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Further Studies on the Dose-Dependent Stimulus Properties of 5-Methoxy-N,N-Dimethyltryptamine

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YOUNG, R., J. A. ROSECRANS AND R. A. GLENNON. *Further studies on the dose-dependent stimulus properties of 5-methoxy-N,N-dimethyltryptamine.* PHARMACOL BIOCHEM BEHAV **25**(6) 1207–1210, 1986.—Twenty-two rats were trained to discriminate either 1.5 mg/kg or 3.0 mg/kg of 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT) from saline in a standard two-lever operant procedure. Once responding was stable, various doses of several serotonin (5-HT) antagonists, i.e., cyproheptadine (CYP), methysergide (UML), cinanserin (CIN), and methergoline (MCE), were administered in combination with 5-OMe DMT, to assess the ability of each antagonist to attenuate each 5-OMe DMT-stimulus. The 5-OMe DMT-stimulus at 1.5 mg/kg was completely antagonized by CYP, and was partially attenuated by CIN and MCE. UML had negligible effects on 5-OMe DMT-appropriate responding. In the 3.0 mg/kg 5-OMe DMT-trained rats, UML and MCE partially blocked the 5-OMe DMT-stimulus; CYP and CIN had no significant effect on 5-OMe DMT-appropriate responding. The results suggest that until the *in vivo* effects and mechanism of action of 5-OMe DMT and certain 5-HT antagonists are better understood, caution is advised when conclusions are drawn from studies employing these agents.

5-OMe DMT Serotonin Serotonin antagonists Drug discrimination

THE hallucinogenic tryptamine analog 5-methoxy-N,N-dimethyl-tryptamine (5-OMe DMT) appears to exert many of its effects, at least in part, through an interaction(s) with serotonin (5-HT) neuronal systems [1, 3, 6, 7]. We have recently reported [15] that antagonism of the discriminative stimulus (DS) properties of 5-OMe DMT by the purported 5-HT antagonist pizotifen (pizotyline, BC-105) is 5-OMe DMT dose-dependent. That is, BC-105 significantly attenuated the DS properties produced by the administration of 5-OMe DMT at 1.5 mg/kg (to animals trained to 5-OMe DMT at either 1.5 or 3.0 mg/kg), but not the stimulus properties produced by the administration of 5-OMe DMT at 3.0 mg/kg (to animals trained to discriminate 5-OMe DMT at 3.0 mg/kg) even when 10 times the dose of BC-105 was used.

The purpose of the present study was to determine if a differential DS effect occurs when purported 5-HT antagonists, other than BC-105, are combined with 5-OMe DMT doses. Specifically, rats were trained to discriminate either 1.5 or 3.0 mg/kg of 5-OMe DMT. The ability of the purported 5-HT antagonists cyproheptadine, methysergide, cinanserin, and methergoline to attenuate the stimulus effects of each 5-OMe DMT training dose was then examined.

METHOD

The animals used in this study were 22 male Sprague-Dawley (350–400 g) rats. All animals were housed individually and had unlimited access to drinking water. The animals were maintained at 80% of their free-feeding body weights by partial food deprivation.

Apparatus

Behavioral testing was conducted in standard operant chambers (Lehigh Valley Electronics model 1417). One wall of the chamber contained the intelligence panel, which consisted of two levers with a dipper for delivery of reinforcement (0.01 ml of sweetened milk) centered between the levers. A houselight (28 V), located 24 cm above the dipper, provided illumination to the chamber. Each operant chamber was housed in a sound-insulated chamber (Lehigh Valley Electronics model 132-02). Standard electromechanical and solid-state programming and recording equipment were used.

