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Discriminative Stimulus Properties of CL218872 and Chlordiazepoxide in the Rat

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GARDNER, C. R. *Discriminative stimulus properties of CL218872 and chlordiazepoxide in the rat.* PHARMACOL BIOCHEM BEHAV **34**(4) 711–715, 1989.—Rats were trained to discriminate either CL218872 (5 mg/kg PO) or chlordiazepoxide (5 mg/kg PO) from vehicle in a 2-lever discrimination task on an FR20 schedule. The discriminative cues produced by these two drugs generalised to a range of benzodiazepine receptor agonists and partial agonists. Nitrazepam, diazepam, RU32698 and RU32514 were less potent in substituting for the CL218872 cue than the chloridiazepoxide cue. Zopiclone, RU31719 and RU43028 substituted for both cues with similar potency, whilst zolpidem and CL218872 were clearly more potent in substituting for the CL218872 cue. Chlordiazepoxide substituted only partially for the CL218872 cue, even at doses which decreased the rate of responding. CGS9896 substituted partially for both cues, but was less effective with the CL218872 cue. RU39419 substituted for the chlordiazepoxide cue, but antagonised the CL218872 cue. CGS8216 and FG7142 antagonised both cues. The contributions of benzodiazepine receptor subtypes or partial agonism to the generation of the CL218872 cue is discussed.

Drug discrimination CL218872 Chlordiazepoxide Benzodiazepine receptors Rats

CL218872 was the first compound to distinguish subtypes of benzodiazepine binding sites. CL218872 preferentially interacted with a higher affinity BDZ_1 site rather than a lower affinity BDZ_2 (11). Initial observations indicated that CL218872 did not have muscle relaxant properties (12), although this separation of behavioural properties may not be that distinct (15,16). Based on this separation of behaviours it was proposed that BDZ_1 sites were associated with the anxiolytic activities of benzodiazepines and the BDZ_2 sites were associated with the CNS depressant effects (12). Subsequently, it was observed that CL218872 could antagonise the loss in righting reflex induced by diazepam, and partial agonist properties of CL218872 were suggested (7). This could explain a lack of muscle relaxant properties as these are associated with highest benzodiazepine receptor occupancies with classical benzodiazepines, and might be expected to be the first effects to be weakened in a partial agonist (6, 8, 17).

There has been much debate as to whether there are separable functional correlates associated with the two binding subsites or whether these sites represent different activation states of one population of receptors (4, 13, 22). The behavioural profile of CL218872 does include a "sedative" effect with impairment of a variety of behaviours (6,16). The discovery of zolpidem, another ligand selective for BDZ_1 sites (1), led to a re-evaluation of the potential functional correlates of selective subsite activation. Zolpidem is a particularly sedative compound and has potential hypnotic activity in clinical use (1,15). Based on the behavioural profile of zolpidem it was suggested that BDZ_1 sites are associated particularly with sedative drug effects (19).

Other benzodiazepine receptor partial agonists antagonise a zolpidem cue (18,21) which might suggest that either the cue is associated with a higher occupancy of benzodiazepine receptors (of either type), as are the CNS depressant effects of classical benzodiazepines (8, 14, 17), or that the BDZ_1 subsite is associated with a distinct pharmacological profile. However, pharmacological studies with the zolpidem cue are complicated by the strong depressant effect of the training drug on operant performance (19).

In order to further investigate the mechanisms underlying the behavioural profile of CL218872, rats were trained to discriminate this compound from vehicle and the pharmacology of this discriminative stimulus (cue) was investigated in comparison with that of an established colony of rats trained to discriminate chlordiazepoxide (CDZP).

METHOD

Subjects

Male hooded Lister rats (Olac, Bicester, UK) were used in all these studies. At the beginning of training rats were 180–220 g and they spent their working lives performing these experiments as long as their baseline responding was stable. Therefore, most pharmacological studies were performed with rats weighing 300–500 g.

