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TFMPP May Produce its Stimulus Effects Via a 5-HT_{1B} Mechanism[1,2]

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McKENNEY, J. D. AND R. A. GLENNON. *TFMPP may produce its stimulus effects via a 5-HT_{1B} mechanism.* PHARMACOL BIOCHEM BEHAV **24**(1) 43–47, 1986.—Tests of stimulus generalization were conducted using rats trained to discriminate 1.0 mg/kg of 1-(3-trifluoromethylphenyl)piperazine (TFMPP) from saline in a standard two-lever operant procedure. Generalization of the TFMPP-stimulus was found to occur with fenfluramine and 1-(3-chlorophenyl)-piperazine (mCPP); generalization did not occur with 8-OH DPAT, quipazine, LSD, 5-OMe DMT or 2,5-DMA. Furthermore, the TFMPP-stimulus was not antagonized by pretreatment of the animals with tetrahydrotrazodone (THT). Based on the results of these studies, and on the results of previous binding studies with these same agents, it is suggested that the stimulus properties of TFMPP are mediated primarily via a 5-HT_{1B} mechanism.

TFMPP 5-HT_{1A} 5-HT_{1B} 5-HT_2 Quipazine 8-OH DPAT 5-OMe DMT LSD mCPP Fenfluramine

WE recently demonstrated that 1-(3-trifluoromethylphenyl) piperazine (TFMPP) serves as a discriminative stimulus in animals [9]. Because TFMPP is considered to be a serotonin (5-HT) agonist, there is reason to believe that the TFMPP stimulus may be mediated by a serotonergic mechanism. There are two major populations of central 5-HT binding sites: those labeled with high affinity by [^{3}H]5-HT have been termed 5-HT_1, whereas those sites in the frontal cortex that are labeled by [^{3}H]spiperone or [^{3}H]ketanserin are referred to as 5-HT_2 [14,18]. TFMPP is a non-indolic, 5-HT_1-selective agonist [16]. The results of our discrimination studies are in accord with this finding. That is, TFMPP-stimulus generalization occurred with the purported 5-HT_1 agonist RU-24969 but not with the purported 5-HT_2 agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). In addition, pretreatment of the animals with the 5-HT_2 antagonist ketanserin failed to attenuate TFMPP-appropriate responding [9]. In an attempt to further characterize the TFMPP stimulus, we undertook several additional tests of stimulus generalization and stimulus antagonism.

METHOD

The animals used in this study were eight Sprague-Dawley rats that had been previously trained to discriminate 1.0 mg/kg of TFMPP from saline under a variable interval 15-sec schedule of reinforcement for food (sweetened milk) reward using standard two-lever operant chambers (Coulbourn Instruments, Model E10-10). The discrimination training procedure for these animals has already been described [9].

Stimulus Generalization Studies

Maintenance of the TFMPP/saline discrimination was insured by continuation of the training sessions throughout this phase of the study. That is, training sessions were conducted with TFMPP (1.0 mg/kg) and saline (1.0 ml/kg) during the two days prior to a generalization test such that half of the animals would receive TFMPP whereas the other half would receive saline. After a 2.5-min non-reinforced session, training was continued for an additional 12.5-min period. A 15 min pre-session injection interval was used. Animals not discriminating TFMPP from saline (as determined by the results obtained during the 2.5-min extinction session), i.e., animals making less than 80% TFMPP-appropriate responding when given TFMPP, or making greater than 20% TFMPP-appropriate responding when administered saline, were not used in the immediately following generalization test session. During investigations of stimulus generalization, test sessions were interposed amongst the training sessions. The animals were allowed 2.5-min to respond under non-reinforcement conditions and were then returned to their individual home cages. An odd number of training sessions (not less than three) separated any two test sessions. Doses of the challenge drugs were administered in a random sequence using a 15-min pre-session injection interval (unless otherwise stated). Stimulus generalization was said to occur when the animals, after being administered a given dose of challenge drug, made 80% or greater of their responses on the TFMPP-appropriate lever. Animals making fewer than five responses during the entire 2.5 min extinction session

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TABLE 1

RESULTS OF GENERALIZATION STUDIES USING TFMPP AS TRAINING DRUG

Agent	Dose	N*	TFMPP-Appropriate Responding (±SEM)†	ED_{50} (95% confidence limits)
mCPP	0.2	3/3	11% (5)	
	0.5	4/4	39% (9)	
	0.7	3/4¶	66% (18)	
	0.8	3/4¶	92% (8)	
	1.0	1/3	—‡	0.47 (0.28–0.80) mg/kg
Fenfluramine	1.0	4/4	36% (11)	
	1.5	4/4	53% (19)	
	1.75	2/4¶	84% (16)	
	2.0	3/4¶	93% (6)	1.23 (0.87–1.74) mg/kg
TFMPP	1.0	8/8	98% (1)	0.23 mg/kg§
Saline (1 ml/kg)		8/8	6% (2)	—

*Number of animals responding/number of animals receiving drug.
†Data obtained during 2.5-min extinction session.
‡Disruption of behavior (i.e., no responding).
§ED_{50} value determined in an earlier study [9].
¶In each case indicated, it was the same animal that was "disrupted" (i.e., that failed to meet the criterion of 5 responses during the extinction session).

