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DISCRIMINATIVE STIMULUS PROPERTIES OF THE SEROTONERGIC AGENT 1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI)

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Summary

Using a two-lever drug discrimination procedure, six rats were trained to discriminate 0.5 mg/kg of racemic 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) from saline. Once trained, the animals demonstrated a dose-related decrease in discriminative performance upon administration of lower doses of DOI (ED50 = 0.16 mg/kg). DOI-stimulus generalization occurred with the putative 5-HT_2 agonist DOM (ED50 = 0.49 mg/kg), but not with the 5-HT_{1A} agonist 8-OH DPAT, or the 5-HT_{1B} agonist TFMPP. Furthermore, the DOI stimulus could be antagonized by pretreatment of the animals with the 5-HT_2 antagonist ketanserin. The present results, coupled with the prior demonstration that DOI possesses a significant affinity and selectivity for 5-HT_2 binding sites, suggest that the discriminative stimulus effects of DOI may be 5-HT_2-mediated.

Multiple populations of central serotonin (5-HT) binding sites have been reported. 5-HT_1 sites, which predominate in rat hippocampus, striatum, and frontal cortex, are labeled by [^{3}H]5-HT, whereas 5-HT_2 sites, the highest density of which is found in the frontal cortex, are labeled by [^{3}H]spiperone and [^{3}H]ketanserin (1). 5-HT_1 sites appear to be heterogeneous and have been further subdivided into 5-HT_{1A} and 5-HT_{1B} sites (2). The serotonin agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) display selectivity for 5-HT_{1A} and 5-HT_{1B} sites, respectively (3,4).

Whereas a considerable amount of work has been done on 5-HT_2 antagonists, relatively little is known concerning 5-HT_2 agonists. We have recently reported that certain 1-(2,5-dimethoxyphenyl)-2-aminopropane derivatives might constitute the first examples of 5-HT_2-selective agonists (5,6). Such agents include the 4-methyl derivative DOM and the 4-iodo derivative DOI. Racemic DOM (5-HT_2 Ki = 100 nM) displays a 30-fold selectivity, and racemic DOI (Ki = 19 nM) a 100-fold selectivity, for 5-HT_2 vs 5-HT_1 sites (6). In a continuing effort to develop site-selective serotonergic agents, and correlates between binding and pharmacological activity, we have trained animals to discriminate the 5-HT_{1A} agonist 8-OH DPAT (7) and the 5-HT_{1B} agonist TFMPP (8,9) from saline. In this present study, we examined the ability of the more selective, putative 5-HT_2 agonist DOI to serve as a discriminative stimulus in rats.

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Methods

Subjects: The animals used in this study were six male Sprague-Dawley (225-300 g) rats. All animals were housed individually and had unlimited access to drinking water. The animals were maintained at approximately 80% of their free-feeding weights by partial food deprivation.

Discrimination Training: The animals were trained to lever-respond to a variable interval (VI) 15-sec schedule of reinforcement on both levers of a standard two-lever operant chamber (Coulbourn Instruments Model E 10-10). The reinforcer was reconstituted powdered milk. Training sessions (15 min) were conducted six days per week. Once the animals learned to press the levers, they were administered either 0.5 mg/kg of DOI or 1.0 ml/kg of 0.9% saline via intraperitoneal (i.p.) injection 15 min prior to a session. DOI and saline were administered using a double alternation sequence (i.e. two days DOI, two days saline) with the constraint that no more than two consecutive sessions could occur under the same treatment condition. Thus, a block of sessions would consist of two drug- and two saline-training sessions. Responses on the right lever were reinforced after administration of DOI for half the animals and on the left lever for the other half of the animals. Responses on the opposite lever were reinforced after administration of saline. After approximately eight weeks of training, discrimination learning was occasionally assessed during an initial 2.5-min non-reinforced (extinction) period (followed by a 12.5-min training session). Data collected during the extinction session included total responses (mean responses/min) and percent responding on the DOI-appropriate lever (i.e. number of responses on the DOI-designated lever divided by total number of responses). The animals were considered to have learned the discrimination task when the group consistently made greater than 80% of their responses on the DOI-appropriate lever after administration of DOI, and less than 20% of their responses on this same lever after administration of saline, in three consecutive test (i.e. extinction) sessions (see Fig 1).

