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Mechanistic Studies on DOM as a Discriminative Stimulus

RICHARD A. GLENNON AND AMY E. HAUCK

Department of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia Virginia Commonwealth University, Richmond, VA 23298

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GLENNON, R. A. AND A. E. HAUCK. *Mechanistic studies on DOM as a discriminative stimulus.* PHARMACOL BIOCHEM BEHAV **23**(6) 937–941, 1985.—Ten rats were trained to discriminate racemic DOM (1.0 mg/kg, IP) from saline using a standard two-lever operant procedure. Once responding was stable, these animals were administered doses of lisuride and the purported 5-HT_1 agonist 8-OH DPAT in tests of stimulus generalization. DOM-stimulus generalization occurred with lisuride, but not with 8-OH DPAT. These animals were also administered doses of LY-53,857, ritanserin, CP-52,215, and THT in tests of stimulus antagonism. Each of these agents possesses a significant affinity for 5-HT_2 binding sites, and each effectively attenuated the DOM-stimulus. These results, coupled with our earlier findings, support the hypothesis that DOM may be producing its stimulus effects via a 5-HT_2-related mechanism.

DOM Discriminative stimulus 8-OH DPAT Lisuride Serotonin 5-HT_1 5-HT_2 Stimulus generalization Stimulus antagonism

THE hallucinogenic agent 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) serves as an effective stimulus in animals in tests of discriminative control of behavior [5]. Over the past several years, a part of our research effort has been directed toward elucidating the mechanism of action of DOM and related agents as discriminative stimuli; current evidence supports the idea that these agents may be acting as agonists at central serotonin (5-HT) sites [3]. Based on the structural similarity of DOM and amphetamine, it was once thought that DOM might act via a dopaminergic mechanism. However, Silverman and Ho [23] demonstrated that dopamine antagonists would effectively attenuate the stimulus effects of amphetamine (but not those of DOM), whereas 5-HT antagonists would attenuate the effects of DOM (but not those of amphetamine). Subsequently, we reported that the 5-HT_2-selective antagonists ketanserin and pirenperone were particularly effective in blocking the stimulus effects of DOM [6]. Conversely, in animals trained to discriminate DOM from saline, DOM-stimulus generalization was shown to occur to the 5-HT agonist quipazine, but not to the purported 5-HT_1 agonists 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and RU-24,969 [6]. These studies suggested that DOM may be acting through a 5-HT_2 mechanism. However, in addition to its affinity for 5-HT_2 sites, quipazine also displays some affinity for 5-HT_1 sites [14]. It has also been reported that TFMPP and RU-24,969 may act selectively at a particular subpopulation of 5-HT_1 sites (i.e., 5-HT_{1B} sites) [18,22]. If this is the case (although it should be noted that there is some lack of agreement as to the existence of distinct 5-HT_{1A} and 5-HT_{1B} sites), it is possible that the mechanism of action of DOM may yet involve a 5-HT_1 component. Further, although ketanserin and pirenperone are relatively selective antagonists (for 5-HT_2 vs. 5-HT_1), they are not specific for serotonin. That is, these agents display significant affinities for other (particularly dopaminergic and α-adrenergic) neurotransmitter binding sites [15]; because of the structural similarity between these two antagonists, they possess rather similar binding profiles [11].

The purpose of the present investigation was to further study the role of serotonin in the mechanism of action of DOM as a discriminative stimulus. The effects of lisuride, an agent known to possess serotonergic character, and 8-OH DPAT, a purported 5-HT_{1A} agonist [17], was examined in tests of stimulus generalization using rats trained to discriminate DOM from saline. The effects of several structurally-dissimilar antagonists (that display different binding profiles, but that are all known to interact at 5-HT_2 sites) was also examined in tests of stimulus antagonism.

METHOD

The animals used in this study were ten male Sprague-Dawley rats, and their body weights were maintained at approximately 80% of free-feeding weight by partial food deprivation. Behavioral testing was conducted in standard operant chambers (Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) housed within light- and sound-attenuating outer chambers. One wall of each operant chamber was fitted with two levers and a dipper (centered between the levers) for delivery of reinforcement (0.01 ml of sweetened milk). The recessed area in which the dipper was located was illuminated by a white light when the dipper was activated. Illumination of each chamber was provided by an overhead 28 V houselight. Solid state and electromechanical programming and recording equipment were used, and these were housed in the same room as the operant chambers.

