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ANTAGONISM OF THE EFFECTS OF THE HALLUCINOGEN DOM AND THE PURPORTED 5-HT AGONIST QUIPAZINE BY 5-HT$_2$ ANTAGONISTS

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Rats trained to discriminate 1.0 mg/kg of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) from saline in a two-lever operant choice task were administered doses of mescaline, LSD, 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT), quipazine, TFMPP and RU-24969. The DOM-stimulus generalized to the three hallucinogenic agents and to quipazine, but not to the purported serotonin agonists TFMPP or RU-24969. Pretreatment of the animals with the 5-HT$_2$ antagonists ketanserin and pirenperone antagonized the effect produced by DOM. Pirenperone also blocked DOM-stimulus generalization to mescaline, LSD, 5-OMe DMT and quipazine. The results of this study suggest that the discriminative stimulus effects of DOM, the three hallucinogenic agents to which DOM-stimulus generalization occurred, and quipazine, may involve those sub-populations of serotonin receptors that are labeled by tritiated ketanserin (i.e. 5-HT$_2$ sites).

Serotonin antagonists Discriminative stimulus Hallucinogenic agents Pirenperone Quipazine DOM

1. Introduction

Certain phenalkylamine and indolealkylamine hallucinogens serve as discriminative stimuli in animals; the discriminative stimulus effects produced by these agents can be effectively attenuated by pretreatment of the animals with various serotonin (5-hydroxytryptamine, 5-HT) antagonists (see Glennon et al., 1983a for a review). Of the antagonists studied up to this time, pizotyline (pizotifen, BC-105) is perhaps the most effective (Colpaert et al., 1982; Glennon et al., 1983a). The results of such antagonism studies have been taken as evidence that the discriminative stimulus effects produced by these hallucinogens may be mediated via a serotonergic mechanism, or may be the result of a direct interaction of these agents with central 5-HT receptors.

Several different in vitro biochemical models of 5-HT receptors have been described, these include (i) high-affinity binding sites for [^{3}H]5-HT in brain tissue preparations, and (ii) sites in frontal cortex preparations that are selectively labeled by [^{3}H]spiperone. These sites are referred to as 5-HT$_1$ sites and 5-HT$_2$ sites, respectively (Peroutka and Snyder, 1979). Leysen (1981) has studied these two binding sites and Leysen and coworkers (1981) have reported that a newly developed quinazoline derivative, ketanserin, possesses a high affinity for 5-HT$_2$ sites and is inactive at 5-HT$_1$ sites; [^{3}H]ketanserin may be a more suitable ligand than [^{3}H]spiperone for studying 5-HT$_2$ interactions, particularly where the latter tends to label certain dopamine sites (Leysen et al., 1982). This same group of investigators also found that ketanserin and pirenperone, a structurally related analog of ketanserin that possesses a similar 5-HT binding profile, are both capable of antagonizing mescaline-induced head twitches in rats (Leysen et al.,

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1982). Recently, Colpaert et al. (1982) have reported that pirenperone is a very potent antagonist of lysergic acid diethylamide (LSD) and that, at low doses, it completely antagonizes the effects of LSD in rats trained to discriminate LSD from saline.

The discriminative stimulus effects of the phenalkylamine hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) are antagonized by pizotyline (Young et al., 1981) and by other 5-HT antagonists, but not by the dopamine antagonist haloperidol (Silverman and Ho, 1980). Thus, it was of interest to determine if the new 5-HT_2 antagonists ketanserin and pirenperone would antagonize the effects of DOM. Secondly, in as much as DOM-stimulus generalization occurs to the phenalkylamine hallucinogen mescaline (Silverman and Ho, 1980; Glennon and Young, 1982), and to the indolealkylamine hallucinogens LSD (Glennon et al., 1983b) and 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT) (Young et al., 1981), the ability to block this stimulus generalization would be additional evidence for mechanistic similarity. Finally, if the discriminative stimulus properties of DOM involve a serotonergic mechanism, it might be possible to demonstrate DOM-stimulus generalization to purported 5-HT agonists; moreover, it might be possible to block this generalization with the 5-HT antagonists. The overall purpose of this study, then, was to investigate the possible role of certain sub-populations of 5-HT receptors that might be involved in the discriminative stimulus properties of several hallucinogenic agents, by utilizing two novel agents that have been demonstrated to interact selectively with 5-HT_2 binding sites.