Generalization Studies: Once the animals had learned to discriminate DOI from saline, tests of stimulus generalization were conducted. During these test sessions, the animals were allowed 2.5 min of non-reinforced lever responding and were then returned to their home cages. Training was continued during the generalization studies; animals not meeting criteria (i.e. animals not making at least 80% of their responses on the DOI-appropriate lever after administration of 0.5 mg/kg of DOI, or making greater than 20% of their responses on the same lever after administration of 1.0 ml/kg of saline) were not used in the immediately subsequent generalization test session. Tests of stimulus generalization were conducted once per week; no animal received more than one drug treatment per day. The first generalization tests investigated the dose-response and time-duration parameters of the DOI stimulus. Doses of DOI were administered (i.p.) in a random sequence using a 15-min pre-session injection interval. In the time-course study, 0.5 mg/kg of DOI was administered using pre-session injection intervals of 15 to 480 min; variation of pre-session injection intervals was done in a random manner and in separate experiments over a 6-week period. In the next series of studies, the ability of the DOI-stimulus to generalize to each of several agents was investigated. A 15-min pre-session injection interval was used throughout, and doses of challenge drugs were administered (i.p.) in a semi-random sequence. That is, the sequence was randomized, but if a particular dose of a given agent resulted in disruption of behavior, only lower doses of that agent would be examined in subsequent test sessions. In the final study, 0.5 mg/kg of DOI (or 1.0 ml/kg of saline) was administered to animals pre-treated (45 min prior to administration of DOI or saline) with various doses of ketanserin. All studies were conducted using a 2.5-min extinction session. Animals not making a total of five responses during an extinction session were reported as being disrupted; stimulus generalization was considered to have occurred when DOI-appropriate responding exceeded 80%. Where generalization occurred, ED50

values (i.e. doses at which the animals would be expected to make 50% of their responses on the DOI-appropriate lever) were calculated by the method of Finney (10).

Drugs: Racemic 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) and its optical isomers R(-)- and S(+)-DOI hydrochloride, DOM hydrochloride, and TFMPP hydrochloride were synthesized in our laboratory. 8-OH DPAT hydrobromide was purchased from Research Biochemicals Inc.; ketanserin tartrate and (+)-lysergic acid diethylamide tartrate (LSD) were gifts from Janssen Pharmaceutica and NIDA, respectively. All solutions were prepared fresh daily in 0.9% sterile saline.

Results and Discussion

Six animals were trained to discriminate 0.5 mg/kg of racemic DOI from saline; after thirty blocks of sessions (see learning curve, Fig. 1) the animals consistently made greater than 80% of their responses on the DOI-appropriate lever after administration of DOI, and fewer than 20% of their responses on this same lever after administration of saline. Response rates under DOI or saline conditions were not significantly different (Table 1). DOI, as shown in Fig. 2, is a rather long-acting agent. Ninety minutes after administration of the training dose of the training drug, the animals made 89% of their responses on the DOI-appropriate lever. It is not until 8 hours post-administration that saline-appropriate responding is observed.

The results of the dose-response study are presented in Table 1; an ED50 dose of 0.16 mg/kg was obtained for racemic DOI. In tests of stimulus generalization, the DOI-stimulus generalized to racemic DOM (ED50 = 0.49 mg/kg), but failed to generalize to either 8-OH DPAT or TFMPP (Table 1). DOI-stimulus generalization was also obtained with both optical isomers of DOI, R(-)-DOI (ED50 = 0.15 mg/kg) and S(+)-DOI (ED50 = 0.34 mg/kg), and with (+)-LSD (ED50 = 0.047 mg/kg). Administration of various doses of ketanserin in combination with 0.5 mg/kg of DOI resulted in a dose-related decrease in DOI-appropriate responding. Administration of ketanserin in combination with saline (data not shown) never resulted in greater than 25% DOI-appropriate responding. Response rates were severely depressed after administration of ketanserin; administration of 0.1 mg/kg of ketanserin in combination with either DOI or saline resulted in disruption of behavior. 8-OH DPAT, TFMPP, and DOM are reported to be site-selective 5-HT agonists (see introduction) with selectivity for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 sites, respectively. It is significant that the DOI-stimulus generalized to DOM, but not to either 8-OH DPAT or TFMPP (Table 1). All doses of the latter two agents resulted in saline-appropriate responding (except where disruption of responding occurred).

It might be noted that the doses employed for 8-OH DPAT and TFMPP are reasonable and are in a behaviorally effective range in that 0.2 mg/kg of the former and 0.5 mg/kg of the latter have been used as training doses and serve as effective discriminative stimuli (7,9). Ketanserin is an effective 5-HT_2 antagonist (1,5). Administration of various doses of ketanserin in combination with DOI resulted in a dose-related antagonism of the DOI stimulus (Table 1). Although ketanserin displays an appreciable affinity for dopaminergic, adrenergic and histaminergic binding sites (11), DOI does not (12). These results, coupled with the affinity of DOI for 5-HT_2 binding sites, suggest that the DOI-stimulus might be 5-HT_2 mediated.