Animals were housed in pairs in a colony room maintained at 22°C and with controlled humidity, on a 8.00–18.00, 18.00–8.00 hr light-dark cycle. Water was continuously available in the home cages, but food was restricted to 80% of that consumed by ad lib fed controls. The rats were fed approximately 4 hr after the operant session. Twenty-four and 6 rats were used for the CDZP and CL218872 cues, respectively. Each member of a pair was trained on the same cue drug, and both were tested in different operant chambers, but at the same time.

Apparatus

The behavioural apparatus consisted of identical standard

Skinner boxes (Camden Instruments, London) each with two retractable levers on one wall, with the food dispensing magazine centrally between them. Each apparatus was housed in a light-proof, sound-attenuated, fan-ventilated chamber.

Operation of the behaviour schedules and recording of data were achieved using microcomputers (Acorn series II) via appropriate interfacing (Camden Instruments). Levers were retracted when the rats were placed in the apparatus and each session began when both levers were simultaneously presented.

Discrimination Training

Rats were trained to discriminate between the effect of vehicle and that of either chlordiazepoxide (CDZP) or CL218872 (both 5 mg/kg PO 1 hr prior to the test). The doses were chosen as being at the lower end of the effective dose range in conflict tests with this strain of rat.

Rats were magazine trained and shaped to press the lever for food reinforcement (45 mg pellets, Camden Instruments). Then they were trained to respond on one of the levers following cue drug injection and on the other lever following administration of vehicle (demineralised water, 2 ml/kg). A food pellet was delivered after every 20th press (FR20) on the correct lever. Responses on the incorrect lever (i.e., drug lever after vehicle injection or vehicle lever after cue drug injection) were recorded, but were not reinforced with food pellet reward.

The drug lever was randomly allocated on the right side of the food magazine for half the rats and on the left side for the other half. The position of the drug and vehicle levers remained constant for each rat for all subsequent sessions. The sequence of drug-vehicle injection was different throughout groups of rats to control for a possible olfactory cue and a quasirandom (vehicle-drug-drug-vehicle-vehicle and drug-vehicle-vehicle-drug-drug) sequence of testing was used for each successive two-week, Monday to Friday test block.

Training criterion was reached when the number of presses prior to receiving the first food pellet (FFP) was <24 for the prior two sessions of both drug and vehicle training. This criterion was maintained throughout drug testing as an index of stable baseline responding. In the majority of cases the FFP was 20 under these fully trained conditions.

Drug Testing

Rats reaching the criterion level of performance were repeatedly used in generalisation and antagonism testing. At least one vehicle and one cue drug response at criterion level was required between each such test. Any given drug/dose combination was allocated randomly to rats as they became available for testing. Where necessary the route of vehicle administration was changed if a test compound in generalisation studies was to be given by a different route from the cue drug. In antagonism studies, when both test compound and cue drug were given, an appropriate vehicle injection was given as well as the cue drug in control tests. Vehicle and cue drug test sessions were 10 min in duration whilst tests with non-cue drugs were 5 min in duration. Following the choice in the test sessions reward was available on an FR20 schedule on the lever of choice.

Drugs

All drugs were dissolved or suspended in appropriate vehicle (demineralised water for oral injections, 2 ml/kg and 0.9% saline for intraperitoneal injection, 1 ml/kg), sonicated and continuously stirred until used. All drugs were administered 1 hr prior to test with the exception of FG7142 which was given 30 min prior to test.

We acknowledge the generous gift of zopiclone from May and Baker, England. Standard compounds were obtained commercially and CGS8216, CGS9896, CL218872, zolpidem and the Roussel (RU) compounds used were synthesised by chemists from Roussel Laboratories.

RESULTS

In tests for generalisation a range of agonists or partial agonists at benzodiazepine receptors substituted for the discriminative stimulus of each training drug, although the relative potencies for substitution for the two cues differed from drug to drug. Nitrazepam and diazepam were less potent in substituting for CL218872 than CDZP (Table 1). Full dose-response curves were not performed, but whereas 1 mg/kg nitrazepam was adequate for full substitution for the CDZP cue, 5 mg/kg was required to produce 83% drug lever selection in CL218872-trained rats and this was associated with an incidence of rats not reaching criterion (20 presses on one lever) on test and being designated as non-responders (NR). The rate of responding in those rats reaching criterion was also reduced. CDZP showed dose-related substitution for its own cue, but only partial substitution for the CL218872 cue at doses up to 40 mg/kg when rates of responding were decreased (Fig. 1).

