipine Norepinephrine Temperature regulation Behavior Rat

THE sympathetic nervous system is of crucial importance in the regulation of many diverse aspects of energy and thermal balance (1,13). The adrenergic agonist norepinephrine (NE) is the primary transmitter mediating many of these diverse metabolic and vascular responses to cold stress. NE is a potent thermogenic agent when tested at a thermoneutral ambient temperature (Ta) but, unexpectedly, it disrupts thermal balance (i.e., has thermolytic effects) in the cold (25,26) such that colonic temperature decreases with respect to saline treatment and the behavioral demand for exogenous heat increases. These differential responses as a function of Ta have been termed paradoxical. The basis for the paradoxical effects of NE are not clear, but similar results have been reported for the non-selective β-agonist isoproterenol (ISO) (4,6) and the adrenal hormone epinephrine (EPI) (3). The evidence thus far suggests an α-adrenoceptor involvement in mediating the paradoxical effects of NE and EPI (3,8). The non-selective α-antagonist phentolamine (100 μg/kg) was more effective than the β-antagonist propranolol (100 μ/kg) in blocking the effects of EPI in the cold, but this effect diminished as the dose of phentolamine increased (3). Phentolamine clearly has hypothermic effects attributable to a decrease in metabolic rate in the cold and vasodilatation at a neutral Ta when the dose is on the order of 5–10 mg/kg (14–16). This suggests that dose is a critical variable because a dose of 100 μg/kg can partially block catecholamine-induced thermolytic effects (3) whereas a 5–10 mg/kg dose produces independent thermolytic responses.

Szreder and colleagues have tested a number of selective α_1-antagonists and reported protective effects on pyrogen-induced fever (20,22,23), an inhibition of NE-induced thermogenesis at a neutral Ta (11), and inhibition of metabolism and increased heat loss in the cold (11,21,23). Similar results were obtained with α_2-agonists (23), and this indicates that hypothermic effects are associated with activation of α_2- or inhibition of α_1-adrenoceptors. However, instead of exacerbating the paradoxical effects of NE and EPI in the cold, the prototypic α_1-antagonist prazosin was found to be much more effective than the α_2-antagonist yohimbine in normalizing body temperature, yet the demand for heat remained elevated (3,6). Since prazosin did not normalize the demand for heat in the cold, it is not clear whether it was only partially effective as an antagonist, perhaps because of interactions with specific α-adrenoceptor subtypes, or whether it independently increased the demand for heat due to an effect on heat-loss mechanisms. Given this background, the purpose of the pres-

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ent study was to examine the dose-response influence of the selective α_{1A}-antagonist WB 4101 on thermoregulatory behavior in the cold. In addition, since α-adrenoceptors are linked to changes in intracellular Ca^{2+} (19), Ca^{2+}-channel blockers might also influence responses to NE, particularly as Ca^{2+}-channel blockers have been shown to have similar thermoregulatory effects to those of α_1-antagonists (11,24). For this reason, the effects of the Ca^{2+}-channel blocker nifedipine were also examined.

METHOD

Animals

Eight female Sprague-Dawley rats were obtained from Charles River Laboratories when they were 3 months of age. The animals were maintained individually in hanging wire cages, and fed Purina Chow (5001) and water ad libitum. The colony room was maintained at 22°C with a relative humidity of 50%, and a light:dark cycle of 12:12 (lights on 0700); all tests were conducted during the light phase of the cycle.

Drugs

(-)-Norepinephrine bitatartrate was obtained from Winthrop Pharmaceuticals (New York, NY). The α_{1A}-antagonist WB 4101 was obtained from Research Biochemicals (Natick, MA), and nifedipine was obtained from Sigma (St. Louis, MO). WB and nifedipine were dissolved in ethanol and diluted with saline for use. NE was dissolved in saline. Saline served as the control for the WB trials, and the ethanol vehicle (5%) was the control for the nifedipine trials. Doses of WB and nifedipine were 0.1, 0.5 and 1.0 mg/kg given intraperitoneally (IP). The dose of NE was 250 μg/kg (IP), which was the same dose that had been found previously to produce paradoxical responses in the same experimental situation (26).

Lever-press Apparatus

The test apparatus allowed animals to obtain unlimited heat in a cold environment by pressing a lever in order to activate infrared heat 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 leverpresses and the cumulative duration of heat lamp activation.

Lever-press Procedure

The animals were shaved closely with an Oster clipper the day prior to a test. The reason for shaving the animals was to prevent the sporadic performance that occurs due to piloerection when the fur is intact. The rats were trained to press the lever in order to activate the heat lamps, and then given at least 4 additional trials of 90-min 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 in order 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 measured with a Physitemp (Clifton, N. J.) BAT-12 meter 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 twice per week with 3–4 days intervening between tests.