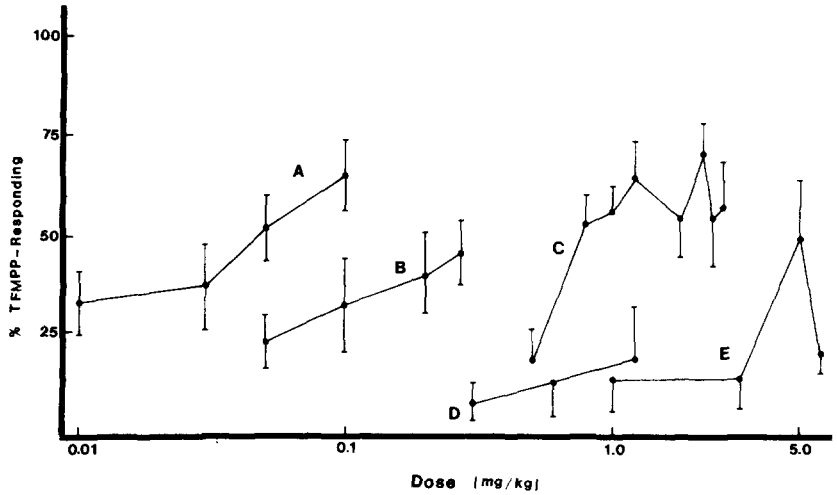

FIG. 1. Effects of several serotonergic agents in animals trained to discriminate 1.0 mg/kg of TFMPP from saline: (A) LSD, (B) 8-OH DPAT, (C) Quipazine, (D) 5-OMe DMT, (E) 2,5-DMA. Each data point reflects the results obtained using 5–7 animals (except for 5-OMe DMT, where 4 animals were used at each dose). Administration of doses higher than those shown resulted in disruption of behavior.

were reported as being disrupted. For those agents where stimulus generalization occurred, ED_{50} values were calculated from the dose-response data by the method of Finney [5]. These ED_{50} values represent doses at which the animals would be expected to make approximately 50% of their responses on the TFMPP-appropriate lever.

Stimulus Antagonism Studies

During the course of these studies, the TFMPP/saline discrimination was maintained as described above. Tests of stimulus antagonism evaluated the effect of THT in combination with TFMPP on TFMPP-appropriate responding. Doses of THT were administered 10 min prior to the administration of 1.0 mg/kg of TFMPP (i.e., 25 min prior to testing). Percent TFMPP-appropriate responding was recorded, as above, during a 2.5-min extinction session. In a separate series of control studies, 1.0 ml/kg of saline was used in place of TFMPP.

Drugs

1-(3-Trifluoromethylphenyl) piperazine and 1-(3-chlorophenyl)piperazine were purchased from Aldrich Chemical

TABLE 2
RESULTS OF STUDIES USING TETRAHYDROTRAZODONE (THT)

Agent (mg/kg)		Pretreatment (mg/kg)		N*	% TFMPP-Appropriate Responding (±SEM)†
TFMPP (1.0)‡	+	Saline (1.0 ml/kg)§		7/7	98% (0.6)
TFMPP (1.0)‡	+	THT§	0.2	3/4	100%
			1.0	4/4	100%
			2.0	2/3	96% (4)
			4.0	3/4	100%
			7.0	3/4	100%
Saline (1.0 ml/kg)‡	+	THT§	0.2	3/3	9% (5)
			1.0	3/3	30% (17)
			2.0	3/3	27% (13)
			4.0	3/3	7% (4)
			7.0	3/4	20% (15)
THT¶	1.0		—	4/4	7% (2)
	5.0		—	3/4	13% (6)
Saline¶ (1.0 ml/kg)			—	7/7	8% (2)

*Number of animals responding/number animals receiving drug.
†Data obtained during 2.5-min extinction session.
‡Administered 15 min prior to extinction session.
§Administered 25 min prior to extinction session.
¶Administered 45 min prior to extinction session.

