Pharmacology Biochemistry & Behavior, Vol. 37, pp. 769–771. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Stimulus Properties of Tiflucarbine: A Novel Antidepressant Agent

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Received 16 January 1990

GLENNON, R. A., J. DE VRY, D. G. SPENCER, JR. AND T. GLASER. *Stimulus properties of tiflucarbine: A novel antidepressant agent.* PHARMACOL BIOCHEM BEHAV **37**(4) 769–771, 1990.—Tiflucarbine is a structurally novel antidepressant that binds at central serotonin (5-HT) binding sites. There is also evidence that this agent is both a 5-HT1 and a 5-HT2 agonist. To further characterize the serotonergic actions of this agent, tiflucarbine was evaluated in groups of rats trained to discriminate the 5-HT1A agonist 8-OH DPAT, the 5-HT2 agonist DOM, and the nonselective 5-HT agonist 5-OMe DMT from saline. Tiflucarbine resulted in partial generalization in the DOM-trained and in the 8-OH DPAT-trained animals. Although two-thirds of the animals were disrupted, 10 mg/kg of tiflucarbine resulted in stimulus generalization in the 5-OMe DMT-trained animals. It is concluded that tiflucarbine is most likely a nonselective 5-HT agonist.

Serotonin 5-HT1A 5-HT2 8-OH DPAT 5-OMe DMT DOM Tiflucarbine Drug discrimination

THE structurally novel antidepressant tiflucarbine (TVX P 4495) has been demonstrated to act primarily via a serotonergic mechanism (2,3). Tiflucarbine binds both at 5-HT1 and 5-HT2 sites and displays little affinity (i.e., K_i >3,000 nM) for adrenergic, dopaminergic, gaba, benzodiazepine, and other binding sites (3). In behavioral studies with rodents, tiflucarbine produces effects indicative of 5-HT1 agonism (such as forepaw treading, hindlimb abduction and flat body posture) and 5-HT2 agonism (such as head-twitch behavior) (3). Whereas its affinity for 5-HT1 sites is not particularly high, tiflucarbine binds at 5-HT2 sites with an affinity (K_i = 115 nM) comparable to that of the 5-HT2 agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (3). Taken together, the available evidence suggests that this agent may be a novel 5-HT2 agonist. In order to further characterize the serotonergic actions of this agent, we conducted drug discrimination studies with groups of rats trained to discriminate the 5-HT1A agonist 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH DPAT), the 5-HT2 agonist DOM, and the nonselective 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT) from saline. This technique has proven to be useful for the investigation of novel serotonergic agents [see Glennon (5) for a review].

METHOD

Discrimination Studies

Three groups of animals were used in the present study. The first group consisted of five male Sprague-Dawley rats that had been previously trained to discriminate 1.0 mg/kg (IP) of DOM from saline using a variable-interval 15-sec (VI 15) schedule of reinforcement for food (sweetened powdered milk) reward. These are the same animals that were used in a previous study, and their training and use have been reported (6). Using a standard two-lever operant chamber (Coulbourn Instruments), training of the animals was maintained throughout this study by the administration of either 1.0 mg/kg of DOM or 1.0 ml/kg of 0.9% saline on a daily basis 15 min prior to testing; training sessions lasted 15 min. Discrimination learning was assessed, under both training-drug and saline conditions, once a week during a 2.5-min extinction session (followed by a 12.5-min training session). Animals not meeting criteria were not used in that particular week's stimulus generalization study. In order to meet criteria, the animals were required to make >80% of their responses on the DOM-appropriate lever after administration of DOM, and <20% of their responses on the DOM-appropriate lever after administration of 0.9% saline, during the 2.5-min extinction session. During the stimulus generalization studies, animals were administered doses of tiflucarbine in 0.9% saline via the intraperitoneal route 15 min (unless otherwise noted) prior to testing. Only one dose of tiflucarbine was tested per week; the animals were allowed 2.5 min to respond under extinction conditions and were then returned to their individual home cages. Animals did not receive DOM or saline on those days tiflucarbine was administered. Data collected during the extinction session included total responses on the DOM-appropriate lever (as a percentage of total responses) and response rate (mean responses per minute). Animals making fewer than 5 total responses during the 2.5-min extinction session were recorded as being disrupted. The stimulus antagonism studies were conducted in essentially the same manner as the stimulus generalization studies. Doses of tiflucarbine were administered 5 min prior to 1.0 mg/kg of DOM; 15 min later, the animals were