Protocol

The first trial examined the effects of WB with or without NE in the leverpress apparatus at a Ta of −8°C. All rats received saline, NE alone (250 μg/kg), WB alone (0.1, 0.5 and 1.0 mg/kg), and the same WB doses followed by the standard NE dose given in a counterbalanced order. At the completion of the WB trials, the animals were tested with vehicle and nifedipine (0.1, 0.5 and 1.0 mg/kg) with or without NE, as above. The order of administration of the doses was counterbalanced. For both trials, the antagonist was given first to permit receptor occupancy prior to administration of the agonist 5 min later. Body weight (± SEM) averaged 315 (± 6.9) g during the WB trials and 339 (± 7.3) g during the nifedipine trails.

Data Analysis

The primary data are the duration of heat lamp activation and the change in Tc resulting from the treatments. Since the amount of heat obtained (s heat/min) could be influenced either by the duration of a response (s heat/R) or the frequency of responding (R/min), these parameters were examined for consistent trends.

Repeated-measures analysis of variance was used to evaluate the effect of drug treatment and dosage on the main variables of post-test Tc and the duration of heat lamp activation. Post hoc paired t-tests were used for specific comparisons either to saline or NE alone. All probabilities are two-tailed.

RESULTS

Figure 1 shows the results for the WB trials. Analysis of variance showed the drug group differences were significant for heat obtained [$F(1,14) = 17.7$, $p < 0.01$], as was the group × dose interaction [$F(3,42) = 3.2$, $p < 0.05$)]. For colonic temperature, groups [$F(1,14) = 20.1$, $p < 0.01$], dose [$F(3,42) = 12.1$, $p < 0.01$], and interaction [$F(3,42) = 8.4$, $p < 0.01$)] were all significant. WB alone produced only a modest increase in the amount of heat obtained, that was significant ($p < 0.05$) at the highest 1.0 mg/kg dose with respect to saline (the zero dose for the WB curve). Pre-injection Tc averaged 38.7 (± 0.1) °C for these trials, and post-test Tc was not affected by any dose of WB. NE alone (the zero dose of the WB + NE curve) produced a substantial 70% increase in the amount of heat obtained as well as a significant decrease in post-test Tc. Pretreatment with WB at the two higher doses (0.5, 1.0 mg/kg) reduced the NE-induced increase in heat influx, and normalized post-test Tc. WB + NE was not significantly different from WB alone for heat influx at the 1 mg/kg dose, and not different for Tc at both the 0.5 and 1.0 mg/kg doses of WB.

The results of the nifedipine trials are shown in Fig. 2. Analysis of variance showed significant group differences for

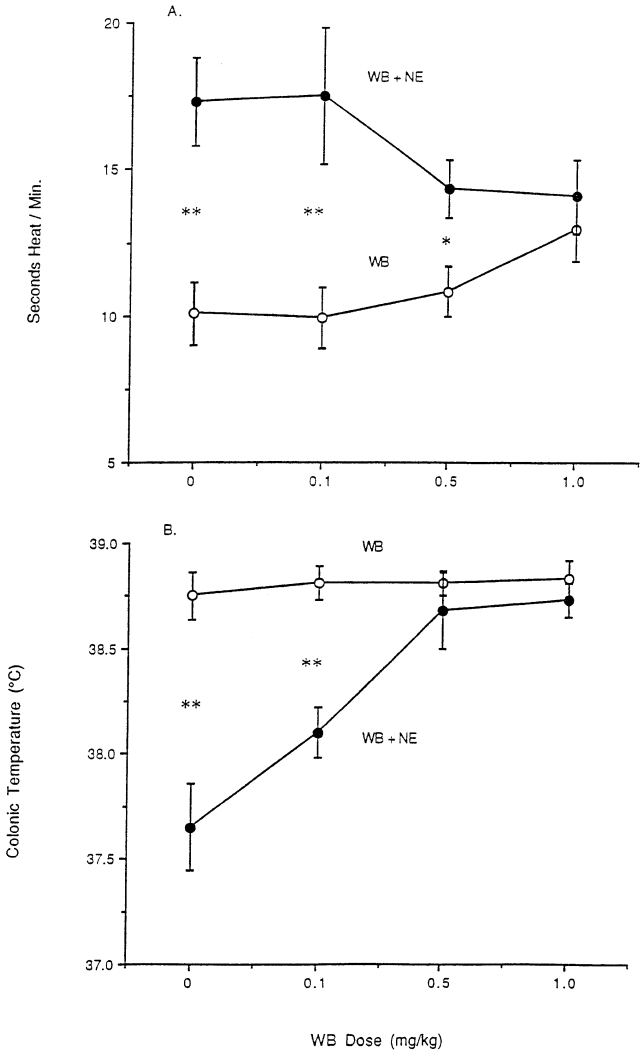

FIG. 1. The effect of WB 4101 (WB) alone or with NE (WB + NE) on: (A) operant responding for heat; (B) post-test colonic temperature. $*p < 0.05$, $**p < 0.01$ WB compared to WB + NE (paired *t*-test). The 0 dose of WB + NE is NE alone.