Co. and were converted to their hydrochloride salts via standard procedures; these salts are referred to as TFMPP and mCPP, respectively. 5-Methoxy-N,N-dimethyltryptamine hydrogen oxalate (5-OMe DMT) and 1-(2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (2,5-DMA) had been previously synthesized in our laboratories. 8-Hydroxy-2-(N-di-n-propylamino)tetralin hydrobromide (8-OH DPAT) was obtained from Research Biochemicals Inc., and (+)-lysergic acid diethylamide tartrate (LSD) was obtained from NIDA. 2-(1-Piperazino)quinoline maleate (quipazine), fenfluramine hydrochloride, and 2-(3-(4-(3-chlorophenyl)-1-piperazinyl)propyl)-s-triazolo[4,3-a]pyridin-3(2H) one hydrochloride (tetrahydrotrazodone; THT) were gifts from Miles Laboratories, A. H. Robins, and Bristol Myers, respectively. Solutions of all agents were prepared fresh daily in sterile 0.9% saline and were administered by intraperitoneal (IP) injection.

RESULTS

The originally-reported [9] TFMPP discrimination was maintained throughout the course of this study such that the animals made greater than 80% of their responses on the TFMPP-appropriate lever after administration of 1.0 mg/kg of TFMPP, and less than 20% of their responses on the same lever after administration of 1.0 ml/kg of saline. As shown in Table 1, the TFMPP-stimulus generalized to mCPP and to fenfluramine; the TFMPP-stimulus did not generalize to any of the other agents examined. Four doses of 5-OMe DMT were evaluated; 1.2 mg/kg of 5-OMe DMT produced a maximum of 19% TFMPP-appropriate responding (Fig. 1), and administration of 1.4 mg/kg resulted in disruption of behavior (i.e., 0 of 4 animals responded). Administration of LSD, 8-OH DPAT, quipazine, and 2,5-DMA (although not necessarily at their highest dose tested) all resulted in partial generalization; maximal TFMPP-appropriate responding for each of these agents was 65% (at 0.1 mg/kg, 46% (at 0.27 mg/kg), 71% (at 2.2 mg/kg), and 49% (at 5.0 mg/kg), respectively (Fig. 1). Administration of higher doses of LSD (0.13 mg/kg), 8-OH DPAT (0.3 mg/kg), quipazine (2.8 mg/kg), and 2,5-DMA (9.0 mg/kg) resulted in disruption of behavior and precluded the further evaluation of these agents. Response rates were not significantly different from those produced by 1.0 mg/kg of TFMPP except: (a) at the highest non-disruption dose of each of the agents tested (where the response rates, i.e., responses per minute, were usually depressed by 50–70%), and (b) where disruption of behavior occurred.

Administration of tetrahydrotrazodone (THT; 0.2–7.0 mg/kg) 10 min prior to the administration of 1.0 mg/kg of TFMPP had no effect on TFMPP-appropriate responding (Table 2); response rates of the animals receiving 7.0 mg/kg of THT in combination with TFMPP were reduced to approximately 40% of that observed after administration of TFMPP alone. Administration of THT 10 min prior to the administration of saline resulted in 7–30% TFMPP-appropriate responding (Table 2). Once again, the animals response rates were not significantly different from (except at 7.0 mg/kg of THT where they were reduced to approximately 40% of) that observed upon administration of saline (or TFMPP) alone. Two doses of THT (1.0 and 5.0 mg/kg) were evaluated in tests of stimulus generalization using a 45-min pre-session injection interval. Both doses produced saline-appropriate responding.

DISCUSSION

Even though TFMPP is considered to be a 5-HT_1 agonist, there is no evidence that the stimulus produced by TFMPP

involves serotonin. Thus, the first goal of this study was to provide some evidence to this effect. That TFMPP-stimulus generalization occurs with fenfluramine, an agent known to release endogenous stores of 5-HT [4], suggests that 5-HT may play a role in the stimulus properties of TFMPP. mCPP is a structural relative of TFMPP where the trifluoromethyl group of the latter has been replaced by a chloro group. Like TFMPP, mCPP has been shown to interact at central 5-HT_1 binding sites but is several-fold less potent in this respect [16]. The results of the stimulus generalization studies (Table 1) are consistent with this finding in that mCPP produces TFMPP-like effects but is several-fold less potent than TFMPP itself.

