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Discriminative Stimulus Properties of CGS 9896: Interactions Within the GABA/Benzodiazepine Receptor Complex

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LEIDENHEIMER, N. J. AND M. D. SCHECHTER. *Discriminative stimulus properties of CGS 9896: Interactions within the GABA/benzodiazepine receptor complex.* PHARMACOL BIOCHEM BEHAV 31(2) 249–254, 1988.—Male rats were trained to discriminate the stimulus effects of CGS 9896 (30.0 mg/kg) from its vehicle. Once trained, discriminative performance was observed to be dose-responsive in the 3.75–30.0 mg/kg range and analysis of the dose-response curve generated an ED_{50} of 6.44 mg/kg. Generalization testing with chlordiazepoxide and pentobarbital produced CGS 9896-appropriate responding, whereas administration of the GABA agonists SL 75 102 resulted in 75% (intermediate) generalization to the CGS 9896 discriminative stimulus. Although full antagonism of the CGS 9896 cue was obtained following administration of Ro15-1788 and pentylenetetrazole, the inverse agonist DMCM failed to provide complete antagonism. These results suggest that the discriminative properties of CGS 9896 are consistent with its activity as a benzodiazepine receptor agonist.

CGS 9896 Drug discrimination Benzodiazepine receptor Chlordiazepoxide SL 75 102 DMCM Ro 15-1788

CGS 9896, a pyrazoloquinoline which displays high affinity for the benzodiazepine (BZ) receptor (14), has been shown to possess an anxiolytic/anticonvulsant profile devoid of the depressant side-effects typically associated with classical anxiolytic and anticonvulsant agents (10). The pharmacology of CGS 9896 has been reviewed (4,5). The anxiolytic potential of CGS 9896 has been assessed in a variety of animal models and its antianxiety efficacy has been found comparable to that of the benzodiazepines (25). Furthermore, the anticonflict activity of both diazepam and CGS 9896 can be antagonized by RO 15-1788 (25) and both drugs possess anticonvulsant properties. Like benzodiazepines, CGS 9896 protects against audiogenic and chemically-induced seizures (6, 7, 15).

In contrast to benzodiazepines, CGS 9896 has been found to be devoid of muscle-relaxing properties in both the rotorod and traction reflex tests and can, in fact, antagonize the muscle-relaxing effects produced by diazepam in these tests (6). Furthermore, ataxia/sedation and CNS depression are absent at doses up to 300 mg/kg in rodents. CGS 9896 potentiation of the depressant effects of ethanol appears to be minimal in contrast to other anxiolytics which profoundly interact with ethanol (6). Finally, the addictive liability of CGS 9896 may prove to be minimal since chronic treatment with this compound in baboons does not appear to result in withdrawal symptoms upon discontinuation (18) and the ability of CGS 9896 to raise the pentylenetetrazole (PTZ) seizure threshold in rats is not altered by chronic treatment (8).

The actions of CGS 9896 are thought to be mediated by the benzodiazepine receptor which is located in a macromolecular receptor complex comprised of receptor sites for not only benzodiazepines but separate binding domains for gamma-aminobutyric acid (GABA) and barbiturates as well (23). The effects of compounds acting on these receptors appear to be mediated by conductance changes associated with a chloride ionophore located within the complex (23). Because of the common end-point mechanism for these receptors, i.e., chloride ionophore modulation, it is not surprising that drugs acting on these different binding sites can exhibit similar behavioral effects. The purpose of the present experiment was to train rats to discriminate CGS 9896 in a drug discrimination paradigm and, subsequently, to test drugs acting at different binding domains within this receptor complex for their ability to either substitute for or antagonize the CGS 9896 discriminative stimulus.

METHOD

Subjects

Experimentally-naive male Sprague-Dawley rats (Zivic-Miller, Allison Park, PA), weighing between 262–325 g at the beginning of the experiment, were used as subjects. These rats were food-deprived and maintained at 80–90% of their free-feeding weights by a restricted diet. Rats were individually housed under a twelve hour light (0600–1800)/dark cycle in a room maintained at 20–22°C.

Apparatus

Ten standard rodent operant chambers (Lafayette Instruments Co., Layfayette, IN), each equipped with two levers and a food receptacle located midway between the levers, were used for training. Each chamber was enclosed in a fan-ventilated, sound-attenuated outer shell. Sessions were controlled and responses were recorded on solid-state equipment (Med Associates, E. Fairfield, VT) located in an adjacent room.

