*European Journal of Pharmacology*, 159 (1989) 41–46
Elsevier



EJP 50589

# Involvement of 5-HT receptor subtypes in the discriminative stimulus properties of mescaline

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Received 19 May 1988, revised MS received 26 July 1988, accepted 11 October 1988

In order to further evaluate the extent to which particular 5-HT receptor subtypes (5-$HT_1$, 5-$HT_2$) might be involved in the behavioral effects of hallucinogenic drugs, rats were trained to discriminate mescaline (10 mg/kg i.p.) from saline and were given substitution (generalization) and combination (antagonism) tests with putatively selective serotonergic and related neuroactive compounds. The mescaline cue generalized to relatively high doses of the 5-$HT_2$ agonists, 2,5-dimethoxy-4-methylamphetamine (DOM), LSD and psilocybin; the extent of generalization to 5-$HT_1$ agonists (8-hydroxy-2-[diethylamino]tetralin (8-OHDPAT), RU-24969 and 8-hydroxy-2-[di-n-propylamino]tetralin (TFMPP)) was unclear. Combinations of the training drug and sufficiently high doses of 5-$HT_2$ antagonists (ketanserin, LY-53857, pirenperone) were followed by saline-lever responding; less selective central 5-HT (metergoline), and DA (SCH-23390, haloperidol) antagonists, did not block the mescaline cue. These data suggest that 5-$HT_2$ receptors are involved in the stimulus properties of mescaline.

Drug discrimination; Hallucinogens; Mescaline; 5-HT receptors; 5-$HT_1$ receptors; 5-$HT_2$ receptors

## 1. Introduction

For some time the effects of indole- and phenyl-alkylamine hallucinogens have been said to be mediated by serotonin (5-HT) receptors primarily because they are blocked by 5-HT antagonists (e.g. Brown and Ho, 1975; Glennon et al., 1983; Kuhn et al., 1978; Tilson et al., 1975; Winter, 1975; 1978) and enhanced by pretreatment with compounds that selectively destroy presynaptic stores of 5-HT (Appel et al., 1970; Brown and Ho, 1975; Commissaris et al., 1980; Joseph and Appel, 1977; Stoff et al., 1976). More recently, it has been concluded that 5-$HT_2$ receptors are particularly important to the expression of hallucinogenic drug effects (Glennon et al., 1984). This hypothesis is supported by: (1) the high correlation that appears to exist between the 5-$HT_2$ binding affinities of a variety of structurally different hallucinogens and both hallucinogenic potency in humans and discriminative stimulus (i.e. 'subjective') properties in animals (Glennon et al., 1984) and (2) reports that putatively selective 5-$HT_2$ antagonists (e.g. ketanserin, pirenperone, ritanserin) block both the discriminative properties of 2,5-dimethoxy-4-methylamphetamine (DOM) (Glennon et al., 1983) and LSD (Colpaert et al., 1985; Cunningham and Appel, 1987; Nielsen et al., 1985) as well as excitatory effects of mescaline (20 mg/kg) on acoustic startle (Davis, 1987) and head twitching (Niemegeers et al., 1983) responses.

In the present experiment, the involvement of 5-$HT_2$ receptors in the effects of hallucinogenic drugs is studied further by analyzing the extent to which the stimulus properties of a relatively low

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dose of mescaline (10 mg/kg) generalize to several putatively selective 5-HT agonists (4-iodo-2,5-dimethoxy-4-methylamphetamine (DOI), DOM, LSD, 8-hydroxy-2-[diethylamino]tetralin (8-OHDPAT), psilocybin, RU-24969, 8-hydroxy-2-[di-n-propylamino]tetralin (TFMPP)) and are blocked by 5-HT and dopamine (DA) antagonists (haloperidol, ketanserin, LY-53857, metergoline, pirenperone, SCH-23390, xylamidine).

## 2. Materials and methods

### *2.1. Subjects*

Experimentally naive, male Sprague-Dawley rats (Charles River), weighing 400–450 g at the beginning of the experiment, were used. Animals were housed individually in a colony of constant temperature (21–23° C) and humidity (40–50%); lighting was maintained on a 12-h light-dark cycle (7:00 a.m.-7:00 p.m.). While food was always available, the amount of water each animal received was restricted to that given during training, after test sessions (10–15 min) and on weekends.

