Pharmacology Biochemistry & Behavior, Vol. 35, pp. 781–784. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Discriminative Stimulus Properties of Flesinoxan

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Received 6 October 1989

YBEMA, C. E., J. L. SLANGEN, B. OLIVIER AND J. MOS. *Discriminative stimulus properties of flesinoxan.* PHARMACOL BIOCHEM BEHAV **35**(4) 781–784, 1990.—Different groups of rats were trained to discriminate either 0.3 mg/kg of flesinoxan (N = 13) or 0.1 mg/kg of 8-OH-DPAT (N = 7) from saline in a two-lever operant drug discrimination task using a fixed ratio 10 schedule of reinforcement. Once trained, animals in both groups displayed a dose-related decrease in discriminative performance upon administration of lower doses of the drug used in training. In generalization tests, flesinoxan generalized to 8-OH-DPAT in 8-OH-DPAT-trained animals and 8-OH-DPAT substituted for flesinoxan in flesinoxan-trained animals. Buspirone substituted partially for both the flesinoxan and the 8-OH-DPAT cue. The results of the present study indicate similarity between the discriminative stimulus effects of flesinoxan and the stimulus produced by the 5-HT_{1A} agonist 8-OH-DPAT. These results, coupled with the finding that flesinoxan has a significant affinity and selectivity for 5-HT_{1A} binding sites, suggest that the stimulus effects of flesinoxan are mediated by a 5-HT_{1A} mechanism.

Drug discrimination Flesinoxan 8-OH-DPAT Buspirone 5-HT_{1A} Rats

THE recently introduced phenylpiperazine derivative flesinoxan represents a new example of a potent and selective 5-HT_{1A} ligand (1). Similar to the selective 5-HT_{1A} agonist 8-OH-DPAT, flesinoxan has been shown to reduce blood pressure and heart rate in several species (3, 4, 8, 11, 14, 23). In pigeons, flesinoxan produced large increases in punished responding, comparable to those found after administration of the 5-HT_{1A} drugs buspirone and ipsapirone (1). The finding that flesinoxan substitutes for the cue of buspirone in a drug discrimination task using pigeons is also consistent with a 5-HT_{1A} mechanism of action of flesinoxan (1,13).

The purpose of the present experiments was to study the discriminative stimulus properties of flesinoxan in rats and to investigate whether the discriminative stimulus effects of flesinoxan in rats are mediated by 5-HT_{1A} receptors. A group of thirteen rats was trained to discriminate flesinoxan (0.3 mg/kg) from the solvent, saline. Another group of seven rats was trained to discriminate 8-OH-DPAT (0.1 mg/kg) from saline. Following acquisition of the task, flesinoxan-trained animals were tested with flesinoxan (0.01–1.0 mg/kg), 8-OH-DPAT (0.01–1.0 mg/kg) and buspirone (1.0–8.0 mg/kg). In 8-OH-DPAT-trained animals several doses of 8-OH-DPAT (0.01–0.1 mg/kg), flesinoxan (0.1–1.0 mg/kg) and buspirone (1.0–8.0 mg/kg) were tested.

METHOD

Subjects

Male Wistar rats, weighing approximately 250 g, were obtained from CPB-TNO (Zeist, The Netherlands). They were housed individually under a nonreversed 12-hr light-dark cycle and a room temperature of 20–22°C. Tap water was freely available. Subjects were maintained at approximately 85% of their expected free-feeding weight by providing them with a diet of 13 g food (Hope Farms) one hour after each daily session. Food was freely available from Friday afternoon until Sunday morning.

Apparatus

Seven ventilated operant chambers (Campden) equipped with two levers and housed in sound-insulated boxes were used. A pellet dispenser delivered 45 mg pellets (Noyes) in a tray placed between the levers. A Digital Equipment Corporation PDP-11 microcomputer with software (SKED) supplied by State Systems Incorporated (Kalamazoo, MI) programmed session events and recorded data.

Training

The training procedure was derived from the one described by Colpaert *et al.* (5). After initial lever-press training, the animals were trained to lever-press according to a fixed ratio 10 (FR 10) schedule of reinforcement. Discrimination training was then begun. A group of thirteen animals was trained to discriminate flesinoxan (0.3 mg/kg) from the solvent saline (0.9% NaCl). Another group of seven rats was trained to discriminate 8-OH-DPAT (0.1 mg/kg) from saline. Depending on the injection condition, reinforcement could be obtained by pressing either the drug-appropriate (D) or the saline-appropriate (S) lever. The position of the D and the S lever was counterbalanced across rats.

Levers were cleaned with 10% ethanol solution between sessions in order to avoid olfactory cues (7). Fifteen minutes before the daily session animals were injected with either drug or saline, according to a two-weekly alternating schedule: (D-S-D-D-S, S-D-S-S-D). For half of the animals the discrimination training started with the first half of the sequence, for the other half training started with the second one. Drug-induced stimulus control was assumed to be present when the animal, in ten consecutive sessions (5 D and 5 S), accurately selected the appropriate lever. The lever on which the rat first emitted ten responses was scored as the selected lever. Accurate lever selection was defined as the accumulation of ten responses on the injection appropriate lever with five or less responses on the inappropriate lever (i.e., number of responses made on both levers before the first reinforcement, between 10 and 15). Generalization tests were started as soon as 6 animals of a group had attained the criterion for drug-induced stimulus control.