Discrimination Training

The animals were each trained to respond to a variable interval (VI) 15-sec schedule of reinforcement on each lever. Lever response training on the VI 15-sec schedule continued until rates of responding stabilized. At this point the animals were divided into two groups and drug discrimination training was begun. The first group of 14 rats was injected IP with either 5-OMe DMT (3.0 mg/kg) or its vehicle (saline), the second group of eight rats received 1.5 mg/kg of 5-OMe DMT or saline. All rats were placed in the operant chambers with

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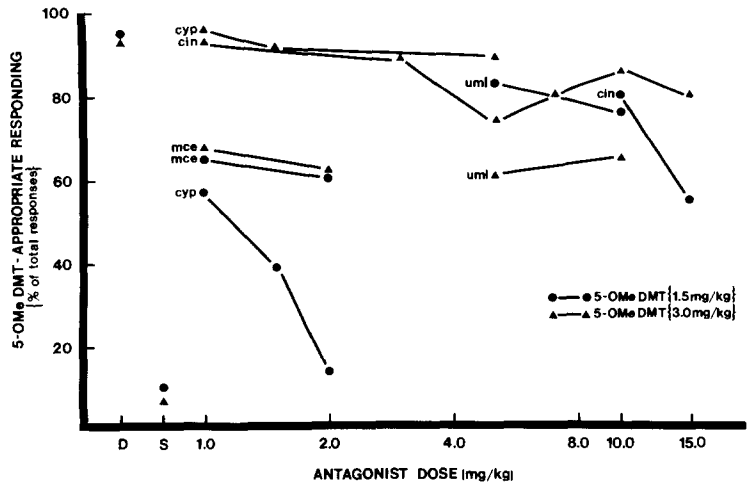

FIG. 1. 5-OMe DMT-appropriate responding after the administration of various doses of 5-HT anagonists (CIN=cinanserin, CYP=cyproheptadine, MCE =methergoline, and UML=methysergide) in combination with each 5-OMe DMT-stimulus. 5-OMe DMT-appropriate responding, after the administration of 5-OMe DMT (D) or saline (S), during discrimination training sessions prior to antagonism tests is also presented.

TABLE 1

RESPONSE RATES (MEAN % OF SALINE RATE) OF RATS AFTER TREATMENT WITH SALINE, 5-OMe DMT (1.5 mg/kg), AND VARIOUS DOSES OF 5-HT ANTAGONISTS IN COMBINATION WITH 5-OMe DMT AT 1.5 mg/kg

Agent	Dose (mg/kg)	n/N*	Mean Response Rate† (% of of saline rate)
Saline (1.0 ml/kg)		8/8	100%
5-OMe DMT	1.5	8/8	95%
Cyproheptadine	1.0	6/6	85%
	1.5	6/6	94%
	2.0	6/6	81%
Methysergide	5.0	7/8	86%
	10.0	4/8	47%
Methergoline	1.0	4/5	70%
	2.0	3/5	44%
Cinanserin	10.0	5/5	93%
	15.0	4/5	45%

*Number of animals responding/number of animals receiving drug.

†Data obtained during 2.5 min extinction session.

both levers present. Training sessions were of 15-min duration. Saline or 5-OMe DMT was administered on a double-alternation schedule (i.e., 2 days saline, 2 days 5-OMe DMT).

On every fifth day, discrimination learning was assessed during an initial 2.5-min nonreinforced (extinction) period followed by a 12.5-min training session. Data collected during the extinction periods included total responses (mean responses/min) and percent appropriate responding on the 5-OMe DMT lever (number of responses on 5-OMe DMT-designated lever/total number of responses). After 40 training sessions, discrimination performance was stable for each group of animals (i.e., 5-OMe DMT, approximately 85%; saline, approximately 10%). Response rates were similar under all treatment conditions. The 5-OMe DMT versus saline discrimination was insured in each group by continuation of training sessions throughout antagonism tests.

Antagonism Tests

In each 5-OMe DMT trained group, the 5-HT antagonists cinanserin (1.0 mg/kg), methysergide (5.0 mg/kg), and cyproheptadine (1.5 mg/kg) were administered 45 min before the training doses of 5-OMe DMT. Methergoline (1.0 mg/kg) was administered 180 min prior to the training doses of 5-OMe DMT [11]. In all antagonist studies the animals were tested for lever-choice response 15 min after the 5-OMe DMT injection, under extinction test conditions. Doses of the antagonists were increased or decreased, in subsequent tests, depending upon the percent 5-OMe DMT response produced by the initial 5-HT antagonist dose in combination with the 5-OMe DMT training doses. However, if an antagonist-agonist combination resulted in greater than 50% suppression of response rate, as compared to saline control response rate, testing of a higher dose with that particular antagonist was not attempted. The results of antagonism tests were classified according to the following criteria [4]: (a) complete antagonism (i.e., ≤20% 5-OMe DMT-appropriate responding), (b) partial antagonism (i.e., 40–70% 5-OMe DMT-appropriate responding), and (c) no antagonism (i.e., >75% 5-OMe DMT-appropriate responding). Control studies of the antagonists in combination with saline (rather than 5-OMe DMT) were also performed. Each data point was determined from the responding of five to eight animals.

