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The D_3 Antagonist PNU-99194A Potentiates the Discriminative Cue Produced by the D_3 Agonist 7-OH-DPAT

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DEPOORTERE, R., GH. PERRAULT AND D. J. SANGER. *The D_3 antagonist PNU-99194A potentiates the discriminative cue produced by the D_3 agonist 7-OH-DPAT.* PHARMACOL BIOCHEM BEHAV **65**(1) 31–34, 2000.—Based on correlations between potencies of various dopamine D_2/D_3 agonists to substitute for the 7-OH-DPAT discriminative cue and their in vitro (mitogenesis test) potencies, it has been suggested that the 7-OH-DPAT cue is mediated by activity at the D_3 subtype. We sought to verify that the 7-OH-DPAT cue could be blocked by PNU-99194A, a commercially available preferential D_3 antagonist. Rats were trained (FR10 two-lever, food-reinforced schedule) to press one lever following 7-OH-DPAT (0.1 mg/kg IP) and the other lever following saline. Rats were then tested with various doses of 7-OH-DPAT alone or in combination with PNU-99194A. 7-OH-DPAT (0.003 to 0.3 mg/kg) engendered dose-dependent substitution; PNU-99194A (1 to 10 mg/kg) failed to antagonize the cue induced by 0.1 mg/kg of 7-OH-DPAT and, at 10 mg/kg, given in combination with 0.003 to 0.1 mg/kg of 7-OH-DPAT, PNU-99194A markedly shifted the 7-OH-DPAT dose–effect curve to the left, i.e., potentiated the 7-OH-DPAT cue. If PNU-99194A is a preferential D_3 antagonist, the present data do not confirm the previous hypothesis that the 7-OH-DPAT cue is mediated by the D_3 subtype. © 1999 Elsevier Science Inc.

7-OH-DPAT Discrimination Dopamine D_3 receptor PNU-99194A

SINCE its cloning in 1990 by Sokoloff and colleagues (21), the dopamine (DA) D_3 receptor has been the object of intensive research [for a recent review, see (14)]. Several laboratories have sought to determine the function of this receptor through the use of correlational studies that compared the potency of a range of agonists—with various levels of selectivity for the DA D_3 receptor—to induce an effect and their in vitro affinity or potency [mitogenesis test: (20)] at the D_3 receptor. On the basis of this work, this subtype of receptor has been linked to the control of locomotion (20), body temperature (16,17), food-reinforced operant behavior (18), cell firing in the ventral tegmental area (13), and the reinforcing efficacy of intravenous cocaine (6) or oral ethanol (8).

Other studies (19,22) have also suggested that the discriminative cue induced by the DA D_2/D_3 receptor agonist 7-OH-DPAT might be mediated by activity at the DA D_3 receptor-subtype. This conclusion was partly based on correlations between the potencies of various DA D_2/D_3 receptor agonists to substitute for the 7-OH-DPAT cue (19,22) and their in vitro potencies to induce mitogenesis in cells transfected with D_3 receptors (20). However, more definite conclusions regarding the implication of the DA D_3 receptor in the 7-OH-DPAT discriminative stimulus would require the demonstration that this stimulus can be selectively and dose dependently blocked by antagonists selective for the D_3 receptor subtype. This verification has been impeded by the scarcity of selective DA D_3 receptor antagonists. At the time when this experiment was conducted, there was only one commercially available DA D_3 receptor antagonist, PNU-99194A (previously referred to as U 99194A) with a reported in vitro preferential affinity for the D_3 (K_i = 78 nM) over the D_2 (K_i = 1572 nM) subtype of DA receptor (11,23). This compound has been shown to increase motor activity in rats (7,23) and mice (10). It has also been used successfully to train rats on a classical food-reinforced FR10 schedule, drug discriminative assay (2,9). To the best of our knowledge, however, this compound (or for that matter, other selective DA D_3 receptor antagonists) has not been used to verify that it can selectively and dose dependently an-

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tagonize the discriminative cue produced by 7-OH-DPAT. In the present study, we sought to verify if in rats the discriminative cue produced by 7-OH-DPAT could be blocked by PNU-99194A.

METHOD

Subjects

Male Wistar rats (IFFA CREDO, L'Arbresle, France) were kept on a 12 L:12 D cycle (lights on 0800–1600 h). Their weights were kept at about 80–85% of their free-feeding values. The experimental protocol was approved by the Synthelabo Recherche Ethical Committee, and was in compliance with current French legislation on animal experimentation.

