Life Sciences, Vol. 35, pp. 1475-1480
Printed in the U.S.A.

Pergamon Press

DISCRIMINATIVE STIMULUS PROPERTIES OF THE SEROTONIN AGONIST 1-(3-TRIFLUOROMETHYLPHENYL)PIPERAZINE (TFMPP)

Richard A. Glennon, J. D. McKenney and Richard Young

Department of Medicinal Chemistry
School of Pharmacy
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298

(Received in final form July 20, 1984)

Summary

Using a standard two-lever drug discrimination procedure, twelve rats were trained to discriminate 1.0 mg/kg of the serotonin (5-HT) agonist TFMPP from saline. Once trained, the animals displayed a dose-related decrease in discriminative performance upon administration of lower doses of TFMPP. Tests of stimulus generalization were performed using the purported 5-HT agonist RU-24,969 and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM). While TFMPP produced stimulus effects similar to those of RU-24,969, these effects seem to be dissimilar to those of DOM. The results of the present study suggest that the discriminative stimulus effects of TFMPP may involve a 5-HT_1-related mechanism.

The use of ligand-binding techniques has allowed the identification of two major types of central serotonin (5-HT) binding sites, i.e. 5-HT_1 and 5-HT_2 sites (1,2). 5-HT_1 sites are labelled with high affinity by tritiated serotonin while ^{3}H-spiperone or ^{3}H-ketanserin are routinely employed to label 5-HT_2 sites (2). 1-(3-Trifluoromethylphenyl)piperazine (TFMPP) has been reported to be a direct-acting central 5-HT agonist (3), and Fuller et al (4) have identified TFMPP as being the most potent member of a series of over two dozen piperazine derivatives with respect to displacing ^{3}H-5-HT binding to rat brain membranes. More recently, Martin and Sanders-Bush (5) have demonstrated that TFMPP binds both to 5-HT_1 (IC_{50} = 38.9 nM) and ^{3}H-spiperone-labelled 5-HT_2 (IC_{50} = 708 nM) sites, although it appears to display some selectivity for 5-HT_1 sites. The pharmacological significance of 5-HT_1 binding sites is poorly understood; furthermore, to date, there have been no reports of selective 5-HT_1 antagonists. The goal of this present study was to determine whether TFMPP, a relatively selective 5-HT_1 agonist, could serve as a discriminative stimulus in rats. If this could be achieved, animals trained to discriminate TFMPP from saline might eventually aid in understanding 5-HT_1-related phenomena and might be useful for identifying potential 5-HT_1 antagonists.

Methods

Subjects: The animals used in this study were twelve male Sprague-Dawley (225-350 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.

Discrimination training: All animals were trained to lever-respond to a variable interval (VI) 15-s schedule of reinforcement on both levers of a standard two-lever operant chamber (Coulbourn Instruments, Model E10-10). The reinforcer was sweetened condensed milk diluted 2:1 with tap water. The session duration was 15-min and sessions were conducted five days a week. Fifteen minutes prior to each session, the rats were injected intraperitoneally (i.p.) with either 1.0 mg/kg of TFMPP or saline on a random schedule with the constraint that no more than two consecutive sessions with the drug or vehicle could occur. For six of the animals in the group, responses on the right lever were reinforced when drug was given and responses on the left lever were reinforced after saline administration; these conditions were reversed for the remaining animals in the group.

On every fifth day of testing, discrimination learning was assessed duran 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 TFMPP lever (number of responses on TFMPP-designated lever/total number of responses). After 70 training sessions, the animals discrimination performance was stable (i.e., TFMPP, approxiamately 98%; saline, approximately 15%). Response rates were similar under each treatment condition. The TFMPP versus saline discrimination was insured by continuation of training sessions throughout generalization tests.

Generalization tests: During generalization investigations, 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 removed from the operant chambers. Animals never received drug (or saline) more than once per day. The first generalization tests investigated the dose-response and time-duration parameters of the TFMPP-stimulus. Dose-response studies assessed the TFMPP-appropriate responding of the rats following the administration of various doses of the training drug. Doses of TFMPP were administered in a random sequence with the standard 15-min injection-time before behavioral testing. The time-course test investigated the effects of decreasing or increasing the time interval between the injection of the TFMPP training dose and the beginning of a test session; in addition to the standard 15-min delay, the effects of various injection-time (i.e. pre-session injection) intervals between 5- and 240-min were studied. Variation of pre-session injection intervals was done in separate experiments during different weeks.