Lever Response/Discrimination Training Procedure

The lever response/discrimination training procedure has been detailed elsewhere (33) and therefore is only briefly described here. Prior to discrimination training, food-deprived rats were trained to press the lever in the operant chamber for food reinforcement (45 mg Noyes pellets). Thirty min prior to the training sessions rats were administered vehicle injections and, upon placement in the operant chamber, were rewarded for responses on the designated vehicle lever only. Initially, rats were trained to respond on the vehicle lever on a fixed ratio (FR) schedule of one, i.e., one correct response resulted in one reinforcement. Over eight training sessions the FR schedule was gradually increased to an FR10, i.e., ten correct responses yielded one reinforcement, where it remained throughout the experiment. Animals were removed from the chamber and returned to home cages after pressing the appropriate lever 400 times and, thus, receiving 40 reinforcements on the FR10 schedule.

Once an FR10 was established on the vehicle lever, training began on the opposite lever (the "CGS lever") following CGS 9896 administration. Thirty min following the administration of an equal volume of vehicle containing CGS 9896 (30.0 mg/kg), rats were rewarded for responses on the CGS lever only. As with vehicle administration, the initial schedule of an FR1 was gradually increased to an FR10 over a period of five training sessions.

Once FR10 responding was established on both levers, discrimination training began. Thirty min prior to daily discrimination training sessions, rats received either vehicle (V) or an equal volume of vehicle containing CGS 9896 (30.0 mg/kg) according to the following two-week, pseudorandom injection schedule: CGS,V,V,CGS,CGS; V,CGS,CGS,V,V. As during lever response training, responses on the CGS lever were rewarded only following CGS 9896 administration; responses on the vehicle lever were rewarded only following vehicle injections. Responses on the inappropriate lever were inconsequential. The first lever upon which ten responses were made was designated the "selected lever" for that session. Daily training sessions were continued until 400 responses were made upon the injection-appropriate lever and, therefore, 40 reinforcements received.

The two-week administration regimen was repeated until the selected lever was correct for the injection received in eight out of ten consecutive training sessions, twice. The number of training sessions required to achieve the criterion is expressed as a sessions-to-criterion value (24). The first sessions-to-criterion indicates the number of sessions required to reach the first session in the first series of eight out of ten correct consecutive sessions. The first session in the second series of eight out of ten correct selections constitutes the second sessions-to-criterion value. Data collection began when all animals had fulfilled this 80% criterion.

Dose-Response Relationship

After reaching criterion performance, the rats were tested in a random order with the following doses of CGS 9896 (IP): 30.0, 22.5, 15.0, 7.5, 3.75 mg/kg. Thirty min following CGS 9896 administration, rats were placed into the discrimination chamber and upon making ten responses on either lever they were immediately removed and returned to their home cages without receiving food reinforcement. During the dose-response testing period, maintenance sessions were interspersed between test sessions such that each CGS 9896 test dose was administered on two occasions, once following a vehicle maintenance day and once following a CGS 9896 (30.0 mg/kg) maintenance day. If at any time during the experiment a rat's discriminative performance fell below the established 80% criterion, i.e., less than eight out of ten correct interspersed maintenance sessions, the data on that rat were dropped from the results for that series of experiments. Once maintenance performance returned to criterion level, data collected from the animal were once again included for periods in which criterion was maintained.

Generalization Experiments

Following establishment of a CGS 9896 dose-response relationship, chlordiazepoxide, pentobarbital and SL 75 102 were each tested for generalization. A test drug was considered to transfer to CGS 9896 if quantal responding was equal to or greater than 80% CGS-appropriate responding. Each dose of the test drugs (TD) was counterbalanced between maintenance days by employing the following test schedule: CGS 9896, TD dose 1, V, TD dose 1, CGS, TD dose 2, V, TD dose 2, CGS, etc. Due to a limited supply, the highest dose of SL 75 102 was administered to only four rats, twice. Test drugs were administered at times and doses determined from the literature; thus, all drugs were administered thirty min prior to test sessions except SL 75 102 (20 min).

Antagonism of the CGS 9896 Cue

Rats were injected with CGS 9896 (30 min) and either Ro 15-1788 (20 min), DMCM (31 min), or PTZ (31 min) prior to being placed into the drug discrimination chamber. Test drug doses presented in Table 2 were selected from drug discrimination literature. The animals were returned to home cages after making ten responses on either lever without receiving reinforcement. Each dose of co-administered drug was counterbalanced between maintenance days as in generalization testing. In addition, all antagonists were co-administered with vehicle to control for any possible effects they may have produced alone.

