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Hypothermia in Mice: D2 Dopamine Receptor Mediation and Absence of Spare Receptors

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MELLER, E., R. HIZAMI AND L. KREUTER. *Hypothermia in mice: D2 dopamine receptor mediation and absence of spare receptors.* PHARMACOL BIOCHEM BEHAV 32(1) 141–145, 1989.—The nonselective dopamine (DA) receptor agonists R(−)apomorphine (APO) and R(−)-N-n-propylnorapomorphine (NPA) elicited dose- and time-dependent hypothermia in mice with ED_{50} values of 300 and 18 μg/kg, respectively. The selective D2 agonist quinpirole (LY 171555) also elicited dose-dependent hypothermia, whereas the selective D1 agonist SKF 38393 had no effect. The selective D1 and D2 antagonists SCH 23390 (1 mg/kg) and sulpiride (200 mg/kg), respectively, did not significantly alter body temperature. The hypothermic effect of a maximal dose of NPA (0.2 mg/kg) was not blocked by SCH 23390 (1 mg/kg) but was significantly attenuated ($p<0.001$) by pretreatment with sulpiride (200 mg/kg). Pretreatment with sulpiride (200 mg/kg) produced a parallel, 40-fold shift to the right of the dose-response curve for NPA. Partial, irreversible DA receptor inactivation by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (2 mg/kg) reduced the maximal hypothermic effect of NPA (to 49% of control) without altering its ED_{50}. Analysis of the data indicated a linear relationship between DA receptor occupancy and hypothermic response. The results demonstrate that DA agonist-induced hypothermia in mice is mediated by D2 receptors and that there is no receptor reserve for this response.

D1 and D2 dopamine receptors Hypothermia Receptor reserve
N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline Sulpiride SCH 23390 N-n-propylnorapomorphine
Apomorphine

DOPAMINE (DA) appears to play an important role in the central regulation of body temperature in mammals (2, 6, 13). The precise neuroanatomic locus for the hypothermia elicited by DA agonists administered either systemically or intracerebrally is not known, although recent evidence suggests that at least one likely site of action is in the preoptic area of the anterior hypothalamus (5, 13, 14). Several recent studies have examined which DA receptor subtype, D1 or D2, is involved in this hypothermic response, with mixed results. Iorio *et al.* (12) found that the selective D1 antagonist SCH 23390 did not block hypothermia produced by the nonselective DA agonist apomorphine (APO), whereas Carboni *et al.* (1) reported that it did. Faunt and Crocker (8), on the other hand, reported a biphasic effect of SCH 23390 alone, an initial hyperthermia followed by a subsequent hypothermia. Its effects on APO-induced hypothermia were complex: both attenuation and potentiation were observed which were dependent on the dose and time of administration of each drug. However, the selective D1 agonist SKF 38393 did not elicit hypothermia, whereas the selective D2 agonist LY 171555 (quinpirole) did. Colboc *et al.* (5) concluded that DA agonist-induced hypothermia was not mediated by D1 receptors. Since there may be substantial species differences for the effects of DA agonists on temperature regulation (13), it should be noted that all these studies were carried out in rats.

We have recently shown (16,17) that D2 dopamine autoreceptors in rat striatum, which mediate local negative feedback inhibition of neurotransmitter synthesis (18), demonstrate a large receptor reserve for full DA agonists such as apomorphine and N-propylnorapomorphine (NPA). In order to examine the relationship between receptor occupancy and response at DA receptors mediating other functional effects, we have investigated the effects of various selective and nonselective DA receptor agonists and antagonists on temperature regulation in male CD-1 mice kept at an ambient temperature of 22°C. The results in this species indicate that DA agonist-induced hypothermia is mediated solely by D2 DA receptors. Furthermore, there appear to be no spare receptors for this physiological response.