Zolpidem and CL218872 were both more potent in substituting for the CL218872 cue, although zolpidem also decreased the rate of responding. The higher doses of each of these drugs that were required to substitute for the CDZP cue also significantly reduced rates of responding (Fig. 1).

Zopiclone, RU31719 and RU43028 all showed dose-related substitution for both cues with similar potencies (Figs. 1 and 2). Zopiclone and RU43028 reduced rates of responding at the doses required for full substitution. Zopiclone depressed rates more in rats trained to discriminate CL218872.

Several other partial agonists at benzodiazepine receptors were all less potent in substituting for the CL218872 cue. Substitution for the CDZP cue was complete with RU32698, but was not complete with RU32514, CGS9896 or RU39419 at the doses tested (Table 1, Fig. 2). Substitution for the CL218872 cue was always less at any given dose and was less than 50% for RU32514, CGS9896 and RU39419. None of this group of compounds affected response rates. Of these compounds RU39419 showed the greatest differential between degree of substitution for the CDZP cue (83% at 20 mg/kg) and degree of substitution for the CL218872 cue (20% at 20 mg/kg). When tested in antagonism studies at this dose RU39419 totally blocked the CL218872 cue (Table 1).

The benzodiazepine antagonist/inverse agonist CGS8216 and the inverse agonist FG7142 both antagonised both cues (Table 1). CGS8216 appeared to be more potent in antagonising the CL218872 cue. Combination of cue doses and either of these drugs tended to decrease response rates. Control experiments showing that neither drug substituted for the CL218872 cue also showed that both drugs tended to decrease response rates when given alone.

Phenobarbitone only partially substituted for either cue (Table 1) at doses which did not markedly impair responding.

DISCUSSION

The discriminative stimulus induced by CL218872 is mediated via benzodiazepine receptors as, similar to the CDZP cue, it is antagonised by the antagonist/weak inverse agonist CGS8216. It represents some form of agonism at benzodiazepine receptors as it is agonists which substitute for the cue, whilst the inverse agonist FG7142 does not substitute but antagonises the cue. The use of

TABLE 1

STIMULUS GENERALISATION AND ANTAGONISM STUDIES USING RATS DISCRIMINATING CL218872 OR CDZP FROM VEHICLE

		CL218872 Cue					CDZP Cue			
				% Rats	Response Rates			% Rats	Response Rates	
Drug	Dose mg/kg	n		Selecting Drug Lever	Vehicle	Drug	n	Selecting Drug Lever	Vehicle	Drug
Nitrazepam	0.5						6	50	78.9 ± 7.9	85.8 ± 11.6
	1						4	100	72.6 ± 13.5	72.1 ± 10.0
	2	5		40	34.5 ± 7	29.7 ± 10.5	10	100	75.7 ± 6.7	66.3 ± 7.0
	5	10	4NR	83.3	50.7 ± 6.4	33.2 ± 8.5*				
Diazepam	5	5		40	48.6 ± 8.3	57.0 ± 7.7	10	100	75.1 ± 7.5	76.1 ± 9.9
Phenobarbitone	10	4		25	54.5 ± 13.7	51.2 ± 16.6	4	25	65.6 ± 8.5	65.9 ± 14.4
	15						6	33.3	77.2 ± 5.5	77.3 ± 6.2
	20	4		50	54.6 ± 8.6	47.7 ± 10.6	4	25	59.8 ± 13.4	44.2 ± 4.0
RU39419	10						6	50	64.3 ± 9.7	74.0 ± 14.1
	20	5		20	42.3 ± 7.1	47.5 ± 9.9	6	83.3	59.0 ± 9.9	68.9 ± 14.6
Cue + RU39419	20	5		0	50.7 ± 6.7	51.8 ± 9.5				
CGS8216	20	6		0	48.6 ± 8.1	37.6 ± 11.2				
Cue + CGS8216	10	5		0	44.0 ± 8.0	33.1 ± 6.3	11	45.5	47.5 ± 7.0	42.8 ± 5.4
	20	6		0	40.8 ± 6.9	29.8 ± 6.1	6	0	58.4 ± 8.4	36.6 ± 8.5*
FG7142	10ip	4		0	50.0 ± 11.9	24.9 ± 4.2*				
Cue + FG7142	10ip	5		0	50.6 ± 8.0	41.6 ± 10.6	6	0	72.4 ± 12.7	28.5 ± 6.5*