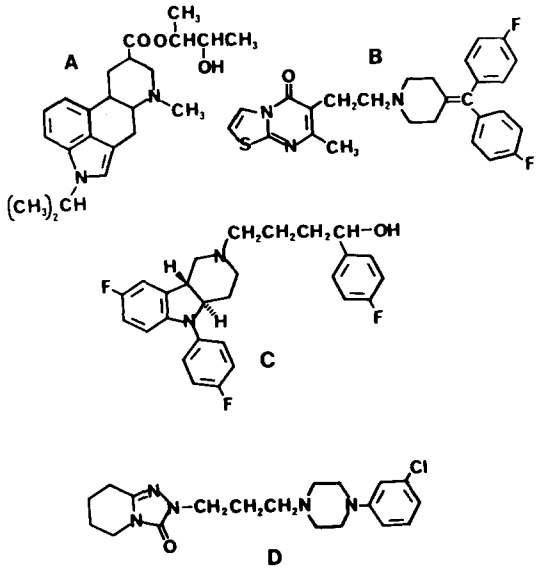

FIG. 1. Structures of antagonists used in the present study: (A) LY-53,857, (B) ritanserin, (C) CP-52,215, (D) THT.

Discrimination Procedures

All rats were trained to respond on both levers for sweetened milk under a variable interval 15-second (VI-15 sec) schedule of reinforcement. After lever-responding was established, each daily session was preceded by an intraperitoneal (IP) injection of either racemic DOM hydrochloride (1.0 mg/kg) or 0.9% saline (1.0 ml/kg). A pre-session injection interval of 15 min was employed; during this period (i.e., following injection) the animals were returned to their individual home cages for 15 min prior to being placed in the operant chamber. Training sessions were of 15 min duration. Responding on one of the levers was reinforced after administration of drug, while responding on the opposite lever was reinforced after administration of saline; treatment conditions were counterbalanced within each group. Saline or drug was administered on a double-alternation schedule (i.e., two days saline, two days drug). On every fifth day, discrimination learning was assessed during an initial 2.5-min non-reinforced (extinction) period, followed by a 12.5-min training session. Data collected during the extinction period included total responses (expressed as mean responses per min) and percent DOM-appropriate responding (i.e., number of responses on the DOM-designated lever as a percent of the total number of responses). After thirty training sessions, discrimination performance was stable under each treatment condition. That is, DOM-appropriate responding was greater than 80% after administration of 1.0 mg/kg of DOM, and less than 20% after administration of 1.0 ml/kg of saline.

Stimulus Generalization Studies

Maintenance of the DOM/saline discrimination was insured in all ten animals by continuation of training sessions throughout the generalization testing period. Discrimination training sessions were conducted with DOM or 1.0 ml/kg of saline during the two days prior to any generalization test. On one of these days, half the animals would receive training drug (i.e., DOM) whereas the other half would be administered saline; after a 2.5-min non-reinforced session, training was continued for an additional 12.5 min. Animals not discriminating drug (i.e., less than 80% DOM-appropriate responding when given the training dose of DOM) from saline (i.e., more than 20% DOM-appropriate responding when given saline) were excluded from the immediately subsequent generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the discrimination training sessions. The animals were allowed 2.5 min to respond under non-reinforcement conditions; the animals were then removed from the operant chamber and returned to their home cages. An odd number of training sessions (not less than three) separated any two test sessions. Generalization tests investigated the ability of the DOM stimulus to generalize to the various challenge drugs. Doses of these agents were administered in a random sequence using a 15-min pre-session injection interval. Stimulus generalization was defined, in this study, as being 80% or greater DOM-appropriate responding. That is, stimulus generalization was said to occur when the animals, after given a dose of challenge drug, made 80% or greater of their total responses on the DOM-appropriate lever. Animals