### *2.2. Apparatus*

The apparatus and general procedure have been described in detail elsewhere (Cunningham and Appel, 1987). Eight commercially available, two-lever operant chambers (BRS/LVE Model 143–25) located in two adjacent rooms were used. Each chamber was equipped with a water-filled (0.1 ml) dipper, mounted equidistant between two response levers on one wall and was housed in a light- and sound-attenuating 'shell' (BRS/LVE Model 132–04). Illumination was provided by a 28 V house light; ventilation and masking noise were supplied by a blower. An Apple IIe microcomputer, located in an adjoining room, was used to program and record all experimental events.

### *2.3. Drugs*

Salts of the following compounds were dissolved in deionized water and were injected i.p. in a volume of 1.0 ml/kg, 15 min prior to behavioral testing: DOI (4-iodo-2,5-dimethoxy-4-methylamphetamine; National Institute on Drug Abuse ((NIDA), Rockville, M.D.), DOM (2,5-dimethoxy-4-methylamphetamine; NIDA), 8-OHDPAT (8-hydroxy-2-[diethylamino]tetralin; Research Biochemicals, Inc., Natick, MA), LSD (NIDA), LY-53857 (6-methyl-1-[1-methylethyl]ergoline-8-carboxylic acid, 2-hydroxy-1-methylpropyl ester [z]-2-butenedioate [1 : 1]; Eli Lilly and Co., Indianapolis, IN), mescaline (Sigma Chemical Co., St. Louis, MO), psilocybin (NIDA), RU-24969 (5-methoxy-3 [1,2,3,6-tetra-hydropyridin-4-yl]-1H indole succinate; Roussel, Romainville France), SCH-23390 (Schering Corp., Bloomfield, NJ), TFMPP (8-hydroxy-2-[di-n-propylamino]tetralin; Aldrich, Milwaukee, WI), xylamidine (Wellcome Research Laboratories, Beckenham, Kent U.K.) In addition, the following compounds were diluted from (5 mg/ml) solutions (in ampules provided by the suppliers) and were given i.p. during combination tests: haloperidol (McNeil Laboratories, Fort Washington, PA), ketanserin (Janssen Pharmaceutica, New Brunswick, NJ), and pirenperone (Janssen); metergoline (Farmitalia, Milan) was dissolved in 1% ascorbic acid.

### *2.4. Procedures*

#### *2.4.1. Preliminary training*

Rats (N = 8) were trained to discriminate 10 mg/kg of mescaline from an equivalent volume of saline (1 ml/kg); one or the other of these substances being administered i.p., 15 min prior to each experimental session. Training began under a schedule of continuous water reinforcement (FR 1) with only the stimulus-appropriate (drug or saline) lever present. To control for the development of cues based on olfactory stimuli (Extance and Goudie, 1981), the position of the correct lever was randomized. For example, half of the animals were reinforced (with water) for responding on the left lever following drug administration and reinforced for responding on the right lever following saline; conditions were reversed for the remaining animals. The order of treatment during training (drug or saline) was also randomized with the restriction that neither condition be present for more than three consecutive sessions. As re-

sponse rates stabilized, the number of responses required for reinforcement (FR 'value') was increased until all rats were pressing the lever reliably under a fixed-ratio (FR 20) schedule of reinforcement under each experimental condition.

*2.4.2. Discrimination training*

Both levers were then presented simultaneously. Rats were required to complete the FR on the drug-appropriate (correct) lever to obtain water; there were no programmed consequences for responding on the incorrect lever. Training sessions (20 min) were conducted at the same time each day, 5 days per week and continued until all animals attained a criterion which required mean individual accuracies of at least 80% correct for 10 consecutive sessions.

*2.4.3. Test procedures*

After each group of animals reached criterion (above), substitution (generalization) tests were initiated; these sessions were conducted 1–2 times per week with training continuing during the 3–4 remaining days. Rats were placed in the chamber as during training except that, once the animal completed 20 responses on either lever or after the session time had elapsed, the house light was turned off and the animal was removed from the chamber without reinforcement; thus, rats were tested during extinction.