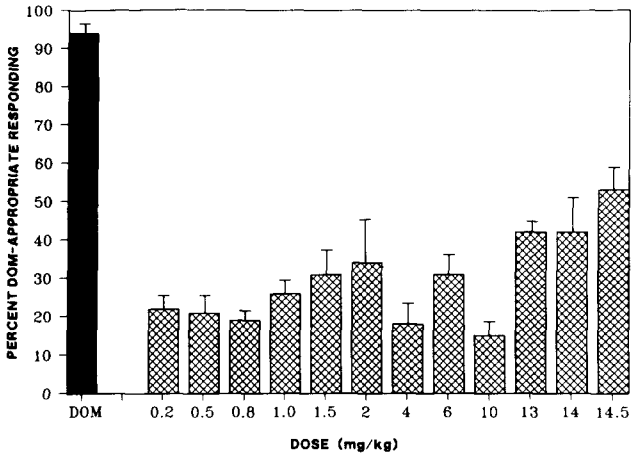

FIG. 1. Results of stimulus generalization studies with doses of tiflucarbine in rats trained to discriminate 1 mg/kg of DOM from saline in a two-lever operant procedure. The bar designated as DOM is the effect of the training dose of DOM (n = 5). Saline (1 ml/kg; results not shown) produced <20% DOM-appropriate responding.

TABLE 1

EFFECT OF TIFLUCARBINE IN RATS TRAINED TO DISCRIMINATE 8-OH DPAT (0.1 mg/kg) and 5-OMe DMT (1.25 mg/kg) FROM SALINE

Agent	Dose (mg/kg)	N*	% Generalization†	Response Rate‡
		8-OH DPAT-Trained Animals		
8-OH DPAT	0.1	19/21	82%	95
Tiflucarbine	1	6/6	17%	112
	2.5	4/6	0%	35
	5	2/6	50%	58
	10	2/6	50%	21
		5-OMe DMT-Trained Animals		
5-OMe DMT	1.25	12/12	100%	105
Tiflucarbine	2.5	5/5	0%	104
	5	8/9	38%	44
	10	4/12	100%	10

*Number of animals responding/number administered drug. †Percent of rats selecting drug lever. ‡Response rate as a percentage of response rate on the preceding saline day.

placed in the operant chamber and allowed to respond for 2.5 min under extinction conditions.

The second group of animals consisted of six male Wistar rats trained to discriminate 0.1 mg/kg (IP) of 8-OH DPAT from saline and the third group included 12 male Wistar rats trained to discriminate 1.25 mg/kg (IP) of 5-OMe DMT from saline. Using standard two-lever operant chambers, these two groups were trained exactly according to the method of De Vry and Traber (1), and Spencer and co-workers (7), respectively, using a fixed-ratio 10 (FR 10) schedule of reinforcement for food (45 mg solid pellet) reward. Following discrimination acquisition, tests of stimulus generalization were conducted. Injection of various doses of tiflucarbine were substituted for either 8-OH DPAT or 5-OMe DMT (in their respective group of animals) 15 min prior to testing. Test sessions were separated from each other by at least three practice sessions in which the training drug or saline were correctly discriminated. Sessions lasted a maximum of ten min but were also terminated upon delivery of 50 reinforcements. During these sessions, the lever on which ten responses first accumulated was rewarded and only responses on that lever were subsequently rewarded. Response rates were calculated as percent saline control response rate; that is, the rate on the test day for each animal was divided by that from the most recent saline session and multiplied by 100.

Drugs

Tiflucarbine or 1-methyl-9-ethyl-4-fluoro-7,8,9,10-tetrahydrothieno[3,2-e]pyrido[4,3-b]indole was used as the lactate salt. 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM) was a gift from the National Institute of Drug Abuse. 5-Methoxy-N,N-dimethyltryptamine (5-OMe DMT) and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT) as their hydrochloride salts were prepared by the Department of Chemistry, Bayer A.G., Wuppertal, F.R.G.

RESULTS

In rats trained to discriminate 1.0 mg/kg of racemic DOM from saline, tiflucarbine produced 15–34% DOM-appropriate responding at doses of up to 10 mg/kg (Fig. 1). At doses of 13–14.5 mg/kg, tiflucarbine produced 42–53% DOM-appropriate responding (Fig. 1) and, at 15 mg/kg, disruption of behavior occurred (i.e., 4 of 5 animals did not respond). Groups of three to four animals were tested at doses below 10 mg/kg and the animals' response rates were comparable to saline control (15% DOM-appropriate responding; 11.2 responses per min). Groups of five animals were tested at doses of 10 mg/kg and greater and here also response rates were comparable to control (except at 14.5 mg/kg; response rate = 5.9 responses per min). Using a dose of 14 mg/kg, the pre-session injection interval was varied from the standard 15 minutes to 60 minutes without any significant difference. With a 60-minute interval, 14 mg/kg of tiflucarbine produced 43% DOM-appropriate responding (n = 5; response rate = 15.6 responses per min).