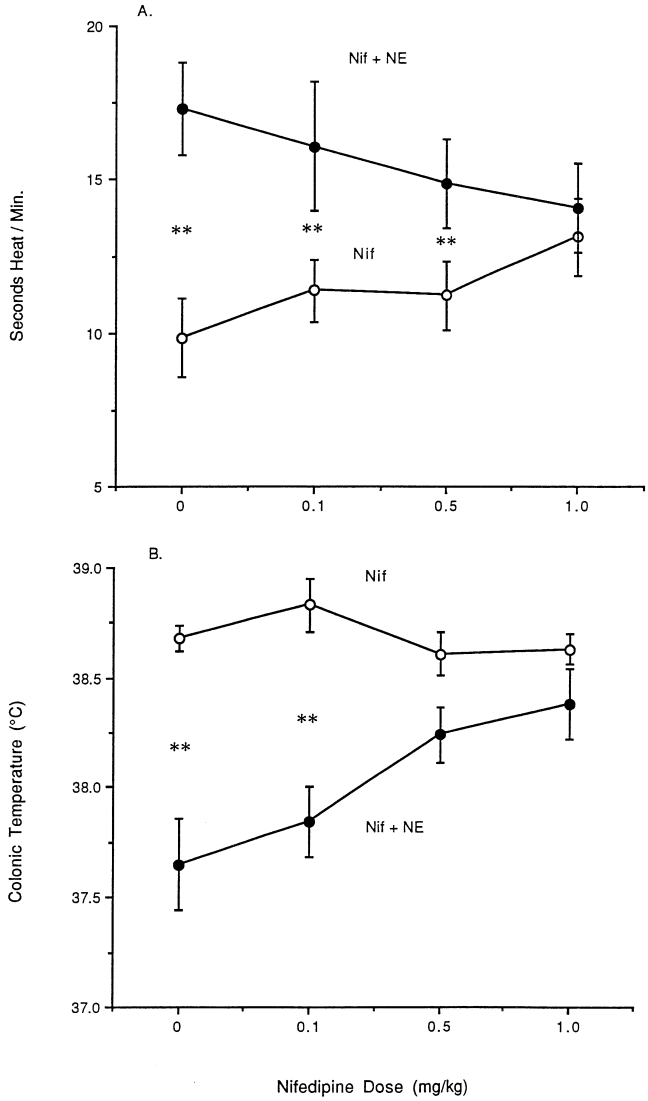

FIG. 2. The effect of nifedipine (Nif) alone or with NE (Nif + NE) on: (A) operant responding for heat; (B) post-test colonic temperature. $**p < 0.01$ Nif compared to Nif + NE (paired *t*-test).

heat obtained [$F(1,14) = 5.7$, $p < 0.05$] and the group × dose interaction [$F(3,42) = 5.81$, $p < 0.01$]. For colonic temperature, drug groups [$F(1,14) = 4.6$, $p < 0.01$] and the group × dose interaction [$F(3,42) = 4.8$, $p < 0.01$] were significant. Nifedipine was similar to WB in that it increased the amount of heat obtained, significantly so ($p < 0.05$) with respect to vehicle at the 1.0 mg/kg dose. There was no significant effect of nifedipine alone on post-test Tc, which did not differ from the pre-injection Tc of 38.7 (± 0.1) °C. When given with NE, nifedipine dose-dependently reduced the demand for heat and normalized post-test Tc.

The increased demand for heat following NE alone was due primarily to an increase in the duration of a response, with little effect on frequency of responding. Response duration increased from 5.9 s/R (saline) to 10.7 s/R (NE), an increase of 80%. WB reduced heat influx in a similar manner by reducing response duration back down to 7.9 s/R at the 1.0 mg/kg dose + NE (a 26% decrease). Nifedipine influenced both frequency of responding and response duration by increasing response rate from 1.6 R/min (NE) to 2.2 R/min at the 1.0 mg/kg dose + NE (a 37% increase) while at the same time reducing response duration to 6.3 s/R (a 41% decrease relative to NE).

DISCUSSION

The effects of NE on thermoregulatory behavior in the cold are paradoxical because NE is the sympathetic neurotransmitter responsible for non-shivering thermogenesis and should, therefore, decrease the demand for radiant heat. Even if it were to simply substitute for endogenous NE release, this should result in no difference in Tc or the demand for heat with respect to saline-treated values. The fact that here, as in previous experiments (25,26), NE increases heat demand is paradoxical, but is due, at least in part, to the fact that exogenous NE in cold-exposed rats inhibits heat production (26).