TABLE 1

RESULTS OF GENERALIZATION STUDIES USING DOM-TRAINED ANIMALS*

Agent	Dose (mg/kg)	N†	DOM-Appropriate Responding (±SEM)	Mean Resp/Min (±SEM)
(±)-DOM	0.2	8/8	21% (4)	15.3(3.3)
	0.4	8/8	36% (8)	12.9(2.5)
	0.6	8/8	59%(10)	12.5(1.8)
	0.8	7/8	75% (8)	12.2(4.8)
	1.0	8/8	93% (3)	13.4(4.7)
			ED_{50}=0.44 (0.28–0.68) mg/kg§	
(±)-8-OH DPAT	0.1	4/4	9% (4)	11.3(3.1)
	0.25	4/4	25% (4)	14.5(5.3)
	0.33	4/5	46% (2)	7.5(3.0)
	0.37	5/5	21% (3)	12.1(4.2)
	0.40	1/4	—‡	
Lisuride	0.08	5/5	36%(15)	11.0(2.8)
	0.16	5/5	40%(16)	14.2(2.0)
	0.32	5/5	46%(10)	10.5(3.1)
	0.48	5/5	55%(26)	11.3(1.5)
	0.56	4/5	73%(16)	11.0(3.1)
	0.64	4/5	92% (6)	10.0(1.1)
			ED_{50}=0.21 (0.09–0.48) mg/kg	
Saline (1.0 ml/kg)	10/10		11% (2)	13.5(2.5)

*Data obtained during 2.5-min extinction session.

†Number of animals responding/number of animals to receive drug.

‡Disruption of behavior (i.e., no responding).

§ED_{50} value followed by 95% confidence limits in parenthesis.

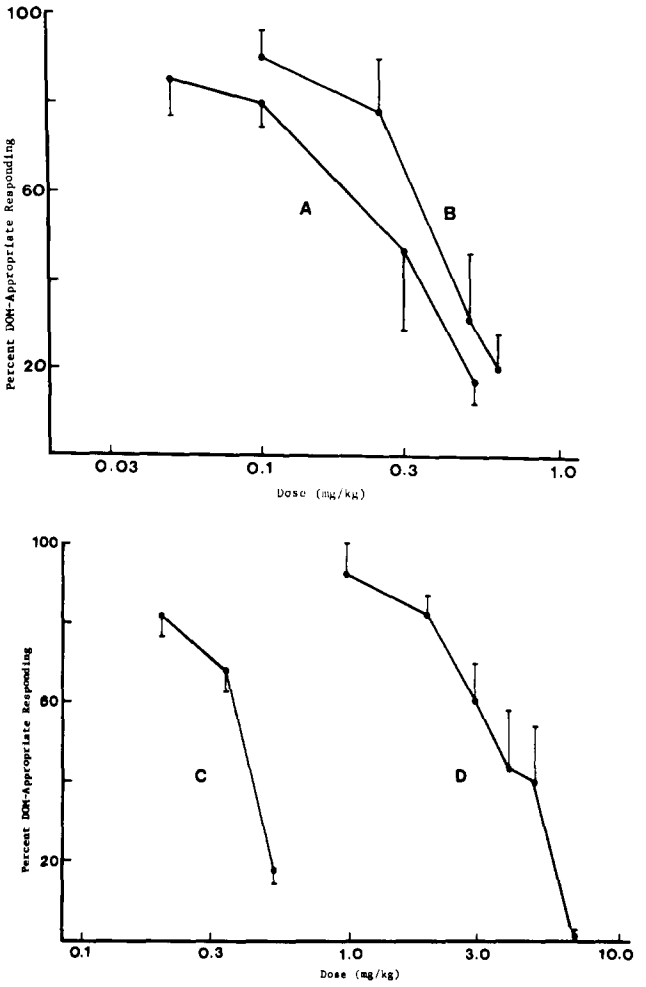

FIG. 2. Dose-response curve for 1.0 mg/kg of DOM in the presence of various concentrations of LY-53,857 (A), ritanserin (B), CP-52,215 (C), and THT (D).

making less than five total responses during the entire 2.5-min extinction session were reported as being disrupted. For those compounds where generalization occurred, ED_{50} values were determined from the dose-response data by the method of Finney [2]. These ED_{50} values are doses at which the animals would be expected to make approximately 50% of their responses on the DOM-appropriate lever.