The final generalization tests investigated the ability of the TFMPP-stimulus to generalize to DOM, RU-24,969, and TFMPP in combination with ketanserin. The dose-response substitution tests of DOM and RU-24969 assessed the percent TFMPP-like response produced by the administration of various doses of these agents. Doses of DOM or RU-24969 were administered in a random sequence with a 15-min injection-time interval prior to the 2.5-min extinction test period. Where generalization occurred, the ED_{50} value was determined from the dose-response data by the method of Finney (6). These ED_{50} values are estimated doses at which the rats would perform 50% appropriate drug-lever responding. Ketanserin was administered 45 min prior to a 2.5-min extinction session (i.e. 30 min prior to administration of either 1.0 mg/kg of TFMPP or 1.0 ml/kg of saline). Doses of ketanserin were administered in a random sequence.

Drugs: 1-(3-Trifluoromethylphenyl)-piperazine was purchased from Aldrich Chemical Co. and was converted to its monohydrochloride salt using standard procedures; the melting point range, after recrystallization from absolute

ethanol, was 235-236°C. 5-Methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU-24 969) was a gift from Centre de Recherches, Roussell-Uclaf, Romainville, France, and racemic 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) was obtained from NIDA, Bethesda, MD. Ketanserin was a gift from Janssen Pharmaceutica, Beerse, Belgium. All solutions were prepared fresh daily in sterile saline and administered by the i.p. route.

Results and Discussion

Twelve animals were trained to discriminate 1.0 mg/kg of TFMPP from saline such that greater than 95% of total responses were made on the drug-designated lever after administration of TFMPP while less than 15% of total responses were made on this same lever after administration of 1.0 ml/kg of saline (Fig. 1). Response rates after administration of 1.0 mg/kg of TFMPP were not significantly different from those produced by saline. The time-course of effects produced by TFMPP is shown in Fig. 2. With a pre-session injection interval of 5 min, 1.0 mg/kg of TFMPP produced 67% drug-appropriate responding. However, drug-appropriate responding after 60 min (92%) was not significantly different from that observed after a 15-min pre-session injection interval. After 60 min, the percent of total responses on the TFMPP-designated lever decreased slowly with time. Discriminative control of responding was demonstrated to be dose related (Table 1); decreasing the dose of TFMPP resulted in a decrease in TFMPP-appropriate responding such that 0.1 mg/kg of TFMPP produced saline-like responding.

In tests of stimulus generalization, the TFMPP-stimulus generalized to RU-24,969 in a dose related manner. Administration of DOM to the TFMPP-trained animals resulted, ultimately, in disruption of behavior. A dose of 0.25 mg/kg of DOM produced saline-like responding; doses of 0.75 to 1.35 mg/kg of DOM resulted in partial generalization and decreased response rates, while a dose of 1.40 mg/kg produced disruption of behavior. In tests of stimulus antagonism, several doses of the 5-HT_2 antagonist ketanserin were administered in combination with 1.0 mg/kg of TFMPP. The highest dose of ketaserin used in this study was 0.75 mg/kg, or four times the ED_{50} dose necessary for the antagonism of 1.0 mg/kg of DOM in DOM-trained animals (11); at this dose, ketanserin had no significant effect on TFMPP-appropriate responding, although the animals' response rates were reduced to approximately 30% of control levels (Table 1). Doses of ketanserin were also administered in combination with saline (data not shown); in these cases, TFMPP-appropriate responding did not exceed 16% and the animals' response rates were not significantly different from those seen after administration of either 1.0 mg/kg of TFMPP alone and/or saline alone.

RU-24,969 is reported to be a potent serotonin agonist that displaces ^{3}H-5-HT from rat membranes with an IC_{50} = 6.8 nM (7,8). RU-24,969 potentiates the behavioral effects of 5-hydroxytryptophan and, like TFMPP, RU-24,969 decreases 5-HT turnover and stimulates prolactin secretion (7). Administration of RU-24,969 to the TFMPP-trained animals resulted in stimulus generalization (Table 1), suggesting that the two agents produce similar stimulus effects.

DOM has been demonstrated to bind to 5-HT sites (9) and recent studies reveal that DOM possesses a 30-fold selectivity for 5-HT_2, as opposed to 5-HT_1, sites (10). DOM (1.0 mg/kg) also serves as a discriminative stimulus in rats, and its effects are attenuated by pre-treatment of the animals with the 5-HT_2 antagonists ketanserin (ED_{50} = 0.18 mg/kg) and pirenperone (ED_{50} = 0.01 mg/kg) (11). Furthermore, DOM-stimulus generalization occurs to R(-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, which possesses greater than

Table 1

Results of generalization studies using TFMPP (1.0 mg/kg) as training drug.