Given this, the increased demand for radiant heat would seem to be an appropriate behavioral response, but it is not sufficient to maintain Tc. In the present study, the animals were demanding only 17 s of heat per min, and could obviously have increased leverpress duration further. The fact that they do not, and allow Tc to drop, shows that NE is thermolytic—i.e., it uncouples behavioral thermoregulation from thermal balance.

Sprague-Dawley rats typically maintain a Tc of about 38.7 (± 0.2) °C when treated with saline in this and previous studies (4,5,7,8). These moderately high temperatures are most likely a compensatory response to the cold Ta, and also a reflection of the fact that rodents select a temperature that is 180° out of phase with their circadian rhythm of body temperature. Thus, they select a warmer gradient temperature and work for more heat in a leverpress apparatus during the day compared to the night (18). It is unlikely that these temperatures reflect the use of a rectal thermocouple probe because the animals are thoroughly adapted to the testing procedure, and the measurements require less than 30 s for a stable reading. Also, the animals show no obvious signs of stress (e.g., vocalization or defecation). In addition, comparable temperatures are obtained when hypothalamic temperature is continuously monitored via implanted thermistors (2).

The present results show that either WB or nifedipine can block the hypothermic effects of NE in the cold, and suggest that α_1-adrenoceptor activation and/or Ca^{2+}-influx appear to mediate the paradoxical effects of NE. However, it is also clear that the two antagonists have effects on thermal balance themselves, but unlike NE produce appropriate behavioral responses such that post-test Tc is no different from saline-treated control animals—i.e., these compounds affect thermal balance, but are not thermolytic. These effects are most obvious at the highest doses tested (1 mg/kg) where the increased radiant heat supplied is sufficient to maintain Tc, and co-administration of NE has no effect on this precise coupling of behavior to the thermoregulatory challenge. In other words, the disruptive effects of NE on thermal balance were negated by both WB and nifedipine.

The ability of both WB and nifedipine to block the thermolytic effects of NE suggests that NE acts via α_1-adrenoceptors to increase Ca^{2+}-influx. Where and how this Ca^{2+}-influx causes thermal balance and thermoregulatory behavior to be disrupted is not known. Likewise, the mechanisms responsible for the effects of the antagonists themselves on thermal balance is not known, although both α_1-antagonists and Ca^{2+}-blockers have been shown to decrease metabolic rate (11,24) and have vasodilator effects (19,22). These effects would exacerbate the thermal deficit in the cold. Either mechanism would explain the compensatory increase in the demand for heat in response to WB and nifedipine, but it is difficult to see how either a decrease in metabolic rate or an increase in heat loss could compensate for the negative thermal balance induced by NE. If anything, vasodilatation and/or reduced heat production due to WB or nifedipine should have potentiated, rather than negated the effects of NE on thermal balance and behavior.

There are two possible, albeit speculative, explanations that might help resolve this dilemma. The first depends on differential vasoconstrictor sensitivity to NE in different tissues, which in turn could depend on the relative proportions of α_1- and α_2-adrenoceptors (9,10), as well as β_2-adrenoceptors. For example, sympathetic nerve stimulation or exogenous NE causes arteriolar constriction in the mesenteric vascular bed via α_1-adrenoceptor activation (12,17), and if this vasoconstrictor effect was more intense than elsewhere, blood would be diverted to more peripheral vascular beds. This would have the effect of transferring heat from core to shell, with a consequent drop in Tc. A similar mechanism has been postulated to account for the protective effect of the β_2-adrenoceptor antagonist ICI 118551 on the paradoxical effects of ISO on thermoregulatory behavior (5,7). If such a vascular re-distribution applies to NE, the selective reversal of mesenteric vasoconstriction by WB and nifedipine could explain why these vasodilators reverse the thermolytic effect of NE.

An alternative, and equally speculative explanation is based on the observation that the responses at the highest dose (1 mg/kg) of WB and nifedipine are identical to those seen when the drugs are combined with NE (Fig. 1 and Fig. 2). It could suggest that rather than blocking the receptor mechanisms for NE, the two antagonists had caused a rapid and complete clearance of NE from the circulation, and the changes in Tc and the demand for heat were simply the same as those seen when the drugs alone were given. There is no evidence in the literature to show that WB or nifedipine affect the disposal of NE, either by increasing degradation or reuptake in synaptic terminals, but a more rapid disposal of injected NE should be seen as a change in the time-course of leverpress activity following injections of NE in the presence and absence of WB or nifedipine—i.e., the initial response to NE should be unaffected, but then attenuate rapidly, whereas if WB and nifedipine were acting as receptor antagonists, the initial NE response should be blunted. This is testable, and is the object of another experiment planned.

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