2. Materials and methods

2.1. Subjects

The animals used in this study were 18 Sprague-Dawley male rats. The animals' weight was reduced to 80% of their free-feeding weights by partial food deprivation. The animals were individually housed and had free access to water.

2.2. Apparatus

Experiments were conducted using commercially available operant chambers (Coulbourn Instruments) that were housed within light and sound attenuated outer chambers. Each operant chamber contained two levers mounted at the same end of one wall; a single dipper that delivered 0.01 ml of sweetened condensed milk (diluted 2 : 1 with tap water) was positioned equidistant between the two levers. During all procedures, the chambers were illuminated by a single overhead 24 V house lamp. All programming and recording of data were done by solid-state and electromechanical equipment located in the same room.

2.3. Discrimination procedure

The drug discrimination training procedure used for these animals is essentially the same as has been previously reported (Young et al., 1981). In essence, the rats were trained to discriminate 1.0 mg/kg of racemic DOM from 1.0 ml/kg sterile saline using the two-lever operant choice task. Administration of DOM or saline 15 min prior to a variable interval 15 s (VI-15) schedule of reinforcement served as the discriminative cue for the correct (reinforced) lever. Occasional periods (extinction sessions) of 2.5 min duration, where responding on neither lever was reinforced, were used to assess the degree of stimulus control exerted by DOM and saline. Once a stable level of performance was attained, i.e. greater than 90% of the animals responses being made on the 'DOM-appropriate' lever after administration of 1.0 mg/kg of DOM and less than 10% of the total responses being made on the same lever after administration of 1.0 ml/kg of saline, generalization and antagonism studies were conducted. The degree of discriminative stimulus control exerted by DOM was assessed routinely throughout the course of the study. Those animals responding less than 90% on the DOM-appropriate lever after administration of a control dose of 1.0 mg/kg of DOM, or greater than 10% on the drug-lever after administration of saline, were not used for the generalization or antagonism studies to be conducted during that particular week.

2.4. Generalization studies

During these generalization (substitution test) studies, test sessions were interposed between discrimination training sessions. During these test sessions, the animals were allowed 2.5 min of non-reinforced lever responding and were then returned to their home cages. We have previously reported that administration of mescaline, LSD and 5-OMe DMT in a dose-related manner to DOM-trained animals results in stimulus generalization. For the purpose of this particular study, these dose response relationships were not replicated, however, those doses previously reported to produce stimulus generalization, i.e. 25 mg/kg for mescaline (Glennon and Young, 1982), 0.1 mg/kg for LSD (Glennon et al., 1983b) and 3.0 mg/kg for 5-OMe DMT (Young et al., 1981), were administered to groups of 5-6 animals, for comparative purposes (data shown in fig. 4 where antagonist concentration is 0). Doses of the 5-HT agonists quipazine and N-(3-trifluoromethylphenyl)-piperazine (TFMPP) were evaluated in a random order, during a 2.5 min extinction session, using, routinely, a 15 min pre-session injection interval (table 1). Where generalization occurred, the ED_{50} value was determined from the dose-response data by the method of Litchfield and Wilcoxon (1949). These ED_{50} values are estimated doses at which the rats would perform 50% appropriate drug-lever responding.

2.5. Antagonism studies

In a typical antagonism experiment, doses of either ketanserin or pirenperone were administered in a random sequence to groups of 4-6 rats, 45 min prior to the administration of drug, or, in the control studies, saline. Fifteen min later, discriminative control of responding was measured, i.e. percent of DOM-appropriate responding of total number of responses made during the 2.5 min extinction session. The time course for pirenperone (0.1 mg/kg) was investigated by varying the pre-session injection interval; 15 min prior to testing, 1.0 mg/kg of DOM was administered and discriminative control of responding was measured as discussed above. Antagonism of the DOM-stimulus generalization response was performed in a manner similar to that used for antagonism of DOM itself, except that either mescaline (25 mg/kg), LSD (0.1 mg/kg), 5-OMe DMT (2.0 mg/kg) or quipazine (3.0 mg/kg) was administered in place of DOM.