NR = Rats not responding to criterion on test.
n = Number of rats completing the drug trial.
* = $p<0.05$ Student's t-test, response rates are shown as mean ± SEM.

low training doses of the cue drugs does not appear to have decreased their selectivity in generalisation studies as phenobarbitone, which produces some behavioural effects similar to either of the cue drugs, only partially substitutes for either cue. The lack of full substitution by CDZP for the CL218872 cue, even at doses depressing response rates, might suggest that the discriminative stimuli produced by these two drugs are not identical. Another classical benzodiazepine, nitrazepam, does achieve 83% substitution at 5 mg/kg PO, although this dose markedly impaired performance. However, the classical benzodiazepines nitrazepam, diazepam and CDZP as well as the partial agonists (5,6) RU32698, RU32514, RU39419 and CGS9896 are all less potent in substituting for the CL218872 cue than they are for the CDZP cue, whether full substitution was observed or not. There is no evidence of a preference for BDZ_1 or BDZ_2 sites with RU32698, RU32514 or RU39419 (6). Reduced maximum substitution observed with some partial agonists (e.g., CGS9896) may be related to lower agonist intrinsic activities (6). RU39419 showed clear substitution for the CDZP cue, but antagonised the CL218872 cue. Zopiclone, RU43028 and RU31719 substituted for both cues with similar potency. Whilst there are no indications of a strong preference for BDZ_1 sites with these compounds (6, 9, 10), these data might

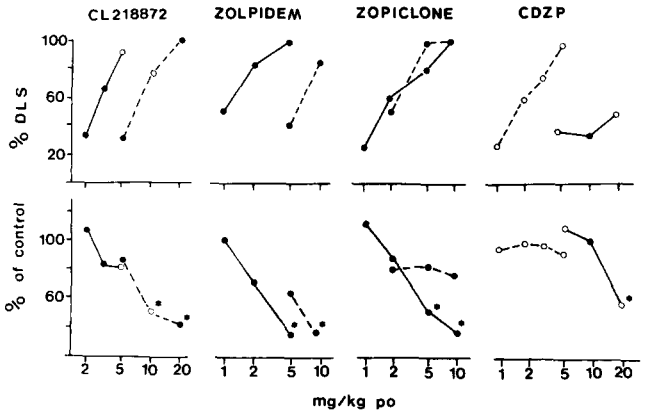

FIG. 1. Ability of some benzodiazepine receptor ligands to substitute for either a CL218872 cue (●—●) or a CDZP cue (●---●). Upper panels show the percentage of rats showing drug lever selection (DLS). Lower panels show the rate of lever pressing during these trials as a percentage of the rates in training of these rats. Doses are shown below the panels. ●: n = 4–7, ○: n = 8–12. *$p<0.05$ Student's t-test.