DOM is a hallucinogenic agent in humans and serves as a discriminative stimulus in animals (13). The DOM-stimulus generalizes with agents that possess a high affinity for 5-HT_2 sites, but not with agents that display selectivity for 5-HT_{1A} or 5-HT_{1B} sites (14,15). Furthermore, the stimulus effects of DOM can be antagonized by any one of several 5-HT_2 antagonists,

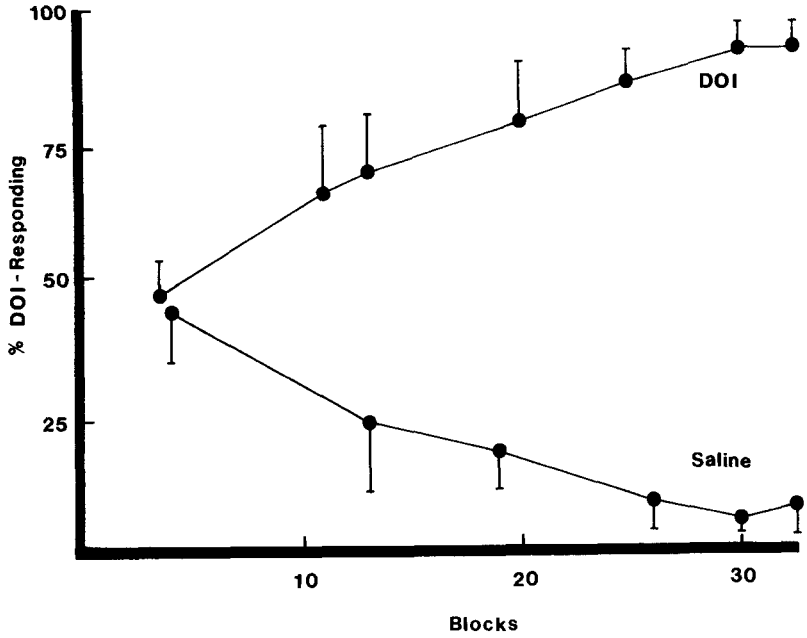

FIGURE 1. Learning curve for the acquisition of the DOI (0.5 mg/kg) vs saline (1.0 ml/kg) (n = 6 animals at each point) discrimination. The Y-axis = responses on the DOI appropriate lever (as a percent of total responses) during 2.5-min tests under extinction conditions; X-axis = blocks of successive double-alternation sessions.

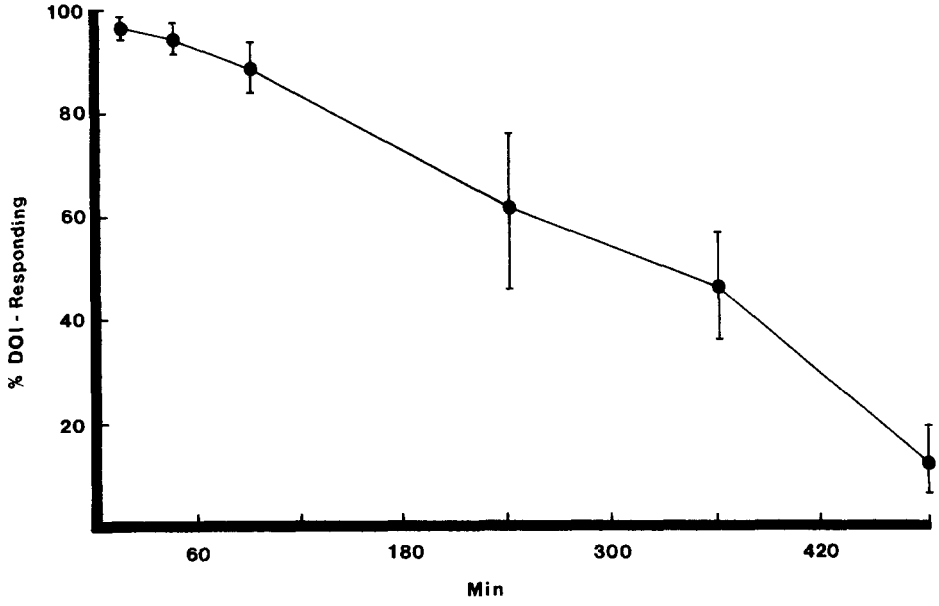

FIGURE 2. Time-course of 0.5 mg/kg of DOI administered to animals trained to discriminate DOI vs saline; min = pre-session injection interval (n = 3-4 animals at each point).

Table 1

Results of stimulus generalization and antagonism studies in animals trained to discriminate racemic DOI (0.5 mg/kg) from saline.

