Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 397–401, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00076-3

Discriminative Effects of Phenazepam and Gidazepam in Rats: Comparison With Other GABA-Related Drugs

T. S. KALININA, T. L. GARIBOVA AND T. A. VORONINA

Institute of Pharmacology of RAMS, Baltiyskaya str. 8, Moscow, 125315, Russia

KALININA, T. S., T. L. GARIBOVA AND T. A. VORONINA. *Discriminative effects of Phenazepam and Gidazepam in rats: Comparison with other GABA-related drugs*. PHARMACOL BIOCHEM BEHAV **64**(2) 397–401, 1999.—The present study assessed the discriminative stimulus effects of phenazepam (PHZ) (2 mg/kg, IP), gidazepam (GDZ) (10 mg/kg, IP), pentobarbital (PB) (10 mg/kg, IP), and buspirone (B) (5 mg/kg, IP) by testing GABA-related drugs in the two-lever liquid reinforced operant discrimination procedure in rats. Diazepam (5–30 mg/kg, IP) dose dependently and completely substituted in GDZ-trained rats and in only 40% PHZ-trained rats. Following phenobarbital (40–100 mg/kg, IP) injections the mean percentages of PHZ- and GDZ-lever responding generally were a monotonically increasing function of dose, but peaked at 39.3 and 52.9%, respectively. The PB discriminative cue was generalized completely to PHZ, GDZ, and phenobarbital. Picrotoxin (2 mg/kg, SC) did not inhibit the PHZ and GDZ discriminations, while it antagonized the PB (10 mg/kg, IP) cue. Calcium valproate (200 mg/kg, IP) failed to produce PHZ effects, and partially substituted for GDZ. B failed to substitute for the discriminative effects of PHZ, GDZ, or PB, producing a maximum 9.3, 18.0, and 33.3% drug lever responding, respectively. These results suggest that the discriminative stimuli of PHZ and GDZ are similar to those of other benzodiazepine agonists. However, the PHZ cue is more selective than that of GDZ. © 1999 Elsevier Science Inc.

Phenazepam Gidazepam Discriminative effects $GABA_A$–BDZ receptor complex Rats

THE interactive relationships between chemical structures, physics-chemical properties, receptor functions, and pharmacological activities were the research strategy that has yielded the development of two clinically effective 1,4 benzodiazepine derivatives phenazepam and gidazepam (1,14,22). Both compounds are benzodiazpine agonists of the $GABA_A$–benzodiazepine receptor chloride channel complex, and demonstrate similar, but not identical, psychotropic profiles (23,25). Phenazepam is a classical benzodiazepine with a powerful anxiolytic effect exceeding those of diazepam and chlordiazepoxide, and strong sedative, anticonvulsive, and hypnotic properties in clinical trails as well as in animal experiments (5,7,23,25). For more than 20 years, phenazepam has been used as an anxiolytic and anticonvulsant in clinical practice in Russia and countries of the former Soviet Union.

Gidazepam is a novel benzodiazepine drug that was introduced into clinical practice 2 years ago, and demonstrates some advantages over classical benzodiazepines such as chlordiazepoxide, diazepam, or phenazepam. In experiments in rats and mice, this compound showed anticonflict activities greater than diazepam, but was less efficacious than phenazepam (25,26). Compared with traditional benzodiazepines, gidazepam demonstrated a wide separation between anxiolytic and sedative dosages. Unlike diazepam or phenazepam, gidazepam did not have a sedative effect on motor activity in the conflict test (25,26). In contrast to classical benzodiazepines, gidazepam did not attenuate learning, and even stimulated the acquisition of active avoidance behavior and improved Sidman avoidance responding (23,24). Based on these facts, gidazepam could be considered as a selective anxiolytic with a nontraditional profile of psychotropic activities.

The present study assessed the specificity of the discriminative stimulus effects of phenazepam and gidazepam by testing GABA-related drugs in rats trained to discriminate these drugs.