*2.4.4. Data analysis*

Accuracy was defined as the percentage of correct responses to total responses before delivery of the first reinforcer during training sessions and, as the percentage of drug-appropriate responses to total responses, during test sessions. Rate (responses/min) is the number of responses emitted before either: (1) completion of an FR 20 on the correct lever (training sessions), or (2) completion of 20 responses on either level (test sessions), divided by the number of minutes taken to com-

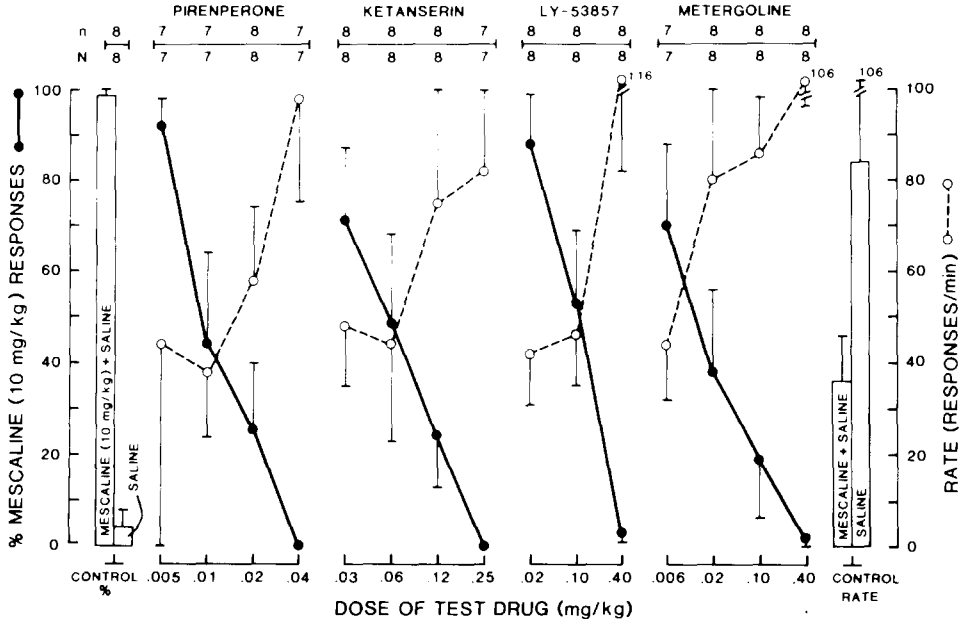


Fig. 1. Results of tests with 5-$HT_2$ antagonist, given 60 min prior to experimental sessions, to rats treated with mescaline (10 mg/kg), 15 min prior to testing (see text). The compounds tested, number of animals responding (n) and number of animals tested (N) at each dose are shown at the top of the figure. Solid circles (connected by solid lines) denote the mean percentage of lever-pressing responses on the drug-appropriate lever (±S.E.M.; left ordinate); open circles (connected by broken lines) denote the mean response rate (±S.E.M.; right ordinate). Pirenperone, ketanserin and LY-53857 reduced drug-appropriate responding significantly ($P < 0.01$). The bar graphs at the left show the mean percentage of drug-appropriate responding following treatment with mescaline and saline during training sessions (±S.E.M.); the bar graphs at the right show the average rates following mescaline and saline (±S.E.M.).

plete the first FR 20 or the test session. A compound was said to have substituted completely for the training drug if the results of statistical analysis (below) were significant ($P < 0.05$) and at least 80% responding occurred on the mescaline-appropriate lever following at lease one dose of that compound (Appel et al., 1982); similarly, antagonism was said to occur only when the results of statistical tests involving combinations of treatments were significant ($P < 0.05$) and no more than 20% drug-appropriate responding occurred after at least one dose of a potential antagonist.

Only data from animals that completed at least 20 responses during substitution or combination tests were analyzed. Because variances across treatments (doses) were not homogeneous and equal numbers of subjects did not always respond at every dose, the data were analyzed non-parametrically (Friedman, 1940; Li, 1964).