Statistics and Measurement

The data collected in the drug discrimination sessions are expressed as both quantal and quantitative measurements as each measurement provides a different indication of lever preference prior to any reinforcement. The quantal measurement is the percentage of rats selecting the CGS lever as their "selected lever," i.e., the lever pressed 10 times first. The quantitative measurement is the number of responses on the CGS lever divided by the total number of responses on both the CGS and vehicle levers at the time the tenth response on either lever is made. This fraction is expressed as a percentage. Unlike the (all-or-none) quantal data, the quantitative measurement accounts for responses on both the selected and unselected lever and, thus, provides a rela-

TABLE 1

DOSE-RESPONSE DATA FOR CGS 9896 AND COMPOUNDS GENERALIZING TO CGS 9896

Drug	Dose (mg/kg)	Percent Responding on CGS 9896 Lever: Quantal	Quantitative (±SD)	Quantal ED_{50} (95% conf. limits)	N
Vehicle	—	3.6	10.8 (5.5)		
CGS 9896	3.75	41.7	41.7 (1.7)		6
	7.5	53.6	50.3 (0.1)		14
	15.0	53.6	54.8 (8.1)	6.4	14
	22.5	78.6	72.7 (7.4)	(3.8–11.6)	14
	30.0	87.3	78.0 (6.8)		14
Pentobar-	2.5	0.0	2.7 (2.2)		9
bital	5.0	55.5	48.2 (2.0)	5.0	9
	10.0	94.4	85.0 (8.3)	(3.4– 7.4)	9
Chlordiaze-	1.0	33.3	34.4 (7.6)		9
poxide	2.5	77.8	63.5 (6.9)	1.4	9
	5.0	100.0	91.5 (4.6)	(0.8– 2.4)	9
SL 75 102	50.0	30.0	42.0 (18.5)		5
	100.0	70.0	62.7 (25.5)		5
	150.0	75.0	64.6 (10.1)		4

tive measure of the magnitude as well as direction of lever preference. The advantages of both types of measurements are discussed by Stolerman and D'Mello (39). ED_{50}s were generated from both the quantal and quantitative data by the Litchfield-Wilcoxon procedure (20). The probit vs. log-dose plots generated from the quantal data were used to test for parallelism between CGS 9896 and test drug dose-response curves. The quantitative data from maintenance and antagonism test sessions were subjected to a paired t-test with $p<0.05$ chosen as the level for significance.

Drugs

CGS 9896 (6.0 mg/ml) was suspended in a 2% by volume solution of Tween 80 by sonification. A 2% Tween 80 (Sigma Chemical, St. Louis, MO) solution was used for vehicle (V) injections. Both vehicle and CGS 9896 injection volumes were 5.0 ml/kg. All test drugs were dissolved or suspended in 2% Tween 80 except chlordiazepoxide and SL 75 102 which were dissolved in distilled water. Drugs were obtained from the following sources: CGS 9896 (CIBA-GEIGY, Summit, NJ), chlordiazepoxide HCl and RO 15-1788 (Hoffmann-La Roche, Nutley, NJ), SL 75 102 (Laboratoires d'Etudes et de Recherces Synthelabo, France, LERS), pentylenetetrazole (Sigma Chemical Company, St. Louis, MO), DMCM (Research Biochemicals Inc., Wayland, MA), and pentobarbital (Bowman Pharmaceuticals, Canton, OH). Injection volumes were constant at 1 mg/kg for all test compounds other than CGS 9896.

RESULTS

Training

The rats required a mean (±SD) of 9.8 (6.4) to reach the first of eight of ten correct consecutive sessions. The mean number of sessions-to-criterion for the second set of eight of ten correct consecutive sessions was 21.2 (7.4). The last animal to reach criterion required 43 training sessions to complete training.

CGS 9896 Dose-Response Relationship

Maintenance trials with 30.0 mg/kg CGS 9896 produced 87.3% quantal responding on the CGS 9896-appropriate lever, whereas vehicle maintenance trials produced 3.6% responding on this lever (or 96.4% vehicle-appropriate responding; Table 1). Administration of various doses of CGS 9896 resulted in a typical dose-response relationship in the dose range of 3.75–30.0 mg/kg and an ED_{50} (generated using the quantitative data) of 7.0 mg/kg; this ED_{50} closely approximated that of 6.4 mg/kg derived from the quantal data as shown in Table 1.