Tests of Stimulus Generalization

Generalization tests were carried out on Wednesday and Friday. On the remaining days the training procedure was continued. Animals not accurately selecting the appropriate lever on Tuesday or Thursday were excluded from the generalization test on the following day. Throughout the generalization session, responding on the selected lever was rewarded according to a FR 10 schedule. Both the training and test sessions ended after fifteen minutes or sooner, i.e., when the animal had obtained fifty reinforcements. If an animal did not respond during the first five minutes of the test, the session was ended and behavior was scored as disrupted. Stimulus generalization was said to occur as at least 80% of the animals, after given a dose of the test compound, selected the drug-appropriate lever. The criterion for accurate drug-lever selection was defined as the accumulation of a maximum of 13 responses on the drug-appropriate lever (i.e., less than four responses were made on the saline-appropriate lever) before the first reinforcement. In the flesinoxan-trained animals each dose was tested in at least 7 different animals and, in most cases, the same dose was administered to the animals twice. For each data point obtained in the generalization tests with the 8-OH-DPAT-trained animals six different animals were used. The same dose of a test drug was administered to these animals at least twice. Response rates were analysed by a two-factor repeated measures general analysis of variance. The first factor (drug) had four levels, the second (dose) three.

Drugs

Flesinoxan-HCl (Duphar), 8-hydroxy-2-(di-n-propylamino)tetralin-HBr (8-OH-DPAT, Research Biochemicals Incorporated) and buspirone-HCl (Bristol-Myers) were dissolved in 0.9% NaCl. All solutions were prepared fresh daily and administered via intraperitoneal injection. In all cases (saline injection as well) the injection volume was 2 ml/kg.

RESULTS

Learning to discriminate between flesinoxan and saline required a mean (±SEM) of 44.7 (4.86) training sessions (range: 31–72 sessions). During the acquisition period two animals in the flesinoxan-trained group died. The mean number of sessions-to-criterion for the rats trained to discriminate 8-OH-DPAT from saline was 95.8 (6.7, range: 42–135). One animal died during the training. The death of the three animals seemed not to be related to the administration of the training substances.

The results of the generalization studies with flesinoxan, 8-OH-DPAT and buspirone in the flesinoxan-trained group are presented in Table 1. Administration of flesinoxan in doses lower than the training dose resulted in a decrease in the percentage animals selecting the drug-appropriate lever. Generalization tests with 8-OH-DPAT and buspirone resulted in a maximum of 90% and 75% flesinoxan-lever selection, respectively.

TABLE 1

RESULTS OF GENERALIZATION STUDIES USING FLESINOXAN (0.3 mg/kg) AS THE TRAINING DRUG

Drug	Dose (mg/kg)	N*	% Rats Selecting DL	Mean Resp/sec (±SEM)†
Saline		12/12	8.3	1.05 (0.13)
Flesinoxan	0.01	9/9	0	0.90 (0.18)
	0.05	13/13	38.5	1.16 (0.13)
	0.1	15/15	33.3	1.27 (0.11)
	0.2	11/11	54.5	1.35 (0.21)
	0.3	19/19	89.5	1.00 (0.12)
	1.0	10/13	80.0	0.28 (0.10)
8-OH-DPAT	0.01	11/11	36.4	1.24 (0.18)
	0.1	16/16	75	1.14 (0.12)
	0.5	10/17	90	0.31 (0.10)
	1.0	0/12	—‡	0
Buspirone	1.0	18/18	50	0.95 (0.25)
	4.0	20/22	75	0.27 (0.09)
	8.0	10/19	60	0.11 (0.08)

*Number of animals responding/number to receive drug.
†Average responses/s (±SEM) during session.
‡Disruption of behavior.

For the analysis of response rates in the flesinoxan-trained animals the data of 8 subjects were used because they showed no missing values. The following conditions were analysed: flesinoxan (0.1, 0.3 and 1.0 mg/kg), 8-OH-DPAT (0.01, 0.5 and 0.1 mg/kg) and buspirone (1.0, 4.0 and 8.0 mg/kg). There was a main effect of the factor drug, $F(3,21) = 11.34$, $p<0.05$, and a main effect of the factor dose, $F(2,14) = 29.99$, $p<0.05$. Post hoc analysis showed that in comparison with saline responding was increased after administration of 0.1 mg/kg of flesinoxan and 0.01 mg/kg of 8-OH-DPAT, $F(1,7) = 7.25$, $p<0.05$ and $F(1,7) = 15.98$, $p<0.05$, respectively. After administration of 1.0 mg/kg of flesinoxan and of 0.5 mg/kg of 8-OH-DPAT the response rate was significantly less than after saline, $F(1,7) = 15.35$, $p<0.05$ and $F(1,7) = 11.83$, $p<0.05$, respectively. Response rate was significantly decreased after administration of 8 mg/kg of buspirone, $F(1,7) = 038.71$, $p<0.05$.