METHOD

Animals

Adult male albino outbred rats weighing 300–350 g were used in these experiments ("Stolbovaja" animal farm; Moscow region). Animals were housed in Plexiglas cages under a

Requests for reprints should be addressed to T. S. Kalinina, Institute of Pharmacology of RAMS, Baltiyskaya str. 8, Moscow, 125315, Russia.

natural light/dark cycle, with food available at all times. Water was freely available at weekend and for 20 min after training sessions.

Apparatus

A six-chamber operant conditioning system (Lafayette Instrument Co., Lafayette, IN) with sound-attenuating cages (model 80001), interface (model 118-01), and a PC Apple IIe was used. Each operant chamber was equipped with two levers, liquid/pellet dispensers, and an electroshock floor. A PC Apple IIe, programmed with original software (6), and a printer (Epson RX-80 F/T+) were used to control schedule contingency and to record data.

Procedure

Drug-discrimination experiments were carried out in the six operant boxes simultaneously. Four group of rats were trained to discriminate between phenazepam (2 mg/kg; $n = 12$), gidazepam (10 mg/kg; $n = 6$), pentobarbital (10 mg/lg; $n = 12$) or buspirone (5 mg/kg, $n = 10$) and vehicle using a two-lever water reinforced drug discrimination procedure. Initially, after 48 h of water deprivation, either lever choice was reinforced with 0.125 ml of tap water, and the ratio of responses per reinforcer was gradually increased to 10. Then rats were trained to press one lever after drug injection and the other following vehicle treatment. To avoid the animals' intrinsic preference for right or left levers, the position of the drug-appropriate and vehicle-like levers was counterbalanced within each group of rats. Training sessions lasted 15 min/5 days per week, and were presented according to an equiprobable alternating sequence of drug and vehicle injections. During the training procedure water was available 1 h after each training session for 20 min and was also available each Friday ad lib in the home cages. Lever selection was defined by the first lever to accumulate 10 responses during the session. Correct lever selection was defined as drug lever selection following drug injection and vehicle lever selection after vehicle administration. Ten consecutive sessions during which the rats showed the accurate lever selection with no less than 80% correct responses was used as the acquisition criterion. After establishment of drug stimulus control, generalization and antagonism tests were performed. In these tests, animals were allowed to respond during 3 min without any reinforcement. Tests occurred one to two times in a week and were separated by three to four training sessions.

Drugs. Phenazepam (Physics-Chemical Institute of Ukrainian Academy of Sciences, Odessa), (gidazepam (Physics–Chemical Institute of Ukrainian Academy of Sciences, Odessa), diazepam (Sigma), buspirone (resythesized by and obtained from Physics-Chemical Institute of Ukrainian Academy of Sciences, Odessa), phenobarbital (Sigma), calcium valproate (Germed, former GDR) and flumazenil (Hoffman–LaRoche) were suspended in a vehicle containing 0.9% saline to which Tween-80 (2 drops/10 ml) was added. Sodium pentobarbital (Sigma) was dissolved in 0.9% saline. Picrotoxin (Sigma) was dissolved in warm (60°C) 0.9% saline. Solutions of bicuculline were prepared with mild acidification. All drugs were administered in an injection volume 2 ml/kg. All training and testing drugs were administered intraperitoneally except bicuculline and picrotoxin, which were given subcutaneously. During training sessions and testing, training drugs were given 20 min before behavioral procedures. In the antagonism tests flumazenil and picrotoxin were given 10 min prior to benzodiazepines or pentobarbital injections and 30 min before testing. Bicuculline and was administered 10 min after administration of the training drugs and 10 min prior to test sessions.

Data Analysis. Experiment events and data collection were controlled by a PC Apple2e operating using a specially developed original behavior software (6). Data recorded were appropriate lever responses (calculated as number of responses on the appropriate lever divided by the total number of responses on both levers), total number of responses (calculated as the total number of responses on both levers), and percent rats selecting the training drug lever. Mean percentage of drug lever responses and percent rats selecting the drug lever were used to obtain generalization profiles. Effects on the number of responses were analyzed by means of a paired Student's *t*-test. Comparisons of effects of drug dosages to training drug control values were made with an exact Fisher's test for data expressed in percent (22).