Generalization Testing

When 5.0 mg/kg chlordiazepoxide was administered to CGS 9896-trained rats it produced 100% of quantal responding on the CGS 9896-appropriate lever. Decreasing doses of chlordiazepoxide produced progressively fewer CGS 9896-appropriate responses (Table 1). An ED_{50} of 1.4 mg/kg was generated from the chlordiazepoxide quantal dose-response data. When the CGS 9896 and chlordiazepoxide dose-response curves were tested for parallelism they were not significantly different in slope (calculated $t=1.17$ < critical $t=2.57$) and, thus, were considered to be parallel (20).

Pentobarbital (10.0 mg/kg) administered to rats trained to discriminate CGS 9896 from vehicle resulted in 94.4% quantal responding on the CGS 9896 lever. A pentobarbital dose-response curve established in the dose range of 2.5–10.0 mg/kg (Table 1) generated a quantal ED_{50} of 5.0 mg/kg. The

TABLE 2
ANTAGONISM OF THE CGS 9896 DISCRIMINATIVE STIMULUS

CGS 9896 (30.0 mg/kg) + Co-Treatment	Antagonist Dose (mg/kg)	Percent Responding on CGS 9896 Lever: Quantal	Quantitative (±SD)	N
PTZ	15.0	0.0	12.3 (2.2)*	5
RO 15 1788	10.0	12.5	17.0 (6.4)*	8
DMCM	0.5	60.0	58.9 (0.3)	5
	0.7	80.0	73.1 (18.5)	5
	1.0	60.0	56.7 (7.1)	5

*Not significantly different from quantitative measurement after vehicle administration.

slope of the dose-response line obtained from this data differed significantly ($p<0.05$) from that of the CGS 9896 dose-response line in the Litchfield-Wilcoxon test of parallelism (calculated $t=5.98$ > critical $t=2.78$) (20).

Administration of SL 75 102 resulted in intermediate generalization to the CGS 9896 discriminative stimulus (Table 1). A limited supply of SL 75 102 precluded testing at doses greater than 150 mg/kg. This dose, however, resulted in 75.0% quantal responding on the CGS 9896 lever and, because of the very strong trend toward generalization, tests for parallelism were performed on the available data. The SL 75 102 dose-response curve was significantly different in slope from the dose-response curve of CGS 9896 (calculated $t=2.96$ > critical $t=2.78$). Although neither SL 75 102 nor pentobarbital dose-response curves were parallel to that of CGS 9896, SL 75 102 and pentobarbital dose-response curves were parallel to each other (20).

Antagonism Studies

When a single dose (chosen from the literature) of either RO 15-1788 or pentylenetetrazole was given in conjunction with CGS 9896, vehicle-like responding resulted (Table 2). However, pretreatment with three doses of DMCM produced only partial antagonism of the CGS 9896 cue. When given with vehicle, antagonism test drugs produced predominately vehicle-like responding (data not shown).

DISCUSSION

Previous work, using CGS 9896 for behavioral control in the discriminative stimulus paradigm, indicates that the CGS 9896 interoceptive cue generalizes to the anxiolytics diazepam and meprobamate, as well as to the more anxioselective compounds tracazolate and CL 218 872 (3). Furthermore, the CGS 9896 cue is antagonized by CGS 8216, a partial inverse agonist at the benzodiazepine receptor. This drug discrimination data is in agreement with extensive in vitro and in vivo studies indicating the effects of CGS 9896 are mediated by the benzodiazepine receptor (4, 6, 15).