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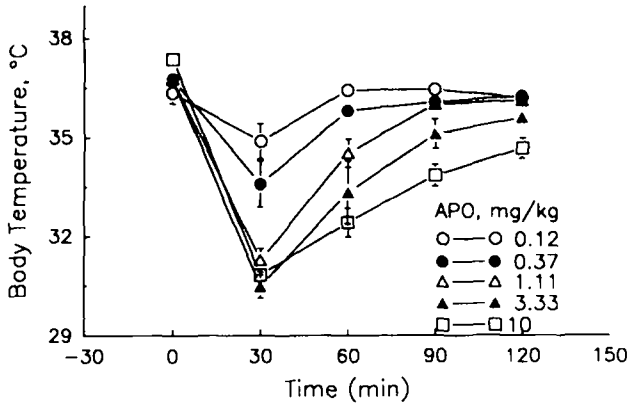

FIG. 1. Dose- and time-dependent hypothermic response to subcutaneous apomorphine. Each point is the mean±SEM of four mice.

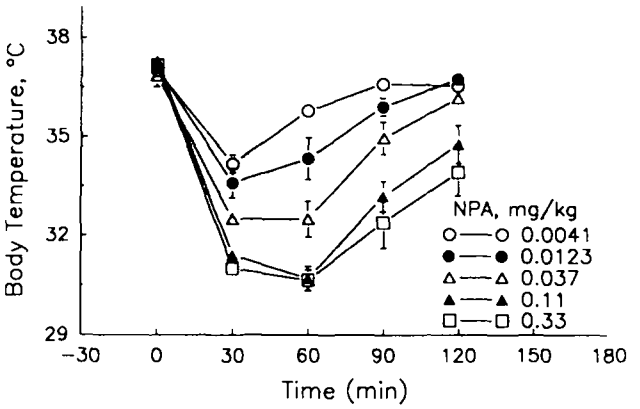

FIG. 2. Dose- and time-dependent hypothermia elicited by NPA. Each point is the mean±SEM of four animals.

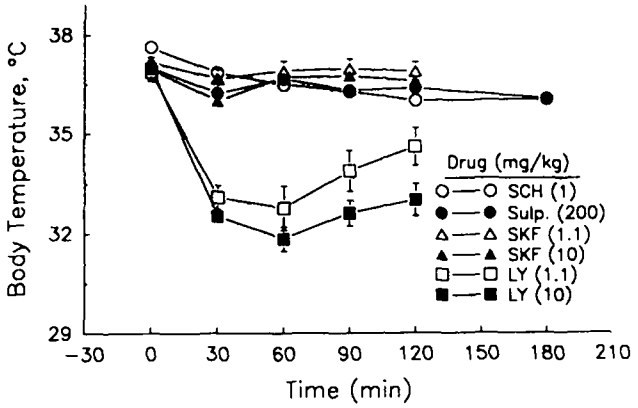

FIG. 3. Effects of selective D1 and D2 agonists and antagonists on body temperature. SCH: SCH 23390; Sulp.: sulpiride; SKF: SKF 38393; LY: quinpirole (LY 171555). N=4 for each dose, except for SCH 23390 and sulpiride, which are the means±SEM of 7 mice.

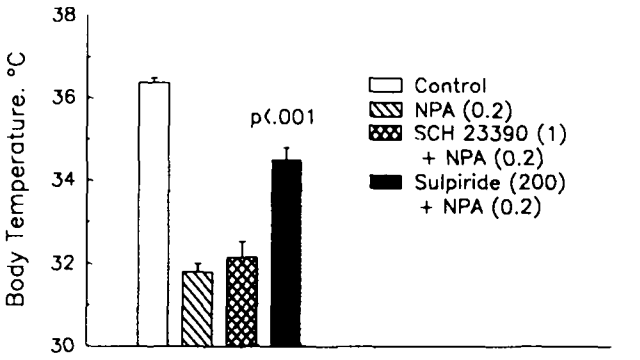

FIG. 4. Effects of pretreatment with selective DA antagonists on NPA-induced hypothermia. Sulpiride was administered 2.5 hr and SCH 23390 30 min before NPA. Maximal blockade of D2 and D1 receptors, respectively, has been shown to occur at these times (15). Temperature was measured 30 min after NPA treatment. The doses of each drug (in mg/kg) are given in parentheses. Each value is the mean±SEM of 7 animals.

METHOD

Animals

Male CD-1 mice (Charles River, Wilmington, MA), 20–22 g, were housed 4–5 per cage under conditions of constant temperature (22±1°C), humidity and light cycle (light/dark 7:00 a.m./7:00 p.m.) for at least one week before use. They were given access to food and water ad lib. Mice were used only twice, allowing at least one week of recovery between treatments, in order to minimize the possibility of tolerance development.