RESULTS

A high level of stimulus control was obtained in all groups of animals under both nondrug and drug training sessions. Acquisition of the discrimination required a mean of 21 (range 14–31) training sessions in phenazepam-trained rats and 29 (range 19–39) training sessions in gidazepam-trained animals, respectively. Pentobarbital-trained rats learned the discrimination criterion after 22 (range 16–33) training sessions. Acquisition of the buspirone discrimination required a mean of 38 (range 17–50) training sessions. Appropriate lever responses average the baseline sessions were 87.2% for the drug and 92.8% for the saline sessions in the phenazepam group, and 94.0% for the drug and 93.8% for the saline sessions in the gidazepam-trained rats. Control tests with pentobarbital-trained rats generally resulted in greater than 86.2% drug-lever responding and saline tests resulted in 95.2% nondrug lever choices. In buspirone-trained rats, these attributes were 87.5 and 90.6%, respectively. Response rates in control tests following phenazepam (77 presses/min), gidazepam (92 presses/min), or buspirone (73 presses/min) did not significantly differ from those on saline sessions. Response rates in control tests following saline were 89 presses/min in phenazepam-trained rats, 78 presses/min in gidazepam-trained rats, and 99 presses/min in buspirone-trained rats. Only in pentobarbital-trained rats were response rates higher after the training dose of pentobarbital (10 mg/kg) compared with saline injections: 120 presses/min vs. 69 presses/min, respectively. These control data indicate that the subjects were under good stimulus control throughout the study. Phenazepam (0.5–2 mg/kg) dose dependently substituted for the phenazepam training dose without significant response rate decreases. Generalization testing with gidazepam (1–50 mg/kg) resulted only in partial substitution for phenazepam, producing a maximum mean of 53% phenazepam-lever responding (Fig. 1). Diazepam (5–30 mg/kg) substituted for phenazepam in dose-dependent manner at an average 83% guidazepam-lever responding with a response of rate-decreasing effects in comparison with vehicle control (Fig. 1). But in this case, criterion required (80% and more of drug-appropriate lever responding) had been met only in 40% of the rats tested.

Full substitution was obtained with diazepam and phenazepam in rats trained to discriminate gidazepam from vehicle. Both drugs in maximum dosages tested produced decreases in overall rates of responding compared to saline control values.

In general, phenobarbital failed to substitute for phenazepam or gidazepam, producing no greater than 39.3 and