In Table 2 the results of the generalization tests with 8-OH-DPAT, flesinoxan and buspirone in the 8-OH-DPAT-trained animals are summarized. Responding to the training drug was dose related in that administration of lower doses of 8-OH-DPAT produced a decrease in the percentage of animals selecting the drug-appropriate lever. The administration of 1.0 mg/kg flesinoxan resulted in a maximum of 91.7% drug lever selection, whereas a maximum of 76.9% drug lever selection was found after 4.0 mg/kg buspirone. For the analysis of the response rates in the 8-OH-DPAT-trained animals the data of six different animals were used because they showed no missing values. The following conditions were analysed: flesinoxan (0.1, 0.3 and 1.0 mg/kg), 8-OH-DPAT (0.01, 0.05 and 0.1 mg/kg) and buspirone (1.0, 4.0 and 8.0 mg/kg). There was a main effect of the factor drug, $F(3,15) = 22.40$, $p<0.05$, and a main effect of the factor dose, $F(2,10) = 10.07$, $p<0.05$. Post hoc analysis showed that in com-

TABLE 2

RESULTS OF GENERALIZATION STUDIES USING 8-OH-DPAT (0.1 mg/kg) AS THE TRAINING DRUG

Drug	Dose (mg/kg)	N*	% Rats Selecting DL	Mean Resp/sec (± SEM)†
Saline		14/14	14.3	0.80 (0.29)
8-OH-DPAT	0.01	17/17	35.3	0.80 (0.23)
	0.025	10/10	30	0.84 (0.28)
	0.05	11/11	63.6	0.93 (0.32)
	0.1	14/14	85.7	0.90 (0.26)
Flesinoxan	0.1	11/11	27.3	0.86 (0.38)
	0.3	13/13	50	0.96 (0.37)
	1.0	12/12	91.7	0.75 (0.37)
	3.0	11/12	81.8	0.31 (0.18)
Buspirone	1.0	10/10	20	0.54 (0.30)
	4.0	13/17	76.9	0.16 (0.12)
	8.0	8/12	75	0.05 (0.07)

For explanation of * and †, see legend to Table 1.

parison with saline responding was increased after 0.3 mg/kg of flesinoxan, $F(1,5) = 17.28$, $p<0.05$. Responding was significantly decreased after administration of buspirone (1.0, 4.0 and 8.0 mg/kg), $F(1,5) = 16.04$, $p<0.05$, $F(1,5) = 16.28$, $p<0.05$ and $F(1,5) = 20.24$, $p<0.05$, respectively.

DISCUSSION

The present study demonstrates that 0.3 mg/kg of flesinoxan can function as a discriminative stimulus in rats. Although the discrimination was difficult to establish in both the flesinoxan- and 8-OH-DPAT-trained animals, the flesinoxan-vehicle discrimination was more readily acquired than the 8-OH-DPAT-saline discrimination, under the present conditions. The finding that cross-generalization occurs between the flesinoxan and the 8-OH-DPAT cue suggests that both cues are qualitatively similar (19). Furthermore, buspirone was found to produce nearly 80% drug-lever selection in both the flesinoxan- and the 8-OH-DPAT-trained animals (i.e., 75 and 76.9% DL selection, respectively). This also indicates that cues of flesinoxan and 8-OH-DPAT may be rather similar. However, in the present study, buspirone doses of between 4 and 8 mg/kg have not been tested. Thus, the possibility remains that complete generalization might have been obtained with intermediate buspirone doses.

Several studies have indicated that the cue of 8-OH-DPAT is probably mediated by the putative 5-HT_{1A} receptor type [i.e., (9, 15, 22)]. Additionally, there are a number of studies suggesting that the discriminative stimulus properties of buspirone are mediated by the 5-HT_{1A} receptor subtype as well (6, 9, 13, 20, 21). Results of binding assays indicate that flesinoxan shares with 8-OH-DPAT and buspirone a high affinity and selectivity for the 5-HT_{1A} binding site (10, 20, 23). Therefore, the similarity between the cues of flesinoxan, 8-OH-DPAT and buspirone found in the present study provides (in vivo) evidence that the discriminative stimulus effect of flesinoxan is mediated via the 5-HT_{1A} receptor type. Several behavioral studies have also indicated that flesinoxan has 5-HT_{1A} agonistic properties. Flesinoxan, like 8-OH-DPAT and buspirone, enhances sexual behavior in male rats (unpublished results), enhances food intake in rats, exerts anxiolytic (1,17) and antiaggressive activity (18). Furthermore, flesinoxan, like 8-OH-DPAT, induces a typically 5-HT_{1A} receptor-related behavioral syndrome and lower-lip retraction, which emerges specifically by activation of the 5-HT_{1A} receptor (2, 12, 16). The present results support the notion that the discriminative stimulus of flesinoxan is mediated via the 5-HT_{1A} receptor. Further work is needed to confirm this hypothesis.

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