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RAPID COMMUNICATION

Discriminative Stimulus Properties of 7-OH-DPAT, a Dopamine D_3-Selective Receptor Ligand

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McELROY, J. F. *Discriminative stimulus properties of 7-OH-DPAT, a dopamine D_3-selective receptor ligand.* PHARMACOL BIOCHEM BEHAV **48**(2) 531–533, 1994.—7-Hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) is a high affinity and selective ligand at dopamine D_3 receptors. Rats were trained to discriminate 7-OH-DPAT (0.05 mg/kg) from drug vehicle using a food-reinforced (FR 10) two-lever operant procedure. Six out of nine rats learned to discriminate 7-OH-DPAT, requiring a mean of 29 training sessions. After the training dose was increased to 0.10 mg/kg the remaining three rats reached discrimination performance criterion. The 7-OH-DPAT stimulus was dose-dependent. Doses of 0.01, 0.03, 0.056, and 0.1 mg/kg 7-OH-DPAT produced 0, 11, 78, and 100% drug-correct lever selection, respectively, with the ED_{50} value calculated to be 0.038 mg/kg. These results demonstrate that the D_3-selective ligand 7-OH-DPAT can control differential operant responding in rats on the basis of its discriminative stimulus properties.

7-OH-DPAT Dopamine D_3 receptor Drug discrimination Dopamine agonist

7-OH-DPAT (7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin) has recently been identified as a high affinity (K_i = 0.78 nM) and selective ligand at dopamine D_3 receptors expressed in Chinese hamster ovary (CHO) cells. Relative to D_3 receptor binding, 7-OH-DPAT binds with 100-, 1000-, and 10 000-fold lower affinity at D_2, D_4, and D_1 receptors, respectively (6). 7-OH-DPAT produces sniffing behavior in mice and rats, increases locomotor activity in rats, induces emesis in dogs, and causes 6-OHDA-lesioned rats to rotate in a contralateral direction (2,7,8). This behavioral profile is similar to that of well-established dopamine agonists such as apomorphine, indicating that 7-OH-DPAT is an agonist at dopamine receptors, in vivo.

Both direct (e.g., apomorphine, quinpirole, SKF38393) and indirect (e.g., amphetamine) agonists at dopamine receptors produce reliable discriminative stimuli (1,3,4,11). The purpose of the present study was to determine whether the dopamine D_3-selective agonist 7-OH-DPAT can similarly control differential operant responding in rats on the basis of its discriminative stimulus properties.

MATERIALS AND METHODS

Nine male Sprague–Dawley rats weighing 177–226 g at the start of the experiment were purchased from Charles River Breeding Laboratories (Wilmington, MA). Animals were housed singly in hanging wire cages in a room maintained at constant temperature (21–23°C) and humidity (50 ± 10%) and illuminated 12 h per day (lights on at 0600). Throughout the study rats were restricted to 12 g of laboratory rodent chow pellets (Bio-Serv, Frenchtown, NJ) per day, while access to water was unlimited. All training and testing was done Monday through Friday of each week.

Model E10-10 Coulbourn operant chambers (28 × 26 × 31 cm) were housed within light-proof, sound-attenuated, and fan-ventilated chambers. Each operant chamber was equipped

TABLE 1

BASELINE DISCRIMINATION ACQUISITION AND RESPONSE RATE RESULTS FOR INDIVIDUAL RATS TRAINED TO DISCRIMINATE 7-OH-DPAT (0.05 mg/kg) FROM DRUG VEHICLE (0.9% saline)

Rat No.	Days to Criterion*	Vehicle Responses Mean†	Vehicle Responses (Range)	7-OH-DPAT Responses Mean†	7-OH-DPAT Responses (Range)	% Control‡
2	33	769	(490–911)	526	(191–865)	68
3	33	666	(490–760)	174	(71–282)	26
6	20	814	(621–1042)	251	(68–543)	31
7	31	1228	(490–1570)	660	(286–1022)	54
9	34	648	(510–732)	382	(121–550)	59
12	20	645	(363–840)	239	(100–567)	37
Mean	29	795		372		46
±SEM	±3	±91		±77		±7
Range	20–34	645–1228		174–660		26–68

*The number of training sessions (including the 10 criterion sessions) to meet the performance criterion of no more than 3 incorrect responses before 10 responses on the injection-appropriate lever in 9 out of 10 consecutive sessions. †The total number of responses per 10-min training session, averaged over the first 10 postcriterion vehicle or 7-OH-DPAT treatments. ‡The mean individual response rate for the first 10 postcriterion 7-OH-DPAT treatments, expressed as a percentage of the corresponding vehicle treatments.

with two nonretractable levers, requiring a downward force equivalent to 15 g (0.15 N), that were mounted 3 cm from the side wall, 3 cm above the metal grid floor, and 5 cm from a centrally placed feeder that delivered one 45-mg food pellet (Dustless Precision Pellets, Bio-Serv). The experimental chambers were connected to a Micro PDP11/73 computer using a LAB LINC interface. A SKED-11 operating system (State System, Kalamazoo, MI) was used to record and control behavior.