2.6. Drugs

Racemic 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) and (+)-lysergic acid diethylamide tartrate monomethanolate were gifts from NIDA, Washington, D.C., while mescaline hydrochloride was obtained from the Psychopharmacology Branch of NIMH. 5-Methoxy-N,N-dimethyltryptamine hydrogen oxalate (5-OMe DMT) was on hand as a result of earlier studies. Quipazine, i.e. 2-(1-piperazino)-quinoline maleate was a gift from Miles Laboratories Inc. and N-(3-trifluoromethylphenyl)-piperazine was purchased from Aldrich Chemical Co. and converted to its hydrochloride salt by standard methods. Pirenperone (R 47,465) and ketanserin (R 41,468) were gifts from Dr. F.C. Colpaert, Janssen Pharmaceutica, Beerse, Belgium. 5-Methoxy-3-(1,2,5,6-tetrahydropyridinyl)-indole (RU-24969) was a gift from Centre de Recherches, Roussell-Uclaf, Romainville, France (via Dr. D. Nelson). All agents were dissolved in sterile saline; solutions were prepared fresh daily and were administered by intraperitoneal injection.

3. Results

Eighteen animals were trained to discriminate DOM from saline such that administration of 1.0 mg/kg of DOM consistently resulted in greater than 90% DOM-appropriate responding. In concert with our previous results, administration of mescaline (25 mg/kg), LSD (0.1 mg/kg) and 5-OMe DMT (3.0 mg/kg) to the DOM-trained animals resulted in stimulus generalization (i.e. greater than 93% DOM-appropriate responding). Administration of the purported 5-HT agonist quipazine also resulted in DOM-stimulus generalization, while TFMPP and RU-24969 produced saline-like effects at 0.5-0.6 mg/kg and disruption

TABLE 1

DOM-stimulus generalization studies employing three purported serotonin agonists.

Agent/dose (mg/kg)	n [a]	% DOM-appropriate responding (±S.E.M.) [b,c]	mean responses min (±S.E.M.) [c]
Quipazine [d]			
1.0	5/5	5 (3.9)	12.0 (1.4)
1.5	5/5	44 (14.7)	14.7 (1.9)
2.0	5/5	65 (17.2)	16.2 (2.1)
3.0	5/5	95 (3.2)	16.5 (3.4)
TFMPP			
0.30	4/4	5 (2.3)	10.2 (1.8)
0.50	5/5	28 (10.6)	7.8 (2.2)
0.75	2/5	–	
1.00	0/3	–	
RU-24969			
0.50	5/5	6 (2.8)	12.0 (1.9)
0.60	3/5	22 (7.6)	7.3 (2.4)
0.65	1/5	–	
0.75	1/5	–	
1.00	0/5	–	
DOM			
1.0	6/6	96 (1.5)	14.6 (3.5)
Saline			
1.0 ml/kg	6/6	6 (1.9)	12.6 (4.4)

[a] Number of animals responding of number of animals receiving a particular dose of drug. [b] Responses on DOM-appropriate lever as a percent of total responses. [c] As measured during the 2.5 min extinction session. [d] ED_{50} value = 1.71 (1.26-2.33) mg/kg.

