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Discriminative Stimulus Properties of mCPP and Alprazolam Are Not Mediated by Anxiety

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GOMMANS, J., T. H. HIJZEN, T. PATTIJ, J. V. D. GUGTEN AND B. OLIVIER. *Discriminative stimulus properties of mCPP and alprazolam are not mediated by anxiety*. PHARMACOL BIOCHEM BEHAV **64**(2) 385–387, 1999.—We investigated whether the interoceptive cues mediated by the anxiolytic benzodiazepine receptor agonist alprazolam and the anxiogenic serotonin $(5\text{-HT})_{1B/2C}$ receptor agonist 1-(3-chlorophenyl)piperazine (mCPP) in rats are related to anxiety. mCPP-induced anxiety in humans can be blocked with alprazolam, and if mCPP drug discrimination is to be used as a model of anxiety, mCPP's stimulus should be blocked by alprazolam. Therefore, two groups of rats were trained to discriminate either alprazolam (2 mg/kg, PO) or mCPP (2 mg/kg, PO) from vehicle in a two-level operant drug discrimination procedure. Cross antagonism tests were performed with alprazolam and mCPP. mCPP did not antagonize alprazolam's stimulus to any extent, but disrupted responding severely. Low and intermediate doses of alprazolam (1.0–4.0 mg/kg, PO) did not antagonize the mCPP discriminative stimulus; only a high dose of 8.0 mg/kg (PO) partially antagonized mCPP but disrupted responding in most of the animals. We conclude that, at best, there is only weak evidence to suggest that the interoceptive cues of alprazolam and mCPP are mediated by modulation of anxiety processes, and that the mCPP drug discrimination as a model for anxiety is unreliable. © 1999 Elsevier Science Inc.

Drug discrimination Anxiety m-Chlorophenylpiperazine (mCPP) Alprazolam

DRUG discrimination techniques have been used widely to characterize psychoactive drugs. It has been shown that the discriminative stimulus properties of drugs can be related to direct modulation of various receptors (8). To relate the stimulus properties to specific, functional effects (e.g., anxiety, analgesia, pain, hunger, etc.) proved a far more difficult task, and has been investigated only incidentally (8). Based on the results with anxiolytic/anxiogenic drugs it was suggested that the pentylenetetrazol (PTZ) cue could function as a model for anxiety (9). Recently, behavioral evidence showed that the PTZ stimulus might indeed be mediated by fear: exposure to a predator [a cat, (2)] or to pheromones of rats that have been shocked (4) engenders almost exclusive PTZ lever-appropriate responding.

It has been suggested that the stimulus properties of the benzodiazepine chlordiazepoxide (CDP) are related to its anxiolytic effects (5). This was based, among others, on the finding that the CDP cue could be antagonized partially by buspirone, and this was ascribed to buspirone's anxiogenic properties. However, according to Griebel (7), reviewing the effects of serotonergic drugs in animal models of anxiety, buspirone demonstrated anxiogenic properties in 9.5%, and anxiolytic effects in 69.5% of the studies reported (7). Buspirone is also used in humans for the treatment of generalized anxiety disorder [(14) for a review]. Therefore, to ascribe the antagonism of CDP by buspirone to its anxiogenic activity is problematic. A better established anxiogenic compound is 1-(3-chlorophenyl)piperazine (mCPP), a $5\text{-hydroxytryptamine}(5\text{-HT})_{1B/2C}$ receptor agonist (11). It has anxiogenic effects in animal models of anxiety and increases anxiety in panic disorder patients and obsessive–compulsive disorder patients (10). In healthy volunteers mCPP increases the subjective ratings of anxiety and increases cortisol, prolactin, and growth hormone (GH) levels (10).

In a recent study it was shown that in human subjects the increase in anxiety, cortisol, and GH levels induced by oral administration of mCPP could be antagonized completely by the triazolobenzodiazepine alprazolam (12). Alprazolam is used to treat generalized anxiety disorder and, unlike most benzodiazpines (e.g., CDP), in particular panic disorder (13). In addition, this compound has anxiolytic effects in a number of animal models (13). If, as suggested recently (15), mCPP drug discrimination could function as a model to assay drugs for human anxiety, it should be sensitive in particular to alprazolam. We, therefore, tested whether the mCPP discriminative stimulus could be antagonized by alprazolam and the

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alprazolam discriminative stimulus by mCPP. In addition, because it was reported that buspirone partially antagonized a CDP cue (4), we tested whether buspirone could antagonize the alprazolam stimulus.