Apparatus and Behavioral Procedures

Rats were trained and tested in two-lever operant chambers (Med-Associates, East Fairfield, VT). Briefly, they were trained to press both levers for food delivery (45 mg pellets) on a FR10 schedule. After stabilization of operant responding, they were trained to press one lever following IP saline injection and the alternate lever following 7-OH-DPAT (0.1 mg/kg IP) injection. Discrimination training (15-min sessions) was from Monday through Friday. Test sessions occurred on Wednesdays and Fridays. Details of the procedure can be found elsewhere (19).

Drugs and Injection Protocols

(±)7-OH-DPAT (7-hydroxy-2-(di-N-propylamino)-tetralin) and PNU-99194A (5,6-dimethoxy-2-(di-n-propylamino)indan) were purchased from RBI (Natick, MA). Drugs were freshly dissolved in saline and administered IP in a volume of 2 ml/kg. 7-OH-DPAT alone and the PNU-99194A/7-OH-DPAT combination were given 30 min pretest; doses are expressed as the weights of the free base.

Data Analysis

Data are presented as the percentage of rats selecting the 7-OH-DPAT–associated lever and as the number of lever presses during the 15-min test session. For the 7-OH-DPAT generalization experiment, the number of lever presses were analyzed by a one-way ANOVA, followed by a Dunnett's test. Controls consisted of the number of presses emitted during the vehicle sessions that immediately preceded each test session. For the experiment where various doses of PNU-99194A were combined with 0.1 mg/kg of 7-OH-DPAT, lever presses were analyzed by a one-way ANOVA. For the experiment in which 10 mg/kg of PNU-99194A was combined with various doses of 7-OH-DPAT, the numbers of lever presses were analyzed by a two-way ANOVA, with the dose of 7-OH-DPAT and the pretreatment (PNU-99194A or saline) as the factors. Analyses were performed with the "GB-Stat" software package (Dynamic Microsystems, Silver Spring, MD).

RESULTS

Effects of Varying the Dose of 7-OH-DPAT

7-OH-DPAT (0.003 to 0.3 mg/kg) engendered dose-related responding on the 7-OH-DPAT–associated lever (Fig. 1). Rates of responding were dose dependently decreased, $F(4, 119) = 17.77$, $p < 0.0001$, with significant effects from the dose of 0.03 mg/kg (the average for all vehicle sessions that immediately preceded each test sessions was 1368 ± 41).

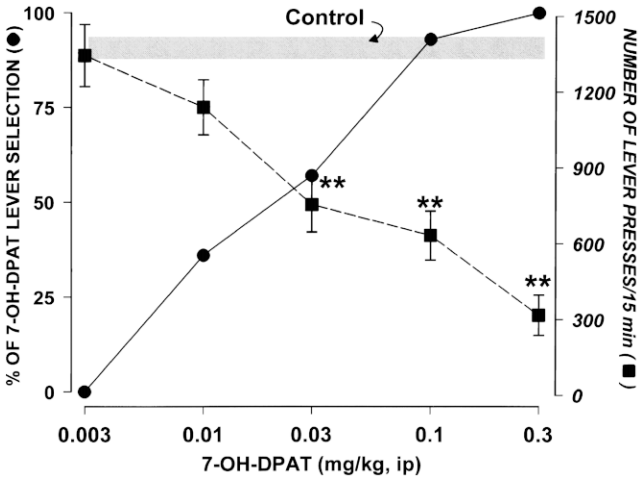

FIG. 1. Left axis: percentage of rats selecting the 7-OH-DPAT associated lever as a function of the dose of 7-OH-DPAT. Right axis: number of lever presses during the test session. Each symbol is the mean (± the SEM for the number of lever presses). $^{**}p < 0.01$, one-tailed post hoc Dunnett's test versus control (average: 1368 ± 41 presses, represented by the shaded area). $n = 11$ to 14 rats per symbol.

Effects of Various Doses of PNU-99194A in Combination With the Training Dose (0.1 mg/kg) of 7-OH-DPAT

PNU-99194A (1 to 10 mg/kg) failed to antagonize the 7-OH-DPAT discriminative cue (Fig. 2). The training dose (0.1 mg/kg) of 7-OH-DPAT substantially reduced the rate of lever pressing (640 ± 126), and this effect also was not antagonized by the three doses of PNU-99194A, $F(3, 36) = 0.77$, $p > 0.05$.

