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Hallucinogenic Agents as Discriminative Stimuli: A Correlation with Serotonin Receptor Affinities

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Abstract. A choice between two levers in an operant chamber was used to train 24 rats, under a variable-interval 15 s schedule of sweetened milk reinforcement, to discriminate a hallucinogenic (psychotomimetic) agent, 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT), from saline administration. The 5-OMe DMT stimulus generalized in a dose-related manner to each of 14 tryptamine related analogs. With the exception of one compound, the effective dose for the 5-OMe DMT response correlated highly ($r = -0.86$) with 5-HT receptor affinity (as determined using an isolated rat fundus preparation).

Key words: Hallucinogens – Discriminative stimulus – Serotonin receptor affinities – 5-OMe DMT – Tryptamines

In a preliminary report, Glennon and Gessner (1975) showed that the affinity for the serotonin (5-hydroxytryptamine, 5-HT) receptors of the isolated rat stomach fundus preparation is approximately ten times higher for 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT) than for N,N-dimethyltryptamine (DMT). More recently, in man 5-OMe DMT has been shown to be about ten times more potent than DMT as a hallucinogenic, psychotomimetic agent (Shulgin, 1978). This promted us to examine the 5-HT receptor affinities and structure activity relationships of a somewhat larger series of compounds, including derivatives of phenalkylamine (Glennon et al., 1978; Glennon and Gessner, 1979). The more potent hallucinogens were found to posses a relatively high affinity for 5-HT receptors; for example, the pA_2 values for (±)-DOB (DOB = 4-Bromo derivative of 2.5-DMA), (±)-DOM (DOM = 4-Methyl derivative of 2.5-DMA see Table 1, firstentry), and 5-OMe DMT are 7.35, 7.12 and 7.08, respectively (i.e., $K_B < 0.1\,\mu M$). Structurally related analogs that are less active, e.g., DMT, or that have little effect on behavior, e.g., 7-OMe DMT, possess lower affinities (i.e., $pA_2 = 6.00$ and 5.33, respectively). In addition, with respect to several chiral hallucinogens, the more behaviorally-potent enantiomers were found to posses a higher affinity than their less active isomers (Glennon, 1979). Furthermore, there is an apparent similarity between rat fundus 5-HT receptors and brain serotonin binding sites with respect to their interaction with tryptamine analogs (Green et al., 1978; Glennon, 1979a).

In a recent publication, we suggested that, within certain constraints, 5-HT receptor affinity might parallel behavioral activity (Glennon and Gessner, 1979). In this present investigation we further examine this hypothesis by studying the ability of 5-OMe DMT to generalize to other hallucinogens, using a discriminative stimulus paradigm with rats as subjects.

Materials and Methods

Drug-Discrimination Procedures. Twenty-four 120-day-old Sprague-Dawley rats (Flow Laboratories; Dublin, VA) were used in this study. The animals' weights were reduced to 80% of their free-feeding weights by partial food deprivation. Animals had free access to water.

Discrimination training was begun by initially training each rat to bar press for food (sweetened condensed milk diluted 2:1 with water) reinforcement using a two-lever operant chamber. After the rats were trained to press both levers, each daily session was preceded by an IP injection of either the drug diluted in normal saline or a 1 ml/kg dose of normal saline. Pressing on one of the levers was reinforced after the administration of drug (5-OMe DMT, 1.5 mg/kg) while responses on the opposite lever were reinforced following saline; all conditions were counterbalanced.

Discrimination training began with eight preliminary training sessions of 15 min duration; 5-OMe DMT was administered on the first 4 days followed by 4 days of saline. Each correct lever press resulted in reinforcement. Subsequent training sessions, also of 15 min duration, were composed of an initial 2.5 min extinction period while lever pressing during the remainder of the session was reinforced according to a variable-interval schedule of 15 s (VI–15 s). The order of drug and non drug training sessions consited of a

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double alternation presentation, which was used throughout the remainder of the study.

After 40 training sessions discrimination performance was stable, and the ability of the 5-OMe DMT stimulus to generalize to challenge compounds was studied during the 2.5-min extinction sessions interspersed between two to four training sessions. Discrimination performance was maintained by continuing training between test sessions using the same double alternation sequence described above. Data were collected only during 2.5-min test sessions and were recorded as percent correct responding on the 5-OMe DMT drug-lever.

Evaluation of Experimental Test Compounds. Dose-response generalization experiments were conducted with each of 14 compounds. These experiments were performed during the extinction sessions described above. Compounds were dissolved in saline and administered IP 15 min prior to a test session. In the studies, six animals were each administered one of four doses of any given compound. In situations where generalization with 5-OMe DMT did not occur or where a compound exhibited only partial generalization ($< 60\%$), a second experiment was conducted using an increased dose-response regimen.