Stimulus Antagonism Studies

The DOM/saline discrimination was maintained as described in the previous section. During the stimulus antagonism studies, a dose of antagonist was administered in combination with 1.0 mg/kg of DOM in order to determine the effect on responding. Doses of LY-53,857, ritanserin and CP-52,215 were administered 45 min prior to administration of DOM; doses of THT were administered 25 min prior to administration of DOM. Fifteen minutes after administration of DOM, the animals were placed in the operant chambers for a 2.5-min extinction session. Each data point represents the responding of 4–6 animals. In a separate series of control studies, the antagonists were examined in combination with 1.0 ml/kg of saline in place of DOM; the pre-session injection intervals were the same as described above. The structures of the four antagonists are shown in Fig. 1. The animals were routinely administered a dose of any given antagonist only once during any one week period.

Drugs

The following agents were received as gifts: (±)-1(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) (NIDA), 8-hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide (8-OH DPAT) (Merrell Dow), lisuride hydrogen maleate (Schering AG), 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6a,7,8,9,10,10a-octahydro-indolo[4,3-fg]quinoline maleate (LY-53857) (Eli Lilly), ritanserin (Janssen Pharmaceutica), 2-(3-(4-(3-chlorophenyl)-1-piperazinyl)propyl)-s-triazolo [4,3-a]pyridin-3(2H)-one hydrochloride (THT) (Bristol Myers), and 8-fluoro-5-(4-fluorophenyl)-2-(4-(4-fluorophenyl)-4-hydroxybutyl)-1a,2,3,4,4a,5-hexahydro-1H-pyrido[4,3-b]indole hydrochloride (CP-52,215) (Pfizer). Solutions of all agents were prepared fresh daily in sterile 0.9% saline and were administered via IP injection.

RESULTS

Ten animals were trained to discriminate 1.0 mg/kg of DOM from saline. The animals response rates after administration of 1.0 mg/kg of DOM (13.4 responses per min) were not different from those observed after administration of 1.0 ml/kg of saline (13.5 responses per min). Although we have previously demonstrated a dose-response relationship for DOM in DOM-trained animals, an ED_{50} was re-calculated using the present data (ED_{50}=0.44 mg/kg) and was found to be identical to that which we had reported earlier [27]. Doses of 8-OH DPAT and lisuride were administered in tests of stimulus generalization (Table 1). 8-OH DPAT produced a maximum of 46% DOM-appropriate responding (at 0.33 mg/kg); 0.37 mg/kg of 8-OH DPAT resulted in saline-appropriate responding, and 0.4 mg/kg resulted in disruption of behavior (i.e., only one of four animals responded). Lisuride produced a rather shallow dose-response curve over the dose range of 0.08 to 0.48 mg/kg, but produced 73% and 92% DOM-appropriate responding at 0.56 and 0.64 mg/kg, respectively. Response rates after administration of lisuride were not significantly different from those obtained after administration of either the training dose of DOM and/or 1.0 ml/kg of saline.

In the stimulus antagonism studies, all four agents, in combination with the training dose of DOM, resulted in dose-related decreases in percent-correct responding such that the highest dose of each agent produced≤20% DOM-appropriate responding (Fig. 2). Response rates were similar to those produced by DOM (or saline) alone, except that 7.0 mg/kg of THT plus 1.0 mg/kg of DOM resulted in a rate of 4.0 (±0.4) responses per min. In the control studies (data not shown), administration of doses of these four agents in combination with saline resulted in DOM-appropriate responding that never exceeded 20%; response rates were similar to those obtained with saline alone, except that 7.0 mg/kg of THT plus 1.0 ml/kg of saline produced a response rate of 5.1 (±1.4) responses per min.

DISCUSSION

Although centrally-acting agents may interact at more

than one amine site, one site may predominate in terms of an animal's ability to discriminate a given agent [4]. There is considerable evidence to suggest that DOM produces its stimulus effects via a serotonergic mechanism [3]; thus, animals trained to discriminate DOM from saline should be able to recognize other agents (barring disruption of behavior) that produce similar serotonergic effects. Lisuride, generally regarded as a dopaminergic agent, binds nearly equally well at 5-HT_1 and 5-HT_2 sites with an affinity comparable to that of LSD [19]. Lisuride also produces "serotonin syndrome" and head-shake behavior in rodents [21], effects thought to be manifestations of 5-HT_1 and 5-HT_2 agonism, respectively [16]. Similar effects are observed in cats [8,26]. As shown in Table 1, the DOM-stimulus generalized to lisuride. Because there is no evidence that DOM produces its stimulus effects via a dopaminergic mechanism, it is assumed that the DOM-trained animals apparently recognize the serotonergic aspects of lisurides actions.