Agent	Dose mg/kg	N[a]	% TFMPP Appropriate Responding[b] (±SEM)	Mean Res/ Min[b] (±SEM)
TFMPP	0.10	5/5	15% (5.9)	12.8 (2.0)
	0.16	7/7	38% (12.7)	18.1 (3.7)
	0.25	5/5	59% (16.8)	21.8 (4.9)
	0.50	5/5	75% (18.2)	17.4 (3.4)
	1.00	8/8	97% (1.0)	11.3 (1.6)
		ED_{50} = 0.23 (0.14-0.38)[c] mg/kg		
RU-24,969	0.10	4/5	28% (12.7)	17.0 (5.3)
	0.30	4/4	74% (14.0)	12.4 (5.8)
	0.50	4/4	91% (4.3)	12.9 (1.6)
		ED_{50} = 0.17 (0.07-0.39)[c] mg/kg		
(±)-DOM	0.25	3/3	16% (6.1)	19.1 (6.3)
	0.75	6/10	53% (14.9)	11.2 (4.4)
	0.90	10/12	48% (9.3)	7.7 (2.2)
	1.05	6/8	46% (5.3)	12.9 (1.4)
	1.20	9/14[d]	70% (7.6)	6.0 (1.2)
	1.30	6/7	68% (13.1)	8.2 (2.7)
	1.35	4/4	42% (12.7)	6.5 (1.6)
	1.40	3/7	-[e]	
TFMPP (1.0 mg/kg)[f]				
+ ketanserin (0.05 mg/kg)		4/4	99% (0.5)	9.0 (5.0)
+ ketanserin (0.20 mg/kg)		3/4	93% (4.1)	8.5 (5.9)
+ ketanserin (0.50 mg/kg)		3/4	96% (4.0)	6.8 (2.0)
+ ketanserin (0.75 mg/kg)		3/4	98% (2.0)	4.0 (1.4)
Saline (1 ml/kg)		8/8	5% (1.3)	12.9 (1.8)

[a] Number of animals responding/number receiving drug.
[b] Data obtained during 2.5-min extinction session.
[c] ED_{50} value followed by 95% confidence limits.
[d] Same seven animals run twice.
[e] Distuption of behavior, i.e. no responding.
[f] Ketanserin was administered 30 min prior to administration of TFMPP

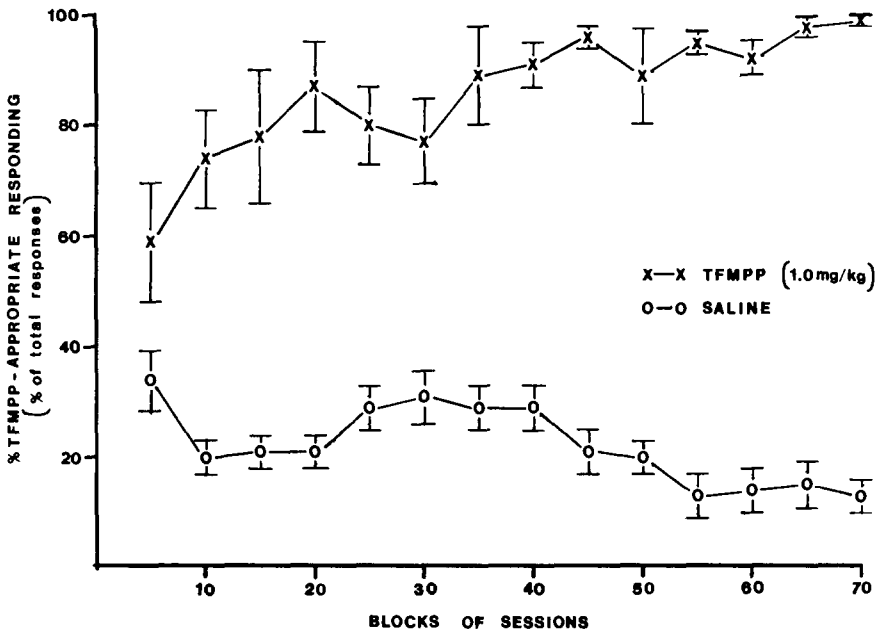

FIGURE 1. Learning curve for the acquisition of the TFMPP (1.0 mg/kg) vs saline (1.0 ml/kg) discrimination; each point reflects the results obtained using all twelve animals.