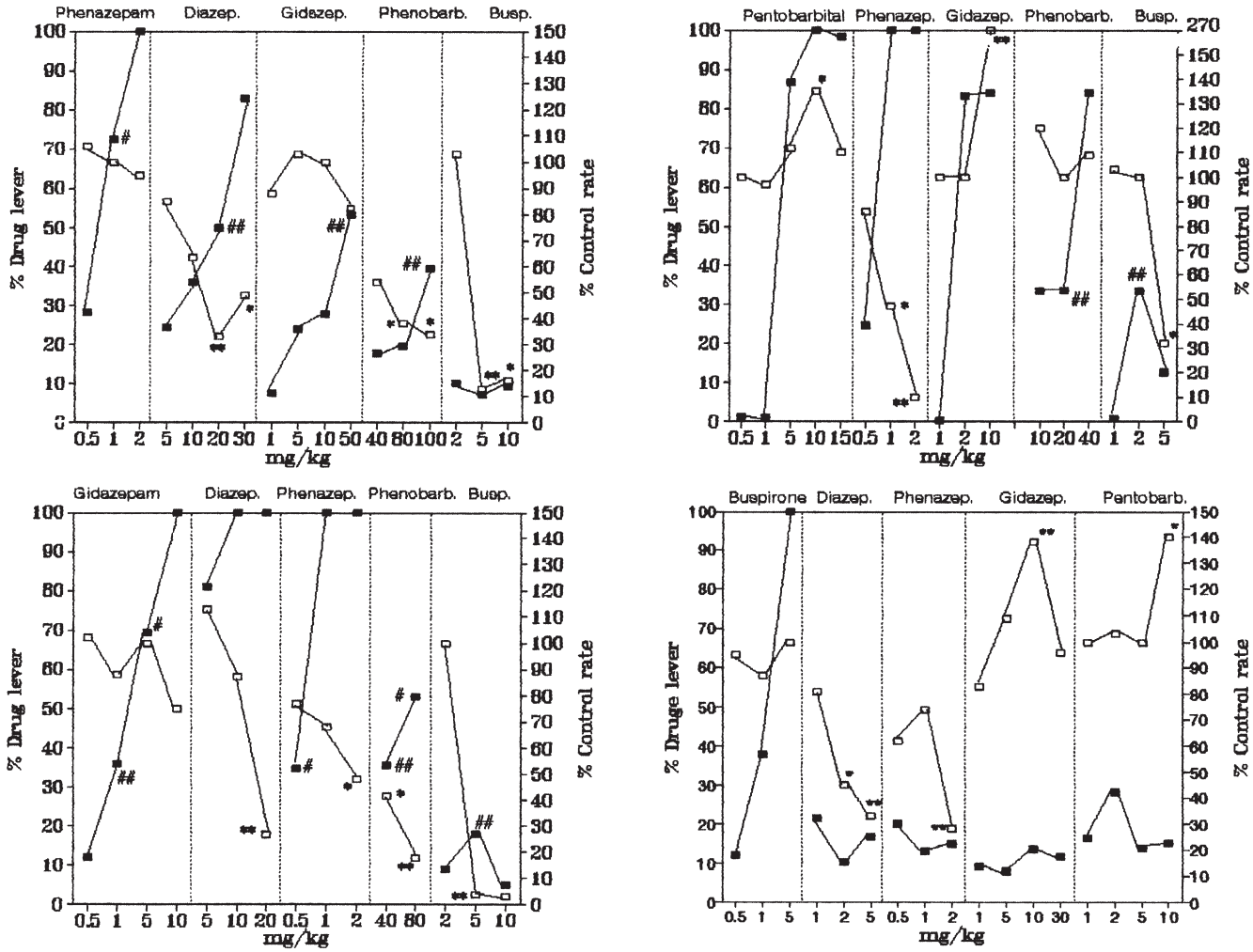

FIG. 1. Generalization test results and overall response rates obtained in substitution test sessions covering 10 responses on one lever or 3 min without reinforcement in rats trained to discriminate phenazepam (2 mg/kg, IP), gidazepam (10 mg/kg, IP), pentobarbital (10 mg/kg, IP), or buspirone (5 mg/kg, IP) from vehicle. The left vertical axes indicate the mean % drug lever responding (■); the right vertical axys indicate overall response rates (as % compared with nondrug sessions) (□); horizontal lines show doses of drugs tested. * and **$p < 0.05$ and $p < 0.01$, respectively, differences from the total responses on both levers obtained during vehicle baseline sessions; # and ##$p < 0.05$ and $p < 0.01$, respectively, differences from drug lever responding obtained from drug baseline sessions.

52.9% phenazepam- and gidazepam-lever responses, respectively (Fig. 1). While following phenobarbital injections, the mean percentages of phenazepam and gidazepam lever choices were a monotonically increasing function of dose (Fig. 1). The pentbarbital discriminative due cue generalized completely to phenazepam, gidazepam, and phenobarbital (Fig. 1). In the substitution test sessions with phenazepam, overall response rates were dose dependently decreased while gidazepam produced a significant increase of the response rates compared with response rates in saline control trials.

The nonbenzodiazepine anxiolytic drug buspirone failed to substitute for the discriminative effects of phenazepam, gidazepam, or pentobarbital, producing a maximum 8.3, 18.0, and 33.3% drug-lever responding in tests for substituting, respectively (Fig. 1). In all groups of $GABA_A$ agonist-trained rats, buspirone produced a significant decrease of the overall response rates (Fig. 1). Phenazepam, gidazepam, diazepam, and pentobarbital did not substitute for buspirone in buspirone-trained rats.

In the antagonism tests, the discriminative stimulus effects of phenazepam and gidazepam were fully antagonized by the selective benzodiazepine antagonist flumazenil in a dose-dependent manner. Following the injections of flumazenil at 15 mg/kg, none of the phenazepam-trained rats selected the phenazepam-appropriate lever (Table 1). Tests of gidazepam in combination with the specific blocker of $GABA_A$ receptor