Drug Treatments

Mice were injected subcutaneously with various doses of drugs in a volume of 0.1–0.2 ml. The drugs used and their sources were: R(−)apomorphine (APO), Sigma Chemical Co., St. Louis, MO; R(−)-N-n-propylnorapomorphine (NPA), Research Biochemicals, Inc., Natick, MA; SCH 23390, Schering-Plough, Bloomfield, NJ; sulpiride, Ravizza, Muggio, Italy; SKF 38393, Smith Kline & French, Philadelphia, PA; quinpirole (LY 171555), Eli Lilly and Co., Indianapolis, IN; N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), Aldrich Chemical Co., Milwaukee, WI.

Temperature Measurements

Colonic temperature was measured at various times after drug treatment by gently inserting a petrolatum-coated thermistor probe (model YSI 402; Yellow Springs Instrument Co., Yellow Springs, OH) 2.5 cm into the rectum. The probe was attached to a digital thermistor thermometer (Digi-Sense, model 8522-10; Cole-Parmer, Chicago, IL); the temperature was recorded after allowing the probe to reach equilibrium (approximately 15 sec). All temperature measurements were made at an ambient temperature of 22±1°C.

Data Analysis

Dose-response curves for DA agonist-induced hypothermia were fit using the ALLFIT computer program (7) as described previously (16,17). Following partial DA receptor inactivation with EEDQ, equieffective agonist doses were utilized to construct double reciprocal plots according to the method of Furchgott and Bursztyn (9) as described previously in detail [(16,17); see also legends to Figs. 7 and 8]. Statistical comparisons were made using the two-tailed Student's *t*-test.

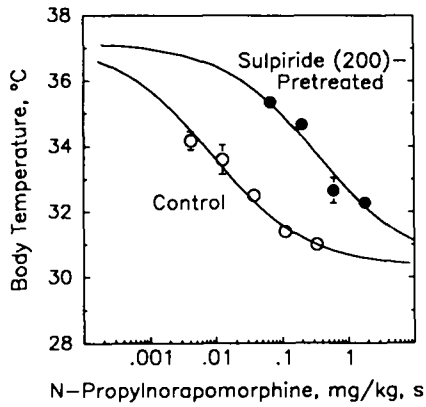

FIG. 5. Effect of sulpiride pretreatment (200 mg/kg, 2.5 hr before) on NPA-induced hypothermia (measured 30 min after NPA). The curves were simultaneously fit by ALLFIT, which indicated that they could share a common slope factor and maximal response without a significant degradation in fit, $F(3,5)=0.62$, $p>0.05$. Sulpiride pretreatment shifted the ED_{50} for NPA approximately 40-fold to the right (control, 9 μg/kg; sulpiride, 341 μg/kg). Each point is the mean±SEM of 4 (control) or 5 (sulpiride-pretreated) mice.

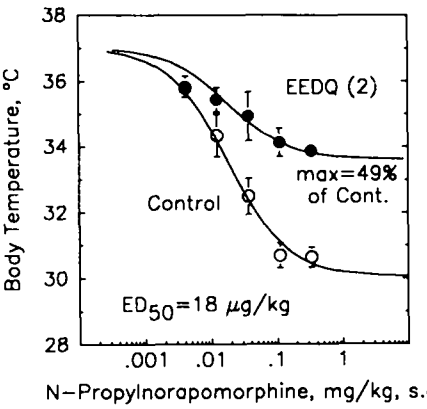

FIG. 6. The effect of EEDQ treatment on NPA-induced hypothermia. Groups of mice were treated with EEDQ (2 mg/kg, SC) or vehicle 24 hr prior to challenge with various doses of NPA. Temperature was measured 60 min after NPA. ALLFIT analysis indicated that the curves could be constrained to share a common slope factor and ED_{50} (but not a common maximal response) without a significant degradation in fit, $F(4,6)=3.51$, $p>0.05$. Each point is the mean±SEM of 4 (control) or 5 (EEDQ) mice. Analysis of the data obtained 30 min after NPA treatment gave similar results.