Tiflucarbine was also examined as an antagonist in the DOM-trained animals (data not shown). Tiflucarbine, at doses of 0.5 and 0.8 mg/kg, had no effect on DOM-appropriate responding after administration of the training dose of the training drug. However, at doses of 1 and 4 mg/kg, administration of tiflucarbine in combination with 1 mg/kg of DOM resulted in disruption of behavior.

In animals trained to discriminate the 5-HT1A-selective agonist 8-OH DPAT from saline, tiflucarbine doses of 1 and 2.5 mg/kg elicited saline-appropriate responding, whereas doses of 5 and 10 mg/kg resulted in disruption of behavior (i.e., 4 of 6 animals failed to respond) (Table 1). In the animals trained to discriminate the nonselective 5-HT agonist 5-OMe DMT from saline (Table 1), 2.5 mg/kg of tiflucarbine elicited saline-appropriate responding with no decrease in response rate as compared to control. At 5 mg/kg, the animals' response rates were decreased by approximately 50%. At 10 mg/kg, only 4 of 12 animals responded; however, all of the animals that responded selected the drug-appropriate lever.

DISCUSSION

In animals, DOM serves as a discriminative stimulus and its stimulus properties appear to be 5-HT2-mediated (5). Tiflucarbine binds at 5-HT1 and 5-HT2 sites and there is evidence that it

is a 5-HT agonist (3). However, DOM-stimulus generalization did not occur with tiflucarbine; partial generalization (42–53% DOM-appropriate responding) was observed at doses of 13 to 14.5 mg/kg, but the animals were disrupted when administered 15 mg/kg. Because both agents bind at [^{3}H]ketanserin-labeled 5-HT2 sites with similar affinity (K_i ca. 100 nM), this result is surprising. As a consequence, tiflucarbine was examined as a potential 5-HT2 antagonist in tests of stimulus antagonism. Doses of 0.5 and 0.8 mg/kg of tiflucarbine had little effect on DOM-appropriate responding; however, doses as low as 1 mg/kg, in combination with 1 mg/kg of DOM, resulted in disruption of behavior.

The stimulus effects produced by 8-OH DPAT are 5-HT1A-mediated (1, 4, 8), whereas those produced by 5-OMe DMT seem to be of a nonselective nature (5,7). In animals trained to discriminate 0.1 mg/kg of the 5-HT1A-selective agonist 8-OH DPAT from saline, tiflucarbine produced saline-like behavior at doses of 1 and 2.5 mg/kg, and disruption of behavior at doses of 5 and 10 mg/kg. With animals trained to discriminate 1.25 mg/kg of 5-OMe DMT from saline, tiflucarbine resulted in stimulus generalization; however, only four of twelve animals responded. Taken together, the results suggest that tiflucarbine may be a 5-HT agonist, but that it is capable of producing a central effect that disrupts the animals' behavior.

Using animals trained to discriminate a nonselective 5-HT agonist, stimulus generalization can occur both with selective and nonselective 5-HT agonists; using animals trained to discriminate a selective 5-HT agonist, stimulus generalization can occur with other agents of similar selectivity and with nonselective serotonergic agents, but not with agents selective for a different population of 5-HT sites (5). For example, DOM-stimulus generalization occurs with other 5-HT2 agonists such as DOB, with the nonselective agonist 5-OMe DMT, and with the indirect-acting 5-HT agonist fenfluramine; however, DOM-stimulus generalization does not occur with the 5-HT1A-selective agonist 8-OH DPAT (5). Tiflucarbine produces only partial generalization in DOM-trained animals suggesting that it may be selective for a different population of 5-HT sites. However, the disruption of behavior observed at the highest dose evaluated makes it difficult to reach this conclusion with any degree of confidence (i.e., had disruption not been evident, stimulus generalization might have occurred at a higher dose). On the basis of the results with the 8-OH DPAT-trained animals, tiflucarbine does not appear to be a 5-HT1A agonist. However, the results with the 5-OMe DMT-trained animals suggest, as do the results with the DOM-trained animals, that tiflucarbine may be a 5-HT agonist that produces central effects that interfere with the animals' responding. In this case, however, disruption of behavior was coincident with stimulus generalization in four animals. There are several possible explanations for these results. Tiflucarbine may be a 5-HT agonist with selectivity for 5-HT sites other than 5-HT1A and 5-HT2 sites. However, there is presently no evidence to support such a suggestion. More likely, tiflucarbine is a nonselective 5-HT agonist. In fact, recent investigations have shown that tiflucarbine is a potent and selective inhibitor of 5-HT reuptake (2). Thus, the nonselective nature of the stimulus effects produced by tiflucarbine may be a direct consequence of its ability to increase synaptic concentrations of 5-HT. This conclusion is also consistent with the effects produced by tiflucarbine in other behavioral studies with rodents (3).

ACKNOWLEDGEMENTS

We wish to thank Bryan Misenheimer for his assistance with some of the discrimination studies.

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