Effects of PNU-99194A (10 mg/kg) in Combination With Various Doses of 7-OH-DPAT

PNU-99194A (10 mg/kg), when given in combination with 0.003 to 0.1 mg/kg of 7-OH-DPAT, increased the percentage

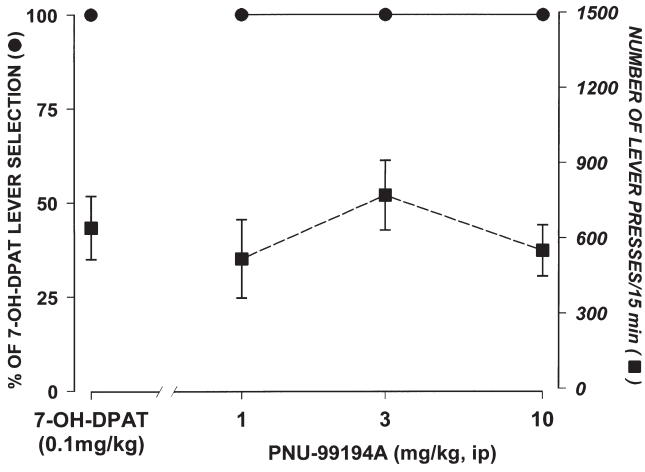

FIG. 2. Effects of PNU-99194A on the percentage of rats selecting the 7-OH-DPAT associated lever (left axis) and on the number of lever presses (right axis). Each symbol is the mean (± the SEM for the number of lever presses). $n = 8$ to 10 rats per symbol.

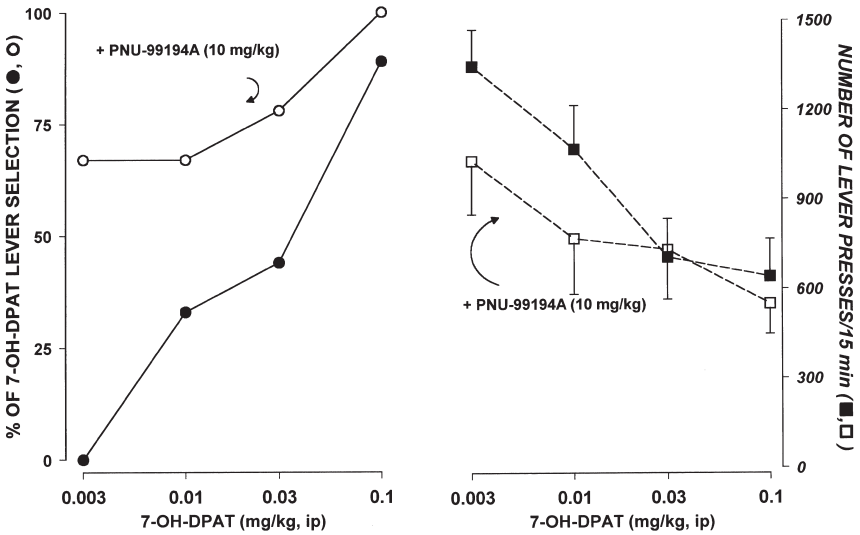

FIG. 3. Left panel: effects of PNU-99194A on the percentage of rats selecting the 7-OH-DPAT associated lever as a function of the dose of 7-OH-DPAT. Right panel: effects of PNU-99194A on the number of lever presses as a function of the dose of 7-OH-DPAT. Each symbol is the mean (± the SEM for the number of lever presses). $n = 9$ to 11 rats per symbol.

of rats selecting the 7-OH-DPAT–associated lever, i.e., PNU-99194A shifted the generalization curve to the left (Fig. 3, left panel). Adding PNU-99194A to 0.003 mg/kg of 7-OH-DPAT resulted in an increase from 0 to 67% of rats selecting the 7-OH-DPAT–associated lever.

PNU-99194A had minimal effect on the rate of responding when compared to treatment with 7-OH-DPAT alone [pretreatment effect: $F(1, 73) = 2.0$, $p > 0.05$ and pretreatment × dose interaction: $F(3, 73) = 0.49$, $p > 0.05$. The factor dose (of 7-OH-DPAT) was found to be highly significant, $F(3, 73) = 7.82$, $p < 0.001$.

DISCUSSION

The main findings of the present study were that the putative DA D_3 receptor antagonist PNU-99194A did not block the discriminative stimulus produced by the DA D_2/D_3 receptor agonist 7-OH-DPAT. It was found instead to shift the 7-OH-DPAT generalization curve to the left, indicating that it potentiated the 7-OH-DPAT discriminative cue.