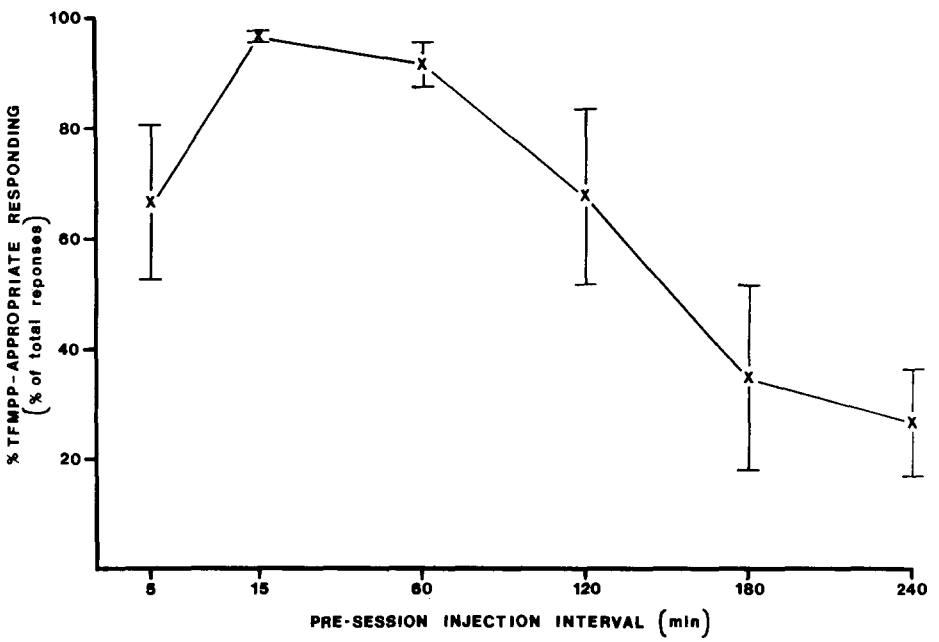

FIGURE 2. Time-course of TFMPP (1.0 mg/kg) administered to TFMPP-trained animals (n=6).

a 200-fold selectivity for 5-HT_2 binding sites (10), but not to TFMPP or RU-24,969 (11). These results have led us to suggest that the stimulus effects produced by DOM may be an expression of 5-HT_2 agonism (11). Administration of DOM to the TFMPP trained animals did not result in complete stimulus generalization, and, at the highest dose evaluated, produced disruption of behavior. Several explanations might be offered to account for the partial generalization produced by doses of 0.75 to 1.35 mg/kg of DOM. TFMPP is selective, but not specific, for 5-HT_1-site interactions, whereas DOM is selective, but not specific, for 5-HT_2 sites. The stimulus produced by TFMPP might be related both to the 5-HT_1 and 5-HT_2 character of TFMPP, or, alternatively, the TFMPP-trained animals might recognize the 5-HT_1 component of DOM. The latter suggestion seems more attractive because, if the TFMPP stimulus involved a 5-HT_2 agonist component, some attenuation of effect would have been noted when the animals were pre-treated with ketanserin.

Taken together, the results of this study demonstrate (a) that at 1.0 mg/kg the serotonin agonist TFMPP serves as an effective discriminative stimulus in rats, (b) that the TFMPP-stimulus generalizes to the purported 5-HT_1 agonist RU-24,969, but not to the purported 5-HT_2 agonist DOM, and (c) that the 5-HT_2 antagonist ketanserin was unable to attenuate the effect of TFMPP under the conditions employed. It is suggested that the stimulus properties of TFMPP may involve a 5-HT_1-related mechanism, although further studies will be necessary to characterize fully this effect.

References

1. S. J. Peroutka and S. H. Snyder, Mol. Pharmacol., 16, 687-699 (1979).
2. J. E. Leysen and J. P. Tollenaere, Ann. Rep. Med. Chem., 17, 1-10 (1982).
3. R. W. Fuller and N. R. Mason, in Serotonin, Current Aspects of Neurochemistry and Function., B. Haber, S. Gabay, M. R. Issidorides and S. G. A. Alivasatos, Eds. (Plenum, New York, 1981), p. 359-368.
4. R. W. Fuller, N. R. Mason and B. B. Molloy, Biochem. Pharmacol., 29, 833-835 (1980).
5. L. L. Martin and E. Sanders-Bush, N. S. Arch. Pharmacol., 321, 165-170 (1982).
6. D. J. Finney, Probit Analysis (Cambridge University Press, London, 1952).
7. P. Hunt and C. Oberlander, in Serotonin, Current Aspects of Neurochemistry and Function., B. Haber, S. Gabay, M. R. Issidorides and S. G. A. Alivasatos, Eds. (Plenum, New York, 1981), p. 547-562.
8. C. Euvrard and J. R. Boissier, Europ. J. Pharmacol., 63, 65-72 (1980).
9. G. Battaglia, M. Shannon, R. A. Glennon and M. Titeler, Soc. Neurosci. Abstr., 9, 1157 (1983).
10. M. Shannon, G. Battaglia, R. A. Glennon and M. Titler, Europ. J. Pharmacol., in press.
11. R. A. Glennon, R. Young and J. A. Rosecrans, Europ. J. Pharmacol., 91, 189-196 (1983).