The results were evaluated by ED_{50} values, determined by the Litchfield and Wilcoxin (1949) technique. One advantage of generalization data generated by the VI–15 s schedule is that individual responses during testing are graded rather than all or none, thus permitting calculations of individual percent generalization values for each dose of each test compound in each subject. These individual data are summarized by the group mean for each dose.

Serotonin Receptor Affinities. Receptor affinity data were obtained using the isolated rat stomach fundus preparation of Vane (1957) and are reported as pA_2 values. These have been reported elsewhere (Glennon et al., 1978; Glennon and Gessner, 1979).

Results

Rats were trained to discriminate between 1.5 mg/kg of 5-OMe DMT and saline. After 40 discrimination training sessions, discrimination responding was relatively stable, correct-lever responding on the 5-OMe DMT lever averaged 86–95% with 5-OMe DMT, while the responding on the same lever after saline administration averaged 17–20%. Response rates under both drug and non-drug conditions were not significantly different. Time duration studies indicated that the peak stimulus control of behavior could be achieved within 30 min following 1.5 mg/kg of 5-OMe DMT.

After the initial training period, these animals were administered various compounds. Generalization of the 5-OMe DMT stimulus to these compounds was observed, in a dose-related manner, for all of the challenge drugs. Generalization of the highest dose tested ranged between 75–85% 5-OMe DMT-correct lever responding for each compound.

Discussion

It is well established that hallucinogenic, psychotomimetic agents can serve as discriminative stimuli in animals (Winter, 1974; Kuhn et al., 1977; Colpaert and Rosecrans, 1978). The discriminative stimulus model is sensitive and specific enough to distinguish differences among the effects produced by members of various classes of hallucinogens (Kuhn et al., 1977; Colpaert and Rosecrans, 1978). On the other hand, two drugs that produce similar behavioral and subjective effects in man often generalize to one another in tests of stimulus control in rats; we may infer that they produce similar interoceptive cues in rats just as they do in man. Evidence that the behavioral effects (interoceptive cues) produced by certain hallucinogenic agents are mediated by serotonergic mechanisms is derived from the finding that these interoceptive cues can be blocked by the administration of 5-HT antagonists. The effects of mescaline, DOM, and LSD have been blocked in such a manner (Winter, 1975, 1978; Browne and Ho, 1975; Kuhn et al., 1978). We have recently demonstrated that 5-OMe DMT can act as a discriminable stimulus in animals when paired with saline. We have also shown that the 5-HT antagonist pizotyline will attenuate the behavioral effects elicited by both 5-OMe DMT and LSD and have concluded that such compounds might be exerting stimulus control by affecting a common neuronal system (Glennon et al., 1979).

Under the hypothesis that the 5-OMe DMT stimulus is mediated by a serotonergic mechanism, it should be possible to demonstrate generalization between this stimulus and those stimuli produced by other hallucinogenic agents for which a similar mechanism has been implicated. Thus, we have shown that DOM will generalize to the 5-OMe DMT stimulus (Glennon et al., 1979). Other factors being equal (or at least inconsequential with respect to behavioral properties) a relationship might exist between the behavioral potency (ability to generalize to 5-OMe DMT) and the 5-HT receptor affinity of a modest series of such compounds. Indeed, this has been found to be the case (Table 1). Among the compounds in Table 1, eleven are N,N-dialkyltryptamines, two are phenylisopropylamines, and one is a α-methyltryptamine. The apparent relationship between affinity and behavior ($r = -0.86$) is further evidence that serotonergic mechanisms may be involved in the stimulus control exerted by these agents.

One of the compounds examined, mescaline, does not appear to fit this relationship. Although mescaline possesses an affinity between that of 6-OMe and 7-OMe DMT (i.e. $pA_2 = 5.65$) its behavioral activity appears to be less than expected. Confirmation that the dose for mescaline is realistic comes from a comparison of the doses of DOM and mescaline necessary to disrupt conditioned avoidance behavior in rats, i.e. 5, and 100 μM/kg, respectively (Nichols et al., 1974). These data compare favourably with those presented in