In this study, the generalization curve for 7-OH-DPAT was quite similar to the one obtained with a previous group of rats trained to discriminate the same compound (19). This dose-dependent generalization indicates that the rats were under appropriate stimulus control. PNU-99194A (1 to 10 mg/kg) failed to antagonize the discriminative cue induced by the training dose (0.1 mg/kg) of 7-OH-DPAT. The highest dose of PNU-99194A tested is behaviorally active, having been used as a discriminative stimulus in a drug discrimination procedure similar to the one used here (2,9), and induces hyperactivity in rats (7,23) and mice (11). PNU-99194A also did not reverse the decrease of operant responding produced by 7-OH-DPAT; in fact, it had a small tendency, at 10 mg/kg, to potentiate this effect. This is in contrast to what is observed with DA D_2/D_3 antagonists such as amisulpride, which was shown to reverse the rate-decreasing effects of 7-OH-DPAT in a previous drug discrimination study (19). Interestingly, PNU-99194A, at 10 and 20 mg/kg, also failed to block the full generalization produced by the same dose (0.1 mg/kg) of 7-OH-DPAT in rats trained to discriminate *d*-amphetamine or cocaine (3).

Additionally, the discriminative cue produced by PD 128907, another putative DA D_3 receptor agonist, could not be reversed by L-745,829 or GR 103,691, two antagonists considered to show selectivity for the D_3 subtype (5). However, it should be noted that the in vivo activity of GR 103,691 has been recently questioned (1). Finally, in rats trained to discriminate apomorphine, it was found that the potency of DA receptor antagonists (haloperidol, UH-232, and AJ76) to block the substitution by PD 128907 was more a function of their affinity for the D_2 than for D_3 receptor subtype (13).

PNU-99194A, at 10 mg/kg, shifted the 7-OH-DPAT generalization curve to the left. Given in combination with 0.003 mg/kg of 7-OH-DPAT (which by itself did not generalize), it engendered 67% generalization. This would indicate that at this dose, PNU-99194A potentiated the discriminative stimulus of 7-OH-DPAT. Unfortunately, due to a limited supply at time of testing, it was not possible to test whether or not PNU-99194A, when given alone, would substitute for the 7-OH-DPAT discriminative cue. In rats trained to discriminate cocaine or *d*-amphetamine, PNU-99194A partially (40–50%) substituted for the discriminative cues (3). Additionally, in rats trained to discriminate PNU-99194A (9), apomorphine and *d*-amphetamine, at the highest doses tested, engendered partial generalization (50%). Thus, data from Baker et al. (3), Franklin and colleagues (9), along with the present results, do not provide any indication that PNU-99194A can block the discriminative stimuli produced by direct and indirect DA receptor agonists. Instead, they tend to indicate that the discriminative stimulus of PNU-99194A could share some commonality with the stimulus produced by these DA receptor agonists. This is inconsistent with the claimed DA D_3 receptor antagonist properties of this compound.

PNU-99194A has been shown to enhance motor activity in both mice (11) and rats (7,23). It was also shown that the stimulant effects of PNU-99194A could be antagonized by the DA receptor antagonist haloperidol (7). One tentative explanation for the present results, therefore, might be that PNU-99194A acts as a DA receptor agonist or partial agonist. Indeed, there is some indication that in vitro, PNU-99194A could possess intrinsic activity at DA D_3 receptors (11). This could explain why in the present study PNU-99194A was found to potentiate the 7-OH-DPAT cue. Alternatively, it is possible that some behavioral effects of PNU-99194A, including the present results, are not mediated by DA D_3 receptors. We recently showed (4) that, in DA D_3 receptor knock-out mice, the increase in locomotor activity produced by this compound was similar to that obtained in wild-type controls, indicating that PNU-99194A acts at other sites.

In summary, the discriminative cue induced by 7-OH-DPAT, supposedly mediated by the DA D_3 receptor, was not antagonized by PNU-99194A, the only commercially available DA D_3 receptor antagonist at the time of testing. Complementary studies using DA D_3 receptor antagonists that have become available more recently, and with better D_3 over D_2 selectivity ratios, are required before drawing more general and definite conclusions regarding the nature of the DA receptors mediating the discriminative cue not only of 7-OH-DPAT, but also of other DA D_2/D_3 receptor agonists. An alternative approach could be to assess whether transgenic mice lacking DA D_3 receptors can still be trained to detect the discriminative cue produced by DA D_2/D_3 receptor agonists.

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