RU-24969, DOM, 8-OH DPAT, quipazine, LSD, 2,5-DMA, and 5-OMe DMT have all been shown to possess activity as serotonin agonists [6,7]. However, whereas the TFMPP-stimulus generalizes to RU-24969, stimulus generalization does not occur with DOM [9]. These findings are also consistent with the results of binding studies in that RU-24969 is a 5-HT_1 agonist, while DOM displays a 30-fold selectivity for 5-HT_2 sites over 5-HT_1 sites [10,19]. The data presented thus far suggest that the TFMPP-stimulus might involve a 5-HT_1 mechanism. 8-OH DPAT is a relatively new serotonin agonist that has been shown to be a potent 5-HT_1-selective agent [17]; thus, it might be anticipated that TFMPP-stimulus generalization should occur with 8-OH DPAT. That this is not the case is shown in Fig. 1. Middlemiss and Fozard have recently demonstrated that 8-OH DPAT binds selectively to a sub-population of 5-HT_1 binding sites (i.e., 5-HT_{1A} sites) [17], whereas, Sills *et al.* [19] have found that TFMPP binds to a different sub-population of 5-HT_1 sites (i.e., 5-HT_{1B} sites). mCPP was also found to be a 5-HT_{1B}-selective agent with approximately half the potency of TFMPP [19]. Serotonin itself apparently binds nearly equally well at both sub-populations of 5-HT_1 sites [19]. Thus, the TFMPP-stimulus may not only be 5-HT_1-mediated, but may be, more specifically, 5-HT_{1B}-mediated. Site-selective interactions have been previously used to explain some of the other pharmacological differences amongst 5-HT agonists [15].

Sills *et al.* [19] also demonstrated that RU-24969 and quipazine are 5-HT_{1B}-selective agents, but that 5-OMe DMT displays selectivity for 5-HT_{1A} sites. This may, in part, explain the observed TFMPP-stimulus generalization to RU-24969 [9], and the lack of generalization to 5-OMe DMT. But it should be noted that unlike RU-24969, 5-OMe DMT and quipazine also bind rather well at 5-HT_2 sites [10,14]. As a consequence, sub-site selectivity (i.e., at 5-HT_1 sub-populations) may not be as behaviorally signficant for agents that demonstrate affinity for 5-HT_2 sites as for agents that are 5-HT_1-selective. LSD is an agent that binds both to 5-HT_1 and 5-HT_2 sites [14,18]. 2,5-DMA also interacts (weakly) at both sites [10]; its affinity for 5-HT_1 sub-populations has not been examined. Each of these latter agents ultimately produced disruption of behavior in the TFMPP-trained animals; the 5-HT_{1A} and/or 5-HT_2 components of these agents may be responsible for this disruption. For example, in tests of stimulus generalization using animals trained to discriminate 1.0 mg/kg of DOM from saline [20], the DOM-stimulus was found to generalize to quipazine (3.0 mg/kg), 5-OMe DMT (3.0 mg/kg), LSD (0.1 mg/kg) and 2,5-DMA (10.0 mg/kg) [7,11]. Doses at which generalization occurred (i.e., values given in parenthesis) are comparable to the doses of these agents that produced disruption of behavior in the TFMPP-trained animals (Fig. 1). Furthermore, the DOM-stimulus did not generalize with the 5-HT_1-selective agents 8-OH DPAT [8], TFMPP and RU-24969 [11].

Another approach to studying the stimulus properties of drugs is to conduct tests of stimulus antagonism. However, to date, there exists no 5-HT_1-specific antagonists. An early report on THT, the tetrahydro derivative of the antidepressant trazodone, suggested that this agent might be a potential 5-HT_1 antagonist [13]. As shown in Table 2, THT did not attenuate the effects of TFMPP at the doses evaluated (higher doses were not evaluated because of the disruptive effects of THT at 7.0 mg/kg); in fact, THT in combination with saline produced up to 30% TFMPP-appropriate responding. Because mCPP has been determined to be a metabolite of trazodone [1,2], it is entirely possible that THT might also be metabolized to this same agent and that the subsequent agonistic effect might mask any possible antagonism of the TFMPP stimulus. However, in tests of stimulus generalization with THT, using a longer (45-min) pre-session injection interval, there was no evidence for this. After our studies had been completed, it was demonstrated that THT actually displays selectivity for 5-HT_2sites [12]. Also, we have since shown that THT can antagonize the stimulus effects of DOM [8].

Shortly after our initial report on TFMPP appeared, Cunningham and Appel published a brief report on the results of their studies with this same agent. They found, consistent with our results, that the TFMPP-stimulus generalized to RU-24969 and to mCPP, but not to LSD or quipazine [3]. Further, they were unable to attenuate the stimulus effect of TFMPP by pretreatment of the animals with any one of several 5-HT_2 antagonists [3].

In summary, the results of our work with TFMPP suggest that TFMPP produces its stimulus effects primarily via a 5-HT_{1B}-related mechanism. The TFMPP-stimulus generalized to those agents that display a high affinity for 5-HT_{1B} sites (i.e., RU-24969, mCPP), but not to those agents that are selective for 5-HT_{1A} sites (i.e., 8-OH DPAT), or agents that, in addition to possessing affinity for 5-HT_{1A} or 5-HT_{1B} sites, also possess a significant affinity for 5-HT_2 sites. The finding that many of the agents examined produced partial generalization is probably a reflection of their selectivity characteristics (i.e., a lack of complete specificity for one site over another).

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