7-OH-DPAT hydrochloride (synthesized by R. K. Ward, DuPont Merck, Wilmington, DE) was dissolved in 0.9% saline and administered SC in a volume of 1 ml/kg body weight 30 min before discrimination training. Doses of 7-OH-DPAT were calculated and are expressed in terms of the free base weight.

Rats were trained to discriminate 7-OH-DPAT from drug vehicle using a standard drug discrimination procedure (9). After habituation to the operant chambers rats were trained to alternate daily between response levers on a fixed-ratio 1 (FR 1) schedule of reinforcement. Once lever pressing was well established the reinforcement contingency was increased incrementally to an FR 10 schedule, while the lever alternation was maintained. Next, the rats were trained to discriminate between 0.05 mg/kg 7-OH-DPAT and drug vehicle (saline). Five of the rats were assigned the left lever as "7-OH-DPAT-correct" and the right lever as "saline-correct." The lever assignments were reversed for the remaining animals. Every 10th response on the 7-OH-DPAT-correct lever was reinforced on days when the rats were pretreated with 7-OH-DPAT, whereas every 10th response on the opposite lever was reinforced after saline injections. In each two-week period there were five drug sessions and five saline sessions, with the constraint that there could not be more than three consecutive drug or vehicle sessions.

After 45 training sessions at 0.05 mg/kg the dose of 7-OH-DPAT was increased to 0.1 mg/kg and discrimination sessions were continued until each rat reached the performance criterion of no more than 3 incorrect responses before 10 responses on the injection-appropriate lever in 9 out of 10 consecutive sessions.

Once drug discrimination was well established at the 0.1-mg/kg training dose, stimulus generalization tests were conducted on Friday of each week, with the training injection replaced by a different dose of 7-OH-DPAT (0.01–0.1 mg/kg). The dose sequence was not systematic. During the test sessions, 10 min in duration, the lever on which the rat first responded 10 times resulted in reinforcement, and subsequent FR 10 reinforcement was made contingent upon pressing this "selected" lever. Training sessions were conducted on Monday

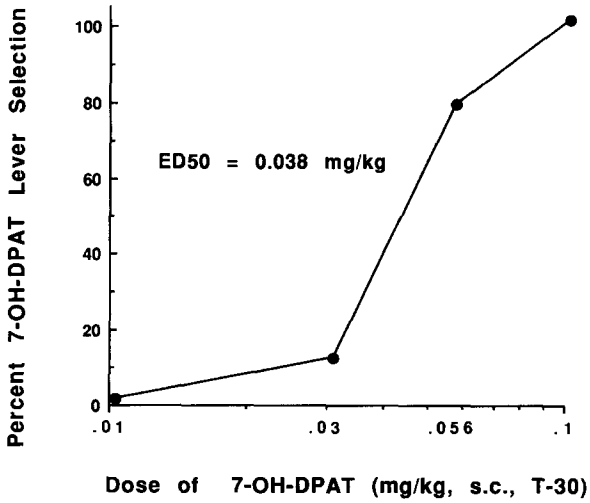

FIG. 1. Dose-response effect. Stimulus generalization gradient as a function of dose of 7-OH-DPAT administered (the training dose was 0.10 mg/kg). All injections were given 30 min before testing and each value represents the percentage of rats selecting the 7-OH-DPAT-appropriate lever.

through Thursday of each week to ensure that discrimination was intact. If any rat failed to demonstrate reliable discrimination (no more than 3 incorrect responses before 10 responses on the injection-appropriate lever), testing with that animal was postponed and discrimination training continued until this performance criterion was attained.

Drug discrimination results are expressed as the percentage of animals selecting the 7-OH-DPAT-appropriate lever. The ED_{50} value for stimulus generalization (i.e., the dose at which half the rats selected the 7-OH-DPAT-appropriate lever) was calculated by nonlinear regression analysis. A repeated-measures *t* test was used to compare the postcriterion response rate after 7-OH-DPAT treatment to the corresponding rate after drug vehicle treatment.

RESULTS

Six out of nine rats learned to discriminate 0.05 mg/kg 7-OH-DPAT from drug vehicle (0.9% saline). Discrimination acquisition for these six rats required 29 ± 3 (mean ± SEM) training sessions (including the 10 criterion sessions) to meet the performance criterion of no more than 3 incorrect responses before 10 responses on the injection-appropriate lever in 9 out of 10 consecutive sessions (Table 1). Averaged for the first 10 postcriterion vehicle treatments, the mean ± SEM response output per 10-min training session was 795 ± 91 responses. For the corresponding 7-OH-DPAT treatments this value was 372 ± 77 responses (46% of the corresponding vehicle treatment value). Thus, 0.05 mg/kg of 7-OH-DPAT produced a highly significant 54% decrease in responding ($p < 0.001$).

Varying the dose of 7-OH-DPAT (0.01–0.1 mg/kg) produced a stimulus generalization gradient (Fig. 1). Doses of 0.01, 0.03, 0.056, and 0.1 mg/kg 7-OH-DPAT produced 0, 11, 78, and 100% drug-correct lever selection, respectively. Nonlinear regression analysis indicated that the dose of 7-OH-DPAT that would produce 50% 7-OH-DPAT lever selection (ED_{50}) was 0.038 mg/kg, approximately one third of the training dose (0.1 mg/kg). The "threshold" dose of 7-OH-DPAT, defined as the lowest dose of 7-OH-DPAT capable of eliciting predominantly training drug-correct lever selection, was 0.056 mg/kg (78% 7-OH-DPAT lever selection).