RESULTS

APO- and NPA-Induced Hypothermia

The nonselective agonists APO and NPA elicited dose- and time-dependent hypothermia in mice (Figs. 1 and 2). Both agonists maximally decreased body temperature about 6–7°C at the highest doses. The maximal effect of APO occurred at 30 min, while that of NPA lasted somewhat longer (30–60 min; Fig. 2). ALLFIT dose-response analysis at the time of peak effect yielded ED_{50} values of 18 and 300 μg/kg for NPA and APO, respectively.

Effects of Selective Agonists and Antagonists

Neither the selective D1 antagonist SCH 23390 (1 mg/kg) nor the selective D2 antagonist sulpiride (200 mg/kg) significantly modified body temperature in mice up to 3 hr after treatment (Fig. 3). These doses of the drugs have previously been shown to completely and selectively block the respective receptors in the brain (15). Similarly, the selective D1 agonist SKF 38393 (1.1 and 10 mg/kg) did not significantly alter body temperature for up to 2 hr after injection. In contrast, the selective D2 agonist quinpirole (1.1 and 10 mg/kg) elicited a long-lasting and dose-dependent hypothermia (Fig. 3). The hypothermia produced by the nonselective agonist NPA was apparently mediated by activation of D2 rather than D1 receptors, since pretreatment with the D1 antagonist SCH 23390 (1 mg/kg) did not modify the effect of a maximal NPA dose (200 μg/kg), whereas pretreatment with the D2 antagonist sulpiride (200 mg/kg) significantly attenuated the response ($p<0.001$; Fig. 4). Furthermore, a comparison of the dose-response curves obtained for NPA-induced hypothermia with and without pretreatment with sulpiride (200 mg/kg) demonstrated an apparent parallel 40-fold shift to the right in the ED_{50} for NPA (Fig. 5), suggesting competitive blockade by sulpiride. Simultaneous ALLFIT analysis of the curves indicated that they could be constrained to share the same slope factor and maximum response without a significant worsening of the fit (legend to Fig. 5). Attempts to share the same ED_{50} resulted in significantly poorer fits. These results demonstrate that DA agonist-induced hypothermia in mice is mediated solely via an interaction at D2 receptors.

Effects of Irreversible DA Receptor Blockade

In contrast to the effects of the reversible antagonist sulpiride (Fig. 5), pretreatment of mice with the irreversible DA receptor antagonist EEDQ (2 mg/kg) (10, 15–17) reduced the maximum hypothermic response to NPA about 50%, without altering the ED_{50} (Fig. 6). Equieffective doses of NPA required to elicit hypothermia in control and EEDQ-treated mice at five different levels of effect (30–70% of the maximal effect obtained in the EEDQ animals) were subjected to a double reciprocal plot as described previously (9, 17, 19). From the resultant linear plot (Fig. 7), the fraction of receptors remaining intact, q, and the pseudo-activation constant of NPA, K_A [in units of dose; see Meller *et al.* (17)] were obtained (Fig. 7). The pseudo-K_A value and the mass-action equation were used to calculate fractional receptor occupancy for each of the experimental doses of the control curve (9, 17, 19). A plot of percent receptor occupancy for each of these doses against observed body temperature is shown in Fig. 8. The relationship is seen to be strictly linear, indicating that there is no receptor reserve for NPA-induced hypothermia (9, 17, 19).

DISCUSSION

The two major findings of this study are: 1) DA agonist-induced hypothermia in CD-1 mice is apparently mediated solely by D2 DA receptors; and 2) there appears to be no receptor reserve for this response as gauged by the linear relationship between receptor occupancy and response for the full DA agonist NPA. This study extends our previous investigations of the relationship between receptor occu-

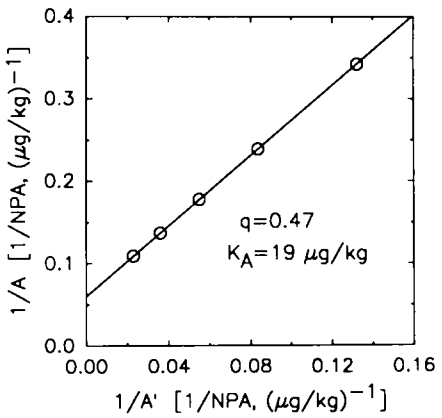

FIG. 7. Double-reciprocal plot of equieffective doses of NPA to elicit hypothermia in control and EEDQ-treated mice. The doses were obtained from the best-fit curves shown in Fig. 6, and corresponded to the doses required to elicit 30, 40, 50, 60 and 70% of the maximal response obtained after EEDQ treatment. The fraction of receptors remaining active, q = 1/slope, and the pseudo-K_A = slope − 1/y-intercept [see (9) and (17)].