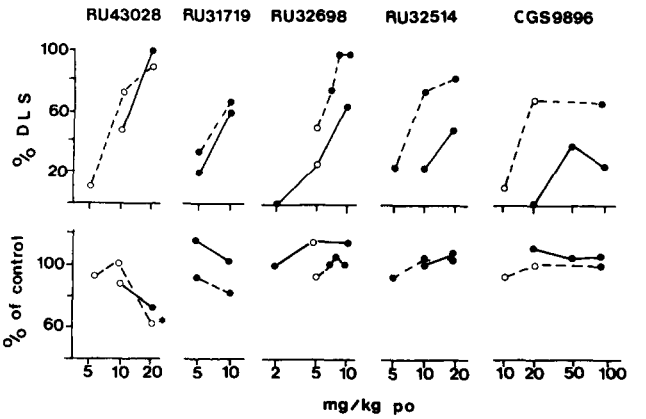

FIG. 2. Ability of some putative partial agonists at benzodiazepine receptors to substitute for either a CL218872 cue (●—●) or a CDZP cue (●---●) (upper panels). Rates of responding are shown in the lower panels. All other details are as described in Fig. 1.

suggest that some preference exists. In contrast, CL218872 and zolpidem, the two drugs which show a preference for BDZ_1 sites (1,11), were clearly more potent in substituting for the CL218872 cue. This difference supports the view that the discriminative stimuli produced by CDZP and CL218872 are not identical.

This potency ratio difference also is not consistent with the hypothesis that the two cues result from different degrees of agonism at the same receptors. If the training dose of CDZP is low then agonists with low intrinsic activity will be detected by their substitution. With higher training doses of CDZP such compounds will not substitute but will antagonise the cue. This has been shown with Ro15-1788 (3). The discriminative stimuli at the different doses of CDZP are not the same but result from different degrees of activation of the same receptor population. However, this hypothesis would predict that, although the degree of substitution would change, the rank order of potency would not. This was not observed in the comparison of the CL218872 and CDZP cues. Furthermore, the fact that CL218872 shows little substitution for the CDZP cue at its own training dose is also inconsistent with different degrees of agonism of the two cue drugs at the same receptor.

Thus, although both cues involve agonism at benzodiazepine receptors and have characteristics in common, as shown by some cross generalisation, the CL218872 cue is not identical to the CDZP cue. The differences may be related to the selectivity of CL218872 (and zolpidem) for BDZ_1 sites (1,11). However, the pharmacological profiles of this CL218872 cue and the zolpidem cue are not the same. Zopiclone substitutes for both these cues, but partial agonists Ro16-6028, Ro17-1812 and ZK91296, which have similar or stronger intrinsic agonism to those substituting for CL218872 in this study (RU32698 and RU43028) (5), were antagonists of the zolpidem cue (18–21). This does not necessarily imply that the mechanisms underlying the two cues are different. The CL218872 cue could result from a lesser degree of activation of the BDZ_1 sites, although Sanger and Zivkovic (20) did not obtain consistent discrimination of lower doses of zolpidem. It has been suggested that CL218872 is a partial agonist at benzodiazepine receptors (7) and it could be a partial agonist at BDZ_1 sites (6). There are presently insufficient partial agonists which have been tested in both cues to determine whether the ranking of potency is the same for both, but this interpretation remains a possibility.

If zolpidem and CL218872 do both induce their discriminative stimuli by interacting with the same benzodiazepine receptor subtype then differences in substitution/antagonism properties of particular agonists are likely to result from the different sensitivities of the cues to different degrees of intrinsic activity. On this basis, some partial agonists may substitute for the CL218872 cue but antagonise the zolpidem cue. Thus, the hypothesis that compounds act "as agonists or partial agonists at one receptor subtype ('chlordiazepoxide-receptor') and as antagonists the other receptor subtype ('zolpidem-receptor')" (19) may not hold. It is more likely that such response characteristics for agonists are determined by the intrinsic activity and dose of the training drug (2,3).

In summary, CL218872 produces an interoceptive stimulus which is mediated via benzodiazepine receptors, but which is not identical to that induced by CDZP. It is possible that the difference may result from a preferential interaction of CL218872 with a functional subtype of benzodiazepine receptors. The particular potency of zolpidem in substituting for the CL218872 cue suggests that this receptor subtype may equate with the BDZ_1 binding site. Differences in the pharmacological profiles of the discriminative stimuli produced by CL218872 and zolpidem may indicate that CL218872 is behaving as a partial agonist.

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