DISCUSSION

The results of this study demonstrate that the D_3-selective agonist 7-OH-DPAT can control differential operant responding in rats on the basis of its discriminative stimulus properties. The threshold dose of 7-OH-DPAT (0.056 mg/kg) agrees quite closely with the minimum dose of 7-OH-DPAT required to produce stereotypical sniffing (0.10 mg/kg) or to cause 6-OHDA-lesioned rats to rotate in a contralateral direction (0.03 mg/kg) (2,7,8). However, a substantially larger dose of 7-OH-DPAT is required to increase open-field locomotor activity (1.0 mg/kg) in rats (8).

Averaged for the first 10 postcriterion 7-OH-DPAT training sessions, 7-OH-DPAT (0.05 mg/kg) decreases food-reinforced operant responding (FR 10 schedule of reinforcement) by 54% relative to the corresponding vehicle (saline) sessions. This 7-OH-DPAT-induced decrease in response rate is consistent with many earlier reports examining the discriminative stimulus properties of other dopamine agonists such as apomorphine, piribedil, and SKF38393 (1,4,11).

The dopamine D_3 receptor is more abundant in brain regions associated with the efficacy of antipsychotic drugs (e.g., limbic dopamine projection areas) than in regions associated with their motor side effects (e.g., dorsal striatum) (5,6,10), suggesting that D_3 receptors might constitute a novel target for the treatment of psychoses or for Parkinson's disease. 7-OH-DPAT, as a potent and selective ligand at the dopamine D_3 receptor, may prove to be a useful tool in furthering our understanding of the functional role of the D_3 subtype dopamine receptor in these and other neuronal and neuroendocrine disorders. However, the precise mechanism by which 7-OH-DPAT produces its discriminative stimulus remains to be established. Stimulus generalization and antagonism studies employing agents highly selective for dopamine and nondopamine receptors may better define the pharmacological basis of the 7-OH-DPAT discriminative stimulus.

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REFERENCES

1. Colpaert, F. C.; Leysen, J. E. M.; Niemegeers, C. J. E.; Janssen, P. A. J. Blockade of apomorphine's discriminative stimulus properties: Relation to neuroleptic activity in neuropharmacological and biochemical assays. Pharmacol. Biochem. Behav. 5:671–679; 1976.
2. Daly, S. A.; Waddington, J. L. Behavioral effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other "D-2-like" agonists. Neuropharmacology 32:509–510; 1993.
3. Harris, R. T.; Balster, R. L. Discriminative control by dl-amphetamine and saline of lever choice and response patterning. Psychonom. Sci. 10:105–106; 1968.
4. Kamien, J. B.; Goldberg, L. I.; Woolverton, W. L. Discriminative stimulus properties of D_1 and D_2 dopamine agonists in rats. J. Pharmacol. Exp. Ther. 242:804–811; 1987.
5. Levant, B.; Grigoriadis, D. E.; DeSouza, E. B. [^{3}H]Quinpirole binding to putative D_2 and D_3 dopamine receptors in rat brain and pituitary gland: A quantitative autoradiographic study. J. Pharmacol. Exp. Ther. 264:991–1001; 1993.
6. Levesque, D.; Diaz, J.; Pilon, C.; Martres, M.-P.; Giros, B.; Souil, E.; Schott, D.; Morgat, J.-L.; Schwartz, J.-C., Sokoloff, P. Identification, characterization, and localization of the dopamine D_3 receptor in rat brain using 7-[^{3}H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc. Natl. Acad. Sci. U. S. A. 89:8155–8159; 1992.
7. McDermed, J. D.; McKenzie, G. M.; Freeman, H. S. Synthesis and dopaminergic activity of (+)-, (+)-, and (−)-2-dipropylamino-5-hydroxy-1,2,3,4-tetrahydronaphthalene. J. Med. Chem. 19:547–549; 1976.
8. McElroy, J. F.; Amy, K. A.; Ward, K. A.; Zeller, K. L.; Cawley, J. F.; Mazzola, A. L. In vivo agonist properties of 7-OH-DPAT, a dopamine D3-selective receptor ligand. Soc. Neurosci. Abstr. 19:1064; 1993.
9. McElroy, J. F.; O'Donnell, J. M. Discriminative stimulus properties of clenbuterol: Evidence for beta adrenergic involvement. J. Pharmacol. Exp. Ther. 245:155–163; 1988.
10. Sokoloff, P.; Giros, B.; Martres, M.-P.; Bouthenet, M.-L.; Schwartz, J.-C. Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 347: 146–151; 1990.
11. Woolverton, W. L.; Kamien, J. B.; Goldberg, L. I. Effects of selective dopamine receptor agonists in rats trained to discriminate apomorphine from saline. Pharmacol. Biochem. Behav. 22: 577–581; 1985.