## 3. Results

### 3.1. Substitution tests

The effects of 5-HT agonists on both percentage of responding on the mescaline-appropriate lever and overall rates of responding are summarized in table 1. Unfortunately, only two doses of each of the putative 5-$HT_1$ agonists could be analyzed because higher doses disrupted responding. While the substitution of RU-24969 for the training drug was statistically significant ($P < 0.01$), the maximum percent of drug-lever responding following treatment with this substance (51%) was considerably below criterion (80%). Since neither 8-OHDPAT nor TFMPP produced significant amounts of responding on the drug-appropriate lever, it cannot be concluded that any of the 5-$HT_1$ agonists mimicked mescaline under the conditions of the present experiment.

While some disruption in rates of lever-pressing also occurring following high doses of 5-$HT_2$

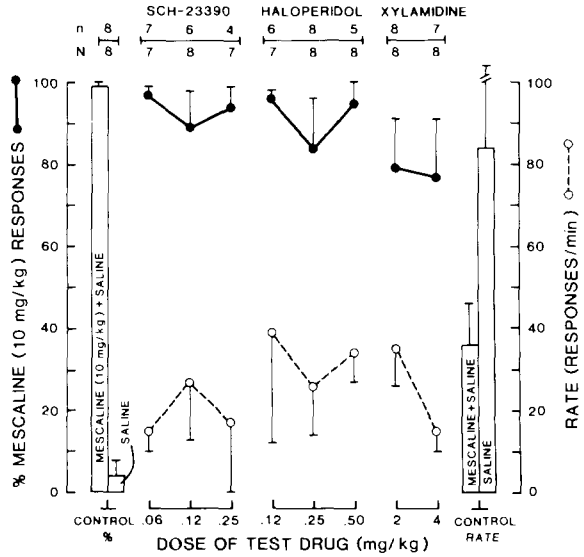


Fig. 2. Results of combination tests with DA, and peripherally active 5-HT antagonists. Only xylamidine had significant effects ($P < 0.05$). See fig. 1 legend for details.

TABLE 1

Results of substitution tests with 5-HT agonists in rats trained to discriminate mescaline (10 mg/kg i.p.) from saline. All drugs were given 15 min prior to testing.

| Compound | Dose (mg/kg) | Percent drug-lever resp. (±S.E.M.) | Rate resp./min (±S.E.M.) | n/N [a] |
|---|---|---|---|---|
| Saline | (control) | 4± 4 | 84±22 | 8/8 |
| *5-$HT_1$ agonists* | | | | |
| 8-OHDPAT | 0.2 | 54± 26 | 21±11 | 4/5 |
| | 0.4 | 39± 17 | 18± 5 | 7/8 |
| RU-24969 [c] | 0.5 | 1± 1 | 50±22 | 6/6 |
| | 1.0 | 51± 12 | 10± 3 | 7/8 |
| TFMPP | 0.2 | 29± 18 | 37±16 | 6/6 |
| | 0.4 | 59± 23 | 7± 3 | 5/7 |
| *5-$HT_2$ agonists* | | | | |
| DOI | 0.125 | 26± 25 | 33±16 | 4/4 |
| | 0.25 | 52± 28 | 43±14 | 4/4 |
| | 0.5 | 96± 4 | 11± 4 | 4/4 |
| DOM [b] | 0.25 | 27± 17 | 22± 5 | 6/6 |
| | 0.5 | 81± 12 | 35±12 | 5/5 |
| | 1.0 | 100 | 28±10 | 4/6 |
| LSD [c] | 0.02 | 10± 10 | 50± 8 | 8/8 |
| | 0.04 | 37± 17 | 59±11 | 8/8 |
| | 0.08 | 95± 5 | 20± 6 | 7/8 |
| | 0.16 | 97± 2 | 6± 2 | 7/8 |
| Psilocybin [c] | 0.125 | 0 | 43±17 | 4/5 |
| | 0.25 | 30± 19 | 57±17 | 6/6 |
| | 0.5 | 84± 14 | 48±14 | 7/7 |
| | 1.0 | 100 | 5± 1 | 3/5 |

[a] n = number of animals responding; N = number of animals tested. [b] $P(Chi_r^2) < 0.05$; [c] $P(Chi_r^2) < 0.01$.

agonists (as well as mescaline itself), three of the four test compounds (DOM, LSD, psilocybin) substituted for the training drug (table 1); the amount of drug-appropriate responding following treatment with the fourth compound, DOI, approached but did not attain statistical significance ($P < 0.09$), perhaps because of the small number of subjects tested ($N = 4$). The amounts of responding on the mescaline lever following all 5-$HT_2$ agonists either approached (DOI, LSD), or reached (DOM, psilocybin) 100%.