METHOD

Subjects

Twenty-four male Wistar rats, weighing approximately 300 g at the start of the training, were obtained from GDL (Utrecht, The Netherlands). Rats were housed individually under a nonreversed 12 L:12 D cycle and a room temperature of 21–23°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 14 g food (Hope Farms, Woerden, The Netherlands) 1 h after each daily session (Monday to Thursday). On Friday afternoon, each animal received 50 g food for the whole weekend.

Apparatus

Twelve ventilated operant chambers (MED associates Inc., East Fairfield) equipped with two levers and housed in sound-insulated boxes were used. A pellet dispenser delivered 45-mg pellets (Noyes Company Inc., Lancaster, NH) in a tray placed between the levers. An IBM-pc using a MED interface and software controlled experimental sessions and recorded data.

Procedure

Rats were trained to lever press according to a tandem variable interval 40-s fixed-ratio 10 (VI 40″, FR10) schedule of reinforcement. Depending on the injection conditions, reinforcement could be obtained by pressing either the drug (D)- or vehicle (V)-appropriate lever. Responding on the inappropriate lever never produced food. The position of D and V levers was counterbalanced across rats. Thirty minutes before the daily sessions the animals were injected with either drug or the used vehicle, according to a two-weekly alternating schedule (D–V–D–D–V, V–D–V–V–D). One group was trained with mCPP (2.0 mg/kg; $n = 12$), an other group with alprazolam (2.0 mg/kg; $n = 12$). Once a week a base-line session was run, during which an extinction period of 2 min preceded the remaining period of 18 min regular training. The lever on which the rats first made 10 responses was scored as the selected lever. Discrimination was considered to be adequately established when animals selected, during 10 consecutive regular training sessions, the injection-appropriate lever and made less than four lever presses on the injection inappropriate lever. In addition, the animals had to make more than 90% of the responses on the injection-appropriate lever during the extinction period of the baseline sessions. Generalization and antagonism tests were carried out on Wednesday and Friday. On the remaining days the training procedure was continued. During testing, an extinction period of 2 min is started as soon the animal presses a lever. If the animal does not respond at all, the session is terminated after 10 min. The results of generalization and antagonism tests are expressed as the mean percentage drug-lever responding during the 2-min extinction. Response rates were analyzed with Wilcoxon's matched-pairs signed-ranks test, using the statistical package of SPSS-PC. Each dose of a test drug was compared to the training dose of mCPP or alprazolam. The level of significance was set at 5%, but in order to control for Type I error, a Bonferroni correction was made.

Drugs

Alprazolam was suspended in vehicle containing gelatin–mannitol (0.5% gelatin, 5% mannitol, dissolved in distilled water). 1-(3-Chlorophenyl)piperazine 2HCl (mCPP) was dissolved in demineralized water for oral administration and in saline for subcutaneous injections. Buspirone HCl was dissolved in saline. Training drugs were administered orally, 30 min before the session. When tested as antagonists, alprazolam was administered orally 45 min before the test session; buspirone and mCPP were injected subcutaneously, 20 min before the test session.

RESULTS

Table 1 shows that the mCPP stimulus was only very marginally antagonized by alprazolam after low and intermediate doses (1.0–4.0 mg/kg, PO). mCPP was only partially antagonized by the highest dose of alprazolam (8.0 mg/kg, PO), a

TABLE 1

THE EFFECTS OF ALPRAZOLAM ON THE mCPP DISCRIMINATIVE STIMULUS IN ANIMALS TRAINED TO DISCRIMINATE 2.0 mg/kg mCPP FROM VEHICLE

Drug	Dose (mg/kg)	*n**	% Drug-Lever Responding† (±SEM)	Resp/s (±SEM)‡
Vehicle	0.0	12/12	3.3 (1.33)	1.6 (0.20)§
mCPP	2.0	12/12	92.2 (5.04)	0.8 (0.26)
mCPP	1.0	6/8	89.1 (8.43)	0.4 (0.16)
(2.0mg/kg)+	2.0	8/11	79.9 (13.20)	0.4 (0.14)
Alprazolam	4.0	11/11	73.5 (10.76)	0.7 (0.20)
	8.0	4/11	55.5 (20.44)	0.2 (0.10)

*Number of animals responding/number of animals tested.
†Percentage of responses made on the drug appropriate lever.
‡Mean number of responses/second.
§Significant compared to mCPP 2.0 mg/kg.