Drugs

Doses of the following drugs were based on the weight of the salt: 5-OMe DMT (Sigma Chemicals), which was converted to the hydrogen oxalate salt, cyproheptadine HCl

TABLE 2

RESPONSE RATES (MEAN % OF SALINE RATE) OF RATS AFTER TREATMENT WITH SALINE, 5-OMe DMT (3.0 mg/kg), AND VARIOUS DOSES OF 5-HT ANTAGONISTS IN COMBINATION WITH 5-OMe DMT AT 3.0 mg/kg

Agent	Dose (mg/kg)	n/N*	Mean Response Rate† (% of of saline rate)
Saline (1.0 ml/kg)		14/14	100%
5-OMe DMT	3.0	14/14	92%
Cyproheptadine	1.0	5/5	87%
	1.5	5/5	94%
	5.0	3/5	40%
Cinanserin	1.0	6/6	81%
	3.0	6/6	75%
	5.0	6/6	87%
	7.0	6/6	81%
	10.0	5/6	71%
	15.0	4/6	47%
Methysergide	5.0	4/5	70%
	10.0	3/5	46%
Methergoline	1.0	6/6	81%
	2.0	3/6	47%

*Number of animals responding/number of animals receiving drug.

†Data obtained during 2.5 min extinction session.

(Merck, Sharp, and Dohme Res. Labs.), cinanserin HCl (Squibb Labs.) and methysergide maleate (Sandoz Pharm.). Cyproheptadine was prepared in distilled water. Methergoline (Farmitalia) was suspended in 0.5% corn starch. All other drugs were dissolved in 0.9% sodium chloride and solutions were prepared immediately before use. All injections were given intraperitoneally.

RESULTS

The results of testing with the 5-HT antagonists in combination with each 5-OMe DMT-training stimulus are shown in Fig. 1. In rats trained to discriminate 5-OMe DMT at 1.5 mg/kg, cyproheptadine (1.0 to 2.0 mg/kg) was the only antagonist which produced dose-related antagonism of 5-OMe DMT-appropriate responding. The administration of the other antagonists resulted in either no significant attenuation (75–95% 5-OMe DMT-appropriate responding) of the 5-OMe DMT-stimulus effect (i.e., methysergide) or partial antagonism (50–70% 5-OMe DMT-appropriate responding) of 5-OMe DMT-appropriate responding (i.e., methergoline and cinanserin). Control doses of the antagonists in combination with saline resulted in saline-like responding (data not shown). Table 1 shows that the response rates of these animals with cyproheptadine in combination with 5-OMe DMT at 1.5 mg/kg were similar to the response rate that occurred after the administration of saline. Response rates showed significant suppression (i.e., <50% of saline response rate), however, with methysergide (10.0 mg/kg), methergoline (2.0 mg/kg), and cinanserin (15.0 mg/kg) in combination with 5-OMe DMT at 1.5 mg/kg.

In the 3.0 mg/kg 5-OMe DMT-trained animals, none of the antagonists completely attenuated 5-OMe DMT-appropriate responding. Partial antagonism of the stimulus effect in these rats was observed with methergoline and methysergide. Control doses of the antagonists in combination with saline resulted in saline-like responding. Table 2 shows that suppression of response rates (i.e., <50% of saline rate) was observed with cyproheptadine (5.0 mg/kg), cinanserin (15.0 mg/kg), methysergide (10.0 mg/kg), and methergoline (2.0 mg/kg) in combination with 5-OMe DMT at 3.0 mg/kg.