Table 1[a]. Generalization data: Comparisons of ED_{50} with pA_2 data

Compound[b]	pA_2	ED_{50}, μmol/kg[c]
(±)-1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane HCl (DOM)	7.12	1.87 (0.89 – 3.90)
5-Methoxy-N,N-dimethyltryptamine HOx (5-OMe DMT)	7.08	1.30 (0.65 – 2.60)
5-Methoxy-N,N-diethyltryptamine HCl (5-OMe DET)	6.94	2.14 (1.01 – 4.55)
Psilocin (4-OH DMT)	6.84	2.36 (1.13 – 5.07)
(±)-2,5-Dimethoxyphenyl-2-aminopropane HCl (2,5-DMA)	6.83	2.55 (1.34 – 4.76)
5-Methoxy-N,N-di-n-propyltryptamine HOx (5-OMe DPT)	6.53	2.14 (0.93 – 4.86)
5-N,N-Trimethyltryptamine maleate (5-TMT)	6.52	2.32 (0.85 – 6.38)
7,N,N-Trimethyltryptamine HOx (7-TMT)	6.29	2.19 (0.89 – 5.51)
(±)-α-Methyltryptamine HCl (α-MeT)	6.25	3.74 (1.75 – 7.91)
4-Methoxy-N,N-dimethyltryptamine HOx (4-OMe DMT)	6.17	3.47 (1.56 – 7.82)
1,N,N-Trimethyltryptamine HOx (1-TMT)	6.02	2.98 (1.02 – 8.66)
N,N-Dimethyltryptamine HOx (DMT)	6.00	2.66 (0.74 – 9.73)
6-Methoxy-N,N-dimethyltryptamine HOx (6-OMe DMT)	5.77	5.06 (2.53 – 10.06)
7-Methoxy-N,N-dimethyltryptamine HCl (7-OMe DMT)	5.33	5.92 (2.62 – 13.29)
Mescaline HCl	5.65	35.32 (19.60 – 63.75)

[a] Correlation and regression formula are as follows: $ED_{50}(x)$ vs. pA_2: $y = -0.36x + 7.45$; $r = -0.86$ ($P < 0.001$) $n = 14$ (mescaline excluded)

[b] Psilocin and DOM were obtained from NIDA: all other compounds were synthesized or obtained as previously described (Glennon and Gessner, 1979). HCl designates hydrochloride salt and HOx, the hydrogen oxalate salt

[c] Generalization doses are followed by 95% confidence limits in parenthesis

Table 1. A possible explanation for this apparent discrepancy is that the mechanism of action of mescaline involves neurotransmitters in addition to serotonin. In disagreement with this, however, is the finding that the behavioral effects of mescaline can be blocked by prior administration of various serotonin antagonists (Winter, 1975; Browne and Ho, 1975). An alternative explanation is that mescaline may be metabolized more rapidly than the other compounds. Browne and Ho (1975a) have reported that the interoceptive cues produced by mescaline do not appear to be produced by a metabolite of mescaline but are produced by the parent compound itself.

Examination of Table 1 reveals that mescaline is the only compound that is not somewhat protected from oxidative deamination by the presence of either an α-methyl or an N-alkyl substituent. We have previously reported that α-methylation has no apparent effect on 5-HT receptor affinity when racemates are examined (Glennon, 1979), and yet α-methylation is known to have an effect on behavioral activity. For example, tryptamine is not considered to be hallucinogenic in man (Brimblecombe and Pinder, 1975) and yet its 5-HT receptor affinity is twice that of DMT. α-Methylation of tryptamine has no effect on 5-HT receptor affinity (i.e. the pA_2 of (±)-α-methyltryptamine, 6.25, is nearly identical to that of tryptamine 6.27), however (±)-α-methyltryptamine is nearly twice as potent as DMT in man (Glennon and Gessner, 1979). Perhaps this same effect is being observed with mescaline. Further study of this effect, by the examination of additional phenalkylamines, tryptamines and related compounds which are not N-alkylated or α-methylated, is planned.

The 5-HT receptor affinities of the compounds examined were determined by treating them as competitive antagonists of 5-HT in an in vitro preparation. In vivo, the behavioral effects of 5-OMe DMT presumably arise via an agonistic interaction with 5-HT receptors (as evinced by blocking experiments). Nevertheless, a relationship exists between the generalization dose of these compounds and their 5-HT receptor affinities as determined using an isolated rat fundus preparation. As we have previously suggested, the 5-HT receptor affinity of a particular compound is not necessarily related to its lipid solubility or rate of metabolism. Therefore, it might be expected that the behavioral activity of certain compounds will not be related to 5-HT receptor affinity. This type of reasoning can be invoked to explain why mescaline does not adhere to the behavior-affinity relationship observed for the other compounds investigated.

A final point of caution should be mentioned. While these data show that there appears to be a correlation between hallucinogenic potency and the ED-50 dose, we do not intend to suggest that this pharmacological model is a predictor of hallucinogenic potential. Rather, it is felt that this 5-OMe DMT model can assist in predicting whether an experimental compound may be interacting with some 5-HT system. We further feel that this model may be a good predictor of postsynaptic agonist activity on 5-HT neurons.

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Note Added in Proof

α-Methylmescaline (i.e. 1-(3,4,5-trimethoxyphenyl)-2-aminopropane hydrochloride) was recently acquired and evaluated. Using the equation from footnote a of Table 1, α-methylmescaline ($pA_2 = 5.60$) is calculated to possess an ED_{50} of 5.14 μmol/kg; employing the same experimental conditions outlined under *Materials and Methods*, this compound is found to have an ED_{50} of 5.59 (2.26–13.72) μmol/kg.