### *3.2. Combination tests*

Treatment with the selective 5-$HT_2$ antagonists (ketanserin, LY-53857, pirenperone) given at the doses indicated (fig. 1), 60 min prior to mescaline (10 mg/kg), reduced drug-appropriate responding significantly ($P < 0.01$) to near zero, while stimultaneously increasing response rates to control (saline) levels (fig. 1); the peripherally acting 5-HT antagonist (xylamidine) also antagonized the mescaline cue ($P < 0.05$) but the amount of drug-appropriate responding following treatment with this compound remained at 77% and, thus, did not attain antagonism criterion (20%).

The less selective 5-$HT_1$ and 5-$HT_2$ antagonist metergoline reduced responding on the mescaline-appropriate lever but this effect did not attain statistical significance ($P < 0.10$). Neither of the compounds that act primarily at DA receptors (SCH-23390, haloperidol) had significant effects on accuracy and, if anything, disrupted response rates (fig. 2).

## 4. Discussion

Interpretation of the present results must be tempered by observations that the data with 5-$HT_1$ agonists are not definitive, since relatively few doses of these substances could be tested, and compounds that may be active at putative 5-$HT_3$ systems were not studied at all. Nevertheless, within these limitations, the results are impressive in that: (1) substances that are known to bind with relatively high affinity to 5-$HT_2$ receptor sites (e.g. DOM) were shown to have stimulus properties that are similar to those of mescaline (table 1) and (2) relatively selective 5-$HT_2$ antagonists (pirenperone, ketanserin, LY-53857) block the mescaline cue to a greater extent than non-selective 5-HT antagonists (metergoline), an effect that does not occur following compounds that act primarily at DA (SCH-23390, haloperidol) receptor sites (figs. 1 and 2). Moreover, the relative potencies of different agents in blocking the mescaline cue (pirenperone > ketanserin > LY-53857 > metergoline) parallel their abilities of preferentially displace 5-$HT_2$ binding (Leysen et al., 1981; Peroutka and Snyder, 1979). Similar results involving the antagonism of a higher dose of mescaline (20 mg/kg) on acoustic startle and head twitching responses by 5-HT antagonists have also been reported (Davis, 1987; Niemegeers et al., 1983).

Thus, these results provide strong evidence that the subjective effects of mescaline, as well as DOM (Glennon et al., 1984) and LSD (Nichols et al., 1986), involve 5-$HT_2$ receptors; they therefore support and extend the hypothesis (Glennon et al., 1984) that actions at these sites mediate the effect of structurally different hallucinogens (indolalkylamines, phenylalkylamines, lysergides). However, this does not mean either that these functionally similar compounds have identical stimulus properties or mechanisms of action. Indeed, LSD and mescaline can be distinguished readily (in rats) by differences in their patterns of generalization, for example, to phenylisopropylamines such as MDA and MDMA (Callahan and Appel, 1987) and it has long been known that these compounds have different electrophysiological effects (on midbrain 5-HT neurons); for example, it has been argued that LSD acts as a direct (post-synaptic) agonist while mescaline acts indirectly (presynaptically), either by facilitating 5-HT release or inhibiting its uptake (Haigler and Aghajanian, 1973).

Nevertheless, since drug discrimination is probably the best available animal model of the 'subjective' effects of hallucinogenic compounds (Glennon et al., 1982), the results of the present study, along with those reported previously, suggest that, while actions at other receptor sites might also be involved, stimulation of 5-$HT_2$ neu-

ronal systems are important to the subjective (as well as other in vivo) effects of all hallucinogenic compounds and perhaps, those conditions under which such effects are most likely to occur 'spontaneously' in humans (e.g. schizophrenia).

## Acknowledgements

This research was supported by USPHS Research Grant RO1 DA02543, from the National Institute on Drug Abuse; a preliminary report of the data was presented at the Society for Neuroscience Annual Meeting (Satellite Session of the Society for the Stimulus Properties of Drugs) New Orleans, 1987.

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