DISCUSSION

Taken together with our earlier report [15], the data presented here indicate that the purported 5-HT antagonists differ in their ability to attenuate the DS properties of 5-OMe DMT. That is, BC-105 and cyproheptadine were the only antagonists to completely attenuate the stimulus effects of 5-OMe DMT at 1.5 mg/kg. Moreover, the antagonism was accomplished without decreasing the animals' response rates. None of the antagonists completely blocked the DS properties of 5-OMe DMT at 3.0 mg/kg. Partial antagonism of the latter stimulus was found by pretreating the animals with methergoline or methysergide; the 5-OMe DMT-stimulus at 1.5 mg/kg was partially attenuated by pretreating the animals with methergoline or cinanserin. While higher doses of those agents may have resulted in complete antagonism, further tests were not attempted because the rats response rates were suppressed by more than 50%.

The different results produced by the antagonists in combination with 5-OMe DMT may be explained, at least in part, by the numerous interactions these agents exert within and between neuropharmacological systems. For example, recent radioligand binding studies have identified two major populations of central 5-HT binding sites, 5-HT_1 and 5-HT_2; 5-HT_1 sites are labelled with high affinity by [^{3}H]5-HT while 5-HT_2 sites are labelled with high affinity by [^{3}H] spiperone or [^{3}H]ketanserin [5, 8, 10, 12, 13]. In addition, spiperone can be used to identify subtypes of 5-HT_1 binding sites. The 5-HT binding sites with high affinity for spiperone have been termed 5-HT_{1A} sites and the binding sites with low affinity for spiperone have been termed 5-HT_{1B} sites [10,12]. 5-OMe DMT binds quite well at both 5-HT_2 sites and 5-HT_1 sites; 5-OMe DMT displays selectivity for 5-HT_{1A} sites [5, 8, 13]. All of the 5-HT antagonists used in the present study bind rather well at 5-HT_2 sites. They also bind, though with a significantly lower affinity, to 5-HT_1 sites: methergoline, cyproheptadine, and cinanserin display equal affinity for 5-HT_{1A} and 5-HT_{1B} sites, while methysergide and BC-105 show selectivity for 5-HT_{1A} sites [13]. Thus, 5-OMe DMT may produce dose-dependent stimulus effects based upon certain interactions between a particular 5-OMe DMT dose and its activity at 5-HT_{1A}, 5-HT_{1B}, and/or 5-HT_2 sites. Antagonism of a 5-OMe DMT-stimulus may then depend on the ability of a 5-HT antagonist to block each component of the stimulus. In addition, the possibility of other neurochemical components in the DS properties of 5-OMe DMT cannot be discounted. Indeed, while cyproheptadine and BC-105 (which is a structural analog of cyproheptadine) have a significant antiserotonin effect, they also have strong antihistamine activity [2,14]. Thus, the possibility of a histaminic (or other neurochemical) component in the DS properties of 5-OMe DMT at 1.5 mg/kg cannot be excluded.

On the other hand, it may be proposed that an antihistaminic (rather than an antiserotonergic) effect predominates at the higher doses of BC-105 and cyproheptadine that would be required to attenuate the 3.0 mg/kg 5-OMe DMT-stimulus. Rather than producing antagonism of the 3.0

mg/kg 5-OMe DMT-stimulus, disruption of behavior (i.e., decreased responding) occurs. In support of this idea, Minnema *et al.* [9] trained rats to discriminate BC-105 (6.0 mg/kg) from saline. The BC-105 stimulus did not generalize to cinanserin, and only partially generalized to methysergide and methergoline. However, complete BC-105-stimulus generalization occurred to cyproheptadine and the phenothiazine antihistamine promethazine, suggesting a possible antihistaminic basis to the 6.0 mg/kg BC-105 DS. Interestingly, promethazine does not antagonize the DS properties of 1.5 mg/kg or 3.0 mg/kg of 5-OMe DMT (unpublished data). Thus it may be suggested that the administration of low doses of BC-105 or cyproheptadine results in a predominantly antiserotonin effect, and consequently, an antagonism is seen of the stimulus properties of 1.5 mg/kg of 5-OMe DMT. However, the administration of higher doses (which would be expected to attenuate the 3.0 mg/kg 5-OMe DMT stimulus) of those agents may result in a predominately antihistamine effect, and consequently, no attenuation is seen of the DS properties of 3.0 mg/kg of 5-OMe DMT. Until further research is conducted to clarify, more fully, the *in vivo* pharmacological profile of these purported serotonin antagonists, and until there is a better understanding of the mechanism of action of 5-OMe DMT, caution is advised when conclusions are drawn from studies employing these agents.

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