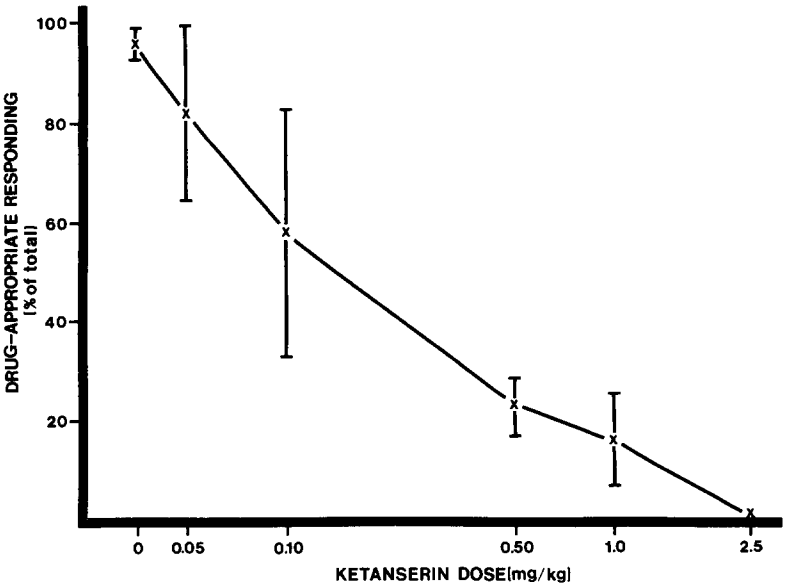

Fig. 1. The effect of various doses of ketanserin on DOM discriminative stimulus control of responding.

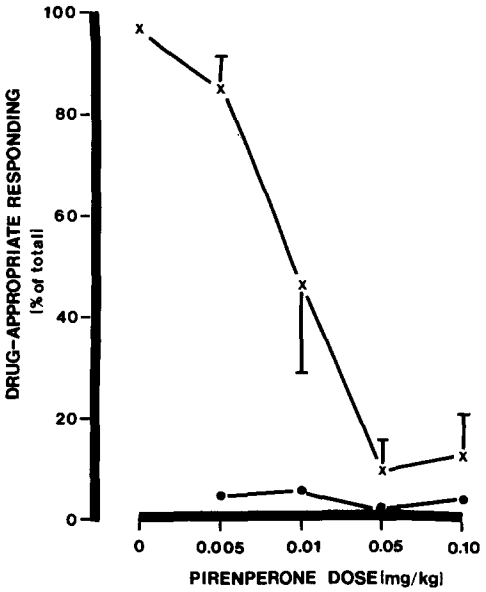

Fig. 2. The effect of various doses of pirenperone on DOM-appropriate responding when administered in combination with 1.0 mg/kg of DOM (×) or 1.0 ml/kg of saline (●).

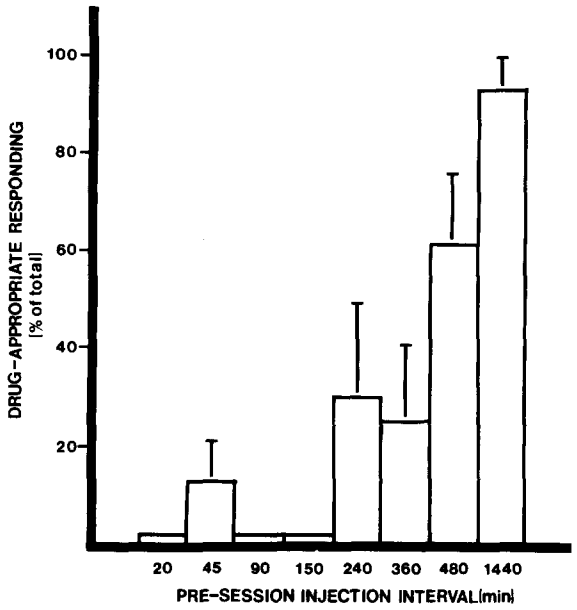

Fig. 3. The effect of various pre-session injection intervals for 0.05 mg/kg of pirenperone given in combination with 1.0 mg/kg of DOM.

of behavior (i.e. no responding) at higher doses (table 1). Using doses of 0.75 mg/kg, the pre-session injection intervals for TFMPP and RU-24969 were varied up to 45 and 120 min, respectively (data not shown), with essentially no difference in effect.