Agent	Dose (mg/kg)	N[a]	DOI-Appropriate Responding (±SEM)[b]	Mean Responses Per Min (±SEM)[b]
(±)-DOI	0.5	6/6	93% (± 4)	17.1 (± 4.6)
Saline (1.0 ml/kg)		6/6	8% (± 3)	18.2 (± 2.6)
(±)-DOI	0.10	3/3	34% (± 6)	14.2 (± 4.2)
	0.25	5/5	62% (± 3)	19.6 (± 7.9)
	0.30	4/4	70% (± 12)	15.1 (± 5.0)
	0.50	5/5	94% (± 4)	18.6 (± 3.9)
		ED50 = 0.16 (0.07 - 0.33)[c] mg/kg		
R(-)-DOI	0.05	3/3	11% (± 6)	10.3 (± 0.2)
	0.10	3/3	39% (± 18)	8.9 (± 0.9)
	0.20	6/6	50% (± 15)	6.0 (± 2.1)
	0.40	3/4	84% (± 9)	5.2 (± 0.4)
		ED50 = 0.15 (0.07 - 0.36) mg/kg		
S(+)-DOI	0.10	3/3	1% (± 1)	12.3 (± 6.1)
	0.20	3/3	38% (± 10)	16.9 (± 8.2)
	0.50	3/3	54% (± 4)	15.3 (± 2.5)
	0.80	3/3	70% (± 3)	8.0 (± 1.5)
	1.10	3/5	86% (± 12)	11.8 (± 2.0)
	1.20	3/3	98% (± 2)	4.4 (± 0.6)
		ED50 = 0.34 (0.14 - 0.84) mg/kg		
(±)-DOM	0.3	3/3	22% (± 7)	9.2 (± 2.3)
	0.6	3/3	67% (± 5)	10.5 (± 4.7)
	1.0	4/4	83% (± 7)	13.2 (± 6.8)
	1.2	4/5	100%	3.5 (± 0.7)
	1.5	1/3	[d]	
		ED50 = 0.49 (0.22 - 0.98)[c] mg/kg		
8-OH DPAT	0.05	4/4	21% (± 5)	18.1 (± 5.7)
	0.10	4/5	4% (± 3)	19.0 (± 3.0)
	0.20	3/3	3% (± 2)	17.6 (± 3.9)
	0.30	1/3	[d]	
TFMPP	0.30	3/3	8% (± 3)	11.3 (± 3.0)
	0.40	2/3	9% (± 5)	6.5 (± 3.3)
	0.50	1/3	[d]	
	0.80	1/3	[d]	
(+)-LSD	0.03	3/4	1% (± 1)	9.3 (± 3.7)
	0.04	5/5	40% (± 11)	12.8 (± 3.2)
	0.06	3/4	83% (± 12)	3.1 (± 0.6)
		ED50 = 0.047 (0.036 - 0.061) mg/kg		
(±)-DOI[e] + KET	0.02	6/6	84% (± 5)	8.0 (± 1.7)
KET	0.03	3/3	51% (± 8)	8.6 (± 3.0)
KET	0.05	4/6	28% (± 7)	3.2 (± 0.6)
KET	0.10	1/3	[d]	

[a] Number of animals responding/number receiving drug. [b] Data obtained during 2.5-min extinction session. [c] ED50 value followed by 95% confidence limits. [d] Disruption of behavior (i.e. no responding). [e] DOI(0.5 mg/kg) administered to animals pretreated with various doses of ketanserin (KET).

including ketanserin, pirenperone, LY 53,857 and ritanserin (14). This has led us to propose that the stimulus effects of DOM and DOM-related agents may be a consequence of 5-HT_2 agonism (5,14). Indeed, there exists a significant correlation between discrimination-derived ED50 values and the 5-HT_2 binding affinities (i.e. Ki values) of a series of such agents (15). DOI possesses a greater affinity and selectivity for 5-HT_2 sites than does DOM (6). As a consequence, it was felt that that DOI might serve as a more selective training drug in tests of stimulus control of behavior in animals.

The DOI-stimulus, like the DOM-stimulus, appears to be 5-HT_2-mediated as determined by stimulus generalization to DOM, lack of stimulus generalization to 8-OH DPAT and TFMPP, and stimulus antagonism by ketanserin. Parallels can also be seen with binding data. For example, the enantiomeric (S/R) potency ratio for the isomers of DOI is 2 in the generalization tests, and 3 with respect to binding at 5-HT_2 sites (12). Furthermore, (+)-LSD possesses approximately ten times the affinity of racemic DOI for 5-HT_2 sites (15) and is about ten times more potent (on a molar basis) than DOI in the DOI-trained animals. As such, animals trained to discriminate DOI from saline might be useful for the evaluation of novel site-selective serotonin agonists and antagonists.

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