In the present study, the CGS 9896 cue was observed to be similar in nature to that produced by benzodiazepines in that it showed complete generalization to chlordiazepoxide, was blocked by the BZ receptor antagonist RO 15-1788 and, like chlordiazepoxide and diazepam, generalized to pentobarbital. Although CGS 9896 generalized to chlordiazepoxide here and diazepam elsewhere (3), CGS 9896 does not consistently produce drug-appropriate responding in rats trained to discriminate benzodiazepines. When administered to rats trained to discriminate chlordiazepoxide, CGS 9896 produced chlordiazepoxide-appropriate responding (31,32). However, rats trained to discriminate diazepam from vehicle have shown both diazepam-appropriate (43) and vehicle-appropriate (35) responding whereas lorazepam-trained rats failed to generalize the lorazepam cue to CGS 9896 (1). The asymmetrical interactions between cues produced by benzodiazepines receptor agonists have been discussed (3, 5, 9, 32). In the discriminative stimulus paradigm, CGS 9896 not only produces an anxioselective cue but also antagonizes the hypnotic cue in zolpidem-trained animals (32), indicating a mixed agonist/antagonist profile of CGS 9896. It is this mixed agonist/antagonist profile of CGS 9896 that may account for the asymmetry between CGS 9896 and benzodiazepine cues.

In the present experiment, pentobarbitol (PB), a compound which relieves anxiety, produced complete (94.4% quantal) generalization to the CGS 9896 cue. Analysis of the CGS 9896 and PB data, however, revealed nonparallel dose-response curves. This indicates that the interoceptive cues of PB and CGS 9896 may be mediated by different mechanisms of action since drugs acting by the same mechanism of action generally display parallel dose-response relationships (19). The difference in mechanism of action of PB and CGS 9896 is supported by several reports indicating that RO 15-1788 blocks benzodiazepine discriminative stimuli but not that produced by pentobarbital (13, 34, 40). These observations are consistent with the demonstration of separate binding sites for BZ and PB within the GABA/BZ complex (23). Furthermore, it has been demonstrated that BZ and PB differentially affect the kinetics of the Cl^- ionophore located within the complex (41). PB is thought to decrease the frequency of ionophore openings and increase open-channel duration, whereas BZ increase the frequency of ionophore openings.

The effects of both BZ and PB are thought to be dependent on GABAergic transmission. Research investigating the role of GABA in anxiety is inconclusive and has been reviewed elsewhere (30). In the drug discrimination paradigm, diazepam does not generalize to the GABA agonists THIP or muscimol nor to the GABA-T inhibitor AOAA (16). Furthermore the $GABA_A$ antagonist bicuculline is ineffective in antagonizing the diazepam discriminative stimulus. In the present experiment, the CGS 9896 cue partially generalized to the GABA receptor agonist SL 75 102. Due to a limited supply of SL 75 102 testing was not continued at higher doses. Whether GABAergic transmission is necessary in the mediation of the anxioselective cue provided by CGS 9896 remains unclear at present.

Pentylenetetrazole (PTZ), a modulator of the TBPS binding site (29), provided complete blockade of the CGS 9896 discriminative stimulus here and it has previously been reported to be highly effective in antagonizing the discriminative stimulus produced by other benzodiazepine receptor agonists (16,21). In contrast to PTZ, the benzodiazepine receptor inverse agonist DMCM was not effective in antagonizing the CGS 9896 discriminative stimulus in this study and, in other studies, has been shown to be inconsistent in antagonizing the discriminative stimuli produced by benzodiazepines. Both DMCM and FG 7142 provided complete

antagonism of the chlordiazepoxide cue (37,38). However, several inverse agonists, including DMCM, have been demonstrated to be only partially effective in antagonizing the diazepam discriminative stimulus (22). The lack of efficacy of inverse agonists in antagonizing diazepam and CGS 9896 mediated effects may be specific to the drug discrimination paradigm as inverse agonists are quite efficacious in antagonizing the effects of benzodiazepines in other paradigms (2, 11, 12, 17, 25–28, 36, 42). It is possible, as previously suggested by Nielsen *et al.* (22), that the chronic treatment regimen of drug discrimination may produce receptor changes that influence the efficacy of ligands for the BZ receptor. Alternative explanations to account for these findings include the binding of β-carboline inverse agonists to a site conformationally distinct from the benzodiazepine binding site, the specificity of the inverse agonists for subtypes of BZ receptors or, simply, the differences in methodology and/or doses of antagonists employed.

In conclusion, CGS 9896 presents a discriminative profile similar to that of the benzodiazepines. The asymmetrical generalization between CGS 9896 and benzodiazepines may reflect the mixed agonist/antagonist properties of the CGS 9896 cue. The possible involvement of GABA in the mediation of the CGS 9896 cue remains unclear; however, future studies utilizing newer GABAergic agonists may help to clarify this issue. Finally, conflicting reports concerning the effectiveness of inverse agonists in antagonizing BZ receptor agonists discriminative stimuli require further investigation.

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