Pretreatment of the animals with ketanserin prior to administration of DOM resulted in a dose-related decrease in DOM-appropriate responding; at a dose of 2.5 mg/kg, ketanserin completely antagonized the effect of 1.0 mg/kg of DOM (fig. 1). Control doses of ketanserin (0.05-2.5 mg/kg) prior to administration of 1.0 ml/kg of saline, in place of DOM, resulted in less than 5% DOM-appropriate responding (data not shown); response rates following administration of ketanserin in combination with either DOM or saline were not significantly different from response rates observed after administration of DOM (or saline) alone. Similar effects were obtained with pirenperone. Pretreatment of the animals with doses of pirenperone greater than 5 μg/kg significantly antagonized the effect produced by 1.0 mg/kg of DOM (fig. 2). Doses of pirenperone in combination with saline produced $\leqslant$ 5% DOM-appropriate responding (fig. 2); pirenperone administration, in combination with either DOM or saline, had no effect on response rate at the doses employed. By varying the pre-session injection interval, it was determined that pirenperone possesses a rather extended duration of action (fig. 3). Even 6 h after administration of 0.05 mg/kg of pirenperone, the animals made less than 30% of their total responses on the DOM-appropriate lever 15 min after the administration of 1.0 mg/kg of DOM; the antagonist effects of pirenperone in combination with DOM were not evident 24 h after the administration of the antagonist.

Pirenperone was examined for its ability to block DOM-stimulus generalization to the hallucinogens mescaline, LSD and 5-OMe DMT. As shown in fig. 4, pirenperone was effective in blocking the stimulus generalization of all three compounds; slopes of the dose-response curves were parallel. Response rates after administration of pirenperone in combination with mescaline, LSD or 5-OMe DMT were not significantly different from that observed after administration of

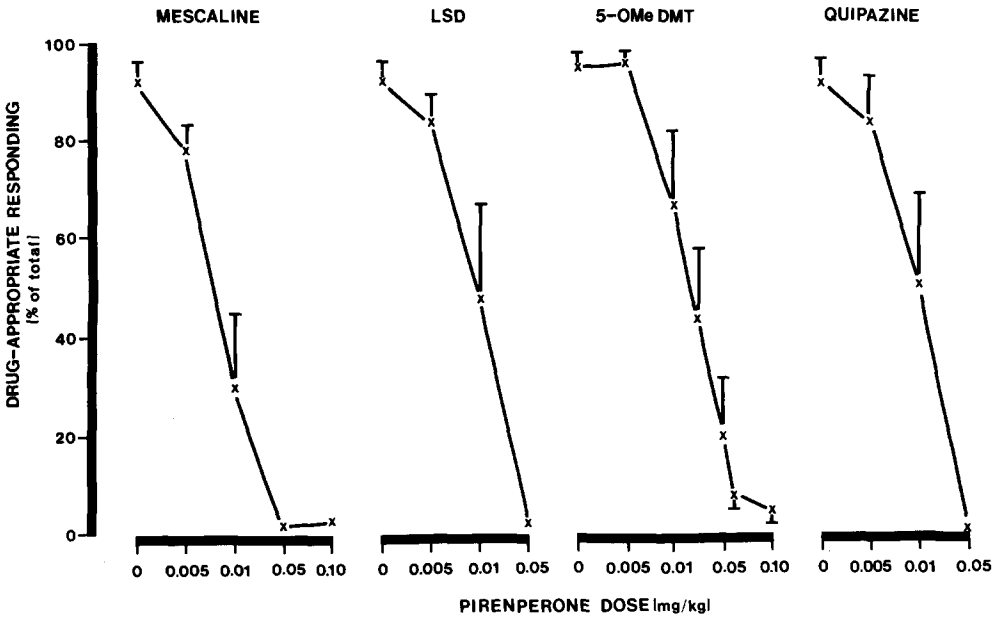

Fig. 4. The effect of various doses of pirenperone on DOM-stimulus generalization to mescaline, LSD, 5-OMe DMT and quipazine.

pirenperone in combination with saline. The ED_{50} doses for pirenperone in blocking the effect of 1.0 mg/kg of DOM is 0.01 mg/kg (95% confidence limit 0.006-0.026 mg/kg), and in blocking the DOM-stimulus generalization to the generalization dose of mescaline, LSD and 5-OMe DMT is 0.01, 0.01 and 0.02 mg/kg, respectively. Pirenperone also effectively antagonized the generalization of the DOM-stimulus to 3.0 mg/kg of quipazine, with an ED_{50} value of 0.01 mg/kg.