TABLE 2

THE EFFECTS OF mCPP AND BUSPIRONE ON THE ALPRAZOLAM DISCRIMINATIVE STIMULUS IN ANIMALS TRAINED TO DISCRIMINATE 2.0 mg/kg ALPRAZOLAM FROM VEHICLE

Drug	Dose (mg/kg)	*n**	% Drug-Lever Responding† (±SEM)	Resp/s (±SEM)‡
Vehicle	0.0	12/12	5.4 (1.73)	1.1 (0.22)§
Alprazolam	2.0	12/12	90.8 (4.43)	1.8 (0.19)
Alprazolam	0.1	10/10	98.7 (0.41)	1.4 (0.19)
(2.0 mg/kg)+	0.3	5/10	97.5 (1.84)	0.2 (0.09)§
mCPP	1.0	4/10	88.9 (5.02)	0.3 (0.15)§
Alprazolam	1.0	7/8	97.1 (1.89)	0.1 (0.04)§
(2.0 mg/kg)+	2.0	6/10	65.2 (16.13)	0.1 (0.05)§
buspirone	3.0	3/10	69.3 (18.51)	0.1 (0.03)§
	4.0	1/10	100	0.0 (0.00)§

*Number of animals responding/number of animals tested.
†Percentage of responses made on the drug appropriate lever.
‡Mean number of responses/second.
§Significant compared to alprazolam 2.0 mg/kg.

dose that disrupted responding severely. mCPP itself reduced response rates compared to water. Table 2 shows that the alprazolam stimulus was to no extent antagonized by mCPP. Alprazolam was partially antagonized by buspirone. Both mCPP (0.3 and 1.0 mg/kg) and buspirone (all doses tested) had pronounced effects on response rate, and after coadministration of 3.0 and 4.0 mg/kg of buspirone there were only three and one subjects left that responded.

DISCUSSION

The discriminative stimulus properties of drugs can often be related to modulation of various receptors (8). The receptor types involved in the discriminative stimuli of mCPP and alprazolam have been investigated before, and offer a clear-cut receptor mechanistic interpretation. The evidence is compelling that the discriminative stimuli of alprazolam and mCPP are mediated by benzodiazepine and 5-$HT_{2C/1B}$ receptors, respectively (1,6,16). In the present study, we investigated whether the discriminative stimuli of mCPP and alprazolam are mediated by the anxiety-related properties of these drugs. If anxiolysis is the main effect on which the alprazolam discrimination is based, it would be expected that mCPP, which is a better established anxiogenic compound than buspirone (7,10), would show the highest level of antagonism. However, mCPP was not able to block the alprazolam stimulus to any extent, and there was only a tendency for buspirone to antagonize alprazolam. The absence of a substantial antagonism of alprazolam by mCPP argues against an interpretation of the discriminative stimulus properties of alprazolam in terms of modulation of anxiety.

mCPP is administered to humans via different routes. In the Sevy et al. study (12) both mCPP and alprazolam were administered orally, and, therefore, in the present study discrimination training with mCPP and antagonism testing with alprazolam were carried out with oral drug administration. If the mCPP discriminative stimulus would be mediated by anxiety, it should be blocked by alprazolam. However, only the highest dose of alprazolam, which disrupted responding in most animals, only partially antagonized mCPP. The fact that in humans the anxiogenic effects of mCPP can by antagonized by alprazolam (12), to our knowledge the only study in which complete antagonism of mCPP's anxiogenic effects in humans has been found, makes the mCPP drug discrimination unsuitable as a model to investigate the putative anxiolytic properties of drugs.

Recent experiments showed that rats can attend to different aspects of a morphine discriminative stimulus (3). Drugs that mimicked one or some aspects of the effects of morphine (e.g., sedation, analgesia, CNS depression) substituted partially in some animals and not in others. It can be speculated that when some aspects of a drug stimulus are antagonized but others not, this might result in partial antagonism. In the case of mCPP and alprazolam, these aspects could be fear, stimulation of hormones, or a variety of other effects. Following such a conceptualization of drug discrimination the present results indicate that the mCPP and alprazolam stimuli are at most partially mediated by modulation of fear.

To conclude, the evidence that the discriminative stimuli of alprazolam and mCPP are mediated by anxiety processes is weak at best, and the use of mCPP drug discrimination is unsuitable as a model to assay putative anxiolytic drugs.

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