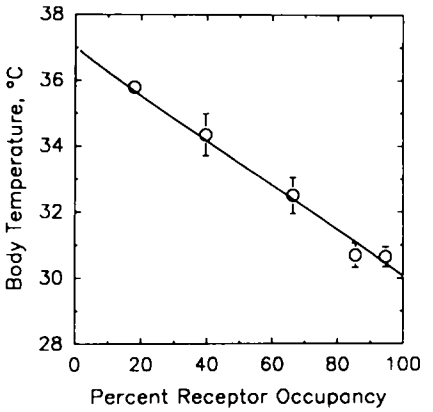

FIG. 8. Percent receptor occupancy vs. response for NPA-induced hypothermia. The pseudo-K_A value (19 μg/kg) and the mass-action equation ($f = [A]/([A] + K_A)$) were used to calculate fractional receptor occupancy (f) for each dose (A) used in the control experiment (Fig. 6). Fifty percent of the maximal hypothermic effect was calculated to require 49% receptor occupancy.

pancy and response for various DA-mediated functional effects. As indicated earlier, we have previously found that D2 DA autoreceptors mediating negative feedback regulation of DA synthesis in rat striatum display a large receptor reserve for DA agonists (16,17). We have also found that DA autoreceptors in rat striatum mediating inhibitory regulation of DA release also possess a substantial receptor reserve for agonists (20). In contrast, postsynaptic striatal D2 receptors which inhibit cholinergic activity appear to have no spare receptors for agonists (17a).

It is instructive to compare the ED_{50} values of NPA for the various functional effects we have thus far examined. In the autoreceptor studies (17) the ED_{50} of NPA was about 1 μg/kg, whereas it was 18 μg/kg in the present study. Furthermore, a value of 19 μg/kg was found in the studies examining NPA-mediated elevation of striatal ACh levels (21). Whereas we have found a substantial receptor reserve at the DA autoreceptor (17), none was found for the latter two functional effects. According to receptor occupancy theory (19), the potency of an agonist should be decreased for the same receptor in the absence of a receptor reserve as compared to its presence. The results are therefore in accord with expectation.

In line with these observations, it is interesting to note that Colboc *et al.* (5) found that the doses of APO required to elicit DA autoreceptor-mediated hypolocomotor activity were substantially lower than those required to elicit hypothermia. Furthermore, we (17) and Clark *et al.* (3) have found that the enantiomers of the DA autoreceptor-selective agonist 3-PPP exhibit different efficacies, with the (+) enantiomer possessing substantially higher efficacy than the (−) enantiomer. Receptor occupancy theory predicts (19) that, in the absence of receptor reserve, an agonist of higher efficacy will elicit a greater degree of response than an agonist of lower efficacy. Interestingly, Hjorth *et al.* (11) reported that (+)3-PPP elicited hypothermia in rats, whereas the (−) enantiomer did not. We have obtained similar results in mice (unpublished observations).

In conclusion, these studies suggest that the method of partial receptor inactivation may be applied to the determination of the relationship between receptor occupancy and response for a physiological function such as DA agonist-mediated hypothermia. This conclusion is based on the virtually identical ED_{50} found for NPA-induced hypothermia and NPA-mediated elevation of ACh levels. Both of these responses appear to be mediated by D2 receptors having no receptor reserve for agonists. Inasmuch as DA agonists are used in the treatment of Parkinson's disease, and selective autoreceptor agonists have been suggested as potential therapeutic agents in schizophrenia (4), the further elucidation of the relationship between receptor occupancy and response for agonists at DA receptors regulating a variety of functional responses could be of significant aid in the design of new psychotherapeutic drugs.

ACKNOWLEDGEMENTS

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