We have previously shown that the DOM-stimulus does not generalize to the 5-HT_1 agonists TFMPP and RU-24969. 8-OH DPAT is a serotonin agonist that demonstrates selectivity for a subpopulation of 5-HT_1 sites (i.e., 5-HT_{1A} sites) [17], and as shown herein, the DOM-stimulus does not generalize to this agent. At 0.33 mg/kg, 8-OH DPAT did produce 46% DOM-appropriate responding suggesting that there might be some similarity between the stimulus properties of these agents. This partial generalization is difficult to interpret and may be the result of a lack of specificity; that is, these agents have been demonstrated to be site-selective, but they are not site-specific. Nevertheless, based on the inability of the DOM stimulus to generalize to 5-HT_1 agonists, it appears unlikely that DOM produces its stimulus effects via a 5-HT_1 mechanism. Conversely, the DOM-stimulus does generalize to R(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, an agent that possess a 250-fold selectivity for 5-HT_2 sites [7].

In the next phase of this study, tests of stimulus antagonism were conducted in order to further investigate a possible 5-HT_2 mechanism. LY-53,857 is a serotonin antagonist that displays minimal affinity for α-adrenergic receptors [1]. Although this agent interacts both at 5-HT_1 and [^{3}H]spiperone-labeled 5-HT_2 sites, it shows more than a 10-fold selectivity for the latter [9]. Ritanserin is a relatively new, and rather potent 5-HT_2 antagonist (IC_{50}=0.19 nM); although it bears some structural resemblence to ketanserin and pirenperone, it reportedly possesses a somewhat different binding profile [12]. CP-52,215, the 4a, 9b-dihydro analog of flutroline, displays a significant affinity for central dopamine and serotonin binding sites. However, whereas its affinity for dopamine sites is in the nanomolar range [20,25], it possess a still greater affinity (by more than two orders of magnitude) for [^{3}H]spiperone-labeled cortical 5-HT_2 sites (IC_{50}=0.1 nM) [20]. In an early report, it was suggested that tetrahydrotrazodone might be a 5-HT_1 antagonist [13]; however, subsequent studies with this agent revealed that it possesses a significant affinity for α-adrenergic and 5-HT_2 sites [10]. Its potency at [^{3}H]spiperone-labeled 5-HT_2 sites in frontal cortex (IC_{50}=80 nM) was about five-fold greater than its affinity for 5-HT_1 sites; in a direct comparison, it was determined to be approximately one-tenth as potent as ketanserin at 5-HT_2 sites [10]. All four of these agents share the property of possessing some degree of selectivity for 5-HT_2 binding sites. Other than for a brief report that CP-52,215 can antagonize the stimulus effects of LSD [24], none of these agents has been previously studied in tests of stimulus antagonism.

As shown in Fig. 2, each of these agents effectively attenuated the stimulus effects of DOM. These findings, coupled with our earlier work with ketanserin and pirenperone, support the hypothesis that DOM may act via a 5-HT_2-related mechanism.

In summary, the DOM-stimulus has been demonstrated to generalize to LSD [6] and lisuride, agents that interact equally well at 5-HT_1 and 5-HT_2 sites. The DOM-stimulus also generalizes to the 5-HT_2-selective agent R(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, but not to the purported 5-HT_1 agonists 8-OH DPAT, TFMPP and RU-24969. DOM itself possesses a significant affinity (Ki=100 nM) and 30-fold selectivity for 5-HT_2 (vs. 5-HT_1) sites [7]. The DOM-stimulus can also be effectively attenuated by pretreatment of the animals with ketanserin, pirenperone [6], pizotyline [27], LY-53,857, ritanserin, CP-52,215 and THT; although these agents display different binding profiles, they have in common the ability to act as 5-HT_2 antagonists. Thus, the results of these studies extend and support our earlier suggestion that DOM may produce its stimulus effects primarily via a 5-HT_2-related mechanism.

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