4. Discussion

Both selective 5-HT_2 antagonists were able to completely antagonize the discriminative stimulus effects of 1.0 mg/kg of DOM. Pizotyline, which also blocks the effects of DOM (Young et al., 1981), has recently been shown to interact selectively with 5-HT_2 sites (Leysen and Tollenaere, 1982). The potencies of pizolytine and ketanserin to antagonize the behavioral effects of DOM are relatively similar, i.e. ED_{50} values are 0.31 (unpublished result) and 0.18 mg/kg, respectively. Pirenperone is more effective than the other two antagonists by at least an order of magnitude, with an ED_{50} value of 0.01 mg/kg. While these studies were nearing completion, Appel and coworkers (1982) reported that pizotyline, ketanserin and pirenperone were all able to antagonize the discriminative stimulus effects of LSD, with pirenperone being the most potent of the three antagonists studied.

Pirenperone was also highly effective in preventing DOM-stimulus generalization to mescaline, LSD and 5-OMe DMT. This is further evidence that these three hallucinogens produce discriminative stimulus effects similar to those of DOM, and, comparing ED_{50} values and the slopes of the curves in fig. 4, that the underlying mechanisms of all four agents are sensitive in a similar manner to the effects of the 5-HT_2 antagonist pirenperone.

Administration of the purported 5-HT agonist quipazine, but not TFMPP or RU-24969, to the DOM-trained animals resulted in stimulus generalization (table 1); in addition, the effects produced by quipazine were completely antagonized by pretreatment of the animals with pirenperone (fig. 4). While, initially, these results appear some-

what puzzling, other investigators have also noted differences amongst the effects produced by various 5-HT agonists; to explain these effects, suggestions have been made that different types of 5-HT agonists might interact with different types of 5-HT receptors. With respect to their ability to bind to 5-HT_1 sites, i.e. to displace [^{3}H]5-HT, RU-24969 and TFMPP are 4-10 times more potent than quipazine (Hunt and Oberlander, 1979; Fuller et al., 1978, 1980). TFMPP appears to display selectivity for 5-HT_1 over [^{3}H]spiperone sites, and similar results were obtained with quipazine (R. Fuller, personal communication); however, Leysen and Tollenaere (1982) have recently reported that quipazine interacts somewhat selectively with [^{3}H]ketanserin (i.e. 5-HT_2) sites. Differences in relative binding might explain the results of the above discrimination studies, which are consistent with the following: (i) both DOM and quipazine produce a characteristic pattern of behavior in animals, i.e. 'serotonin syndrome' (Green et al., 1976; Green, 1981), (ii) phenylpiperazine-type 5-HT agonists, including TFMPP, do not ordinarily produce a 5-HT syndrome identical to that of quipazine (Rokosz-Pelc et al., 1980; Green et al., 1981; Lucki and Frazer, 1982), (iii) the 5-HT syndrome produced by quipazine is characterized by locomotor stimulation while TFMPP produces a dose-related decrease in locomotor activity that is unaffected by pretreatment of the animals with pirenperone (Lucki and Frazer, 1982), and (iv) the serotonin syndrome may be a 5-HT_2 receptor related phenomenon (Peroutka et al., 1981).

The overall results of the present study are (i) that the discriminative stimulus effects of DOM appear to be similar to those produced by mescaline, LSD, 5-OMe DMT and quipazine, (ii) that the effects produced by DOM are probably dissimilar to those produced by TFMPP and Ru-24969, (iii) that the discriminative stimulus effects of DOM can be antagonized by ketanserin and pirenperone, and (iv) that the DOM-stimulus generalization to mescaline, LSD, 5-OMe DMT and quipazine can also be antagonized by pirenperone. Taken together, these results suggest that the discriminative stimulus effects of DOM, the hallucinogens to which the DOM-stimulus generalized, and quipazine, might be due, primarily, to serotonergic interactions involving those subpopulations of serotonin receptors that are labeled by tritiated ketanserin (i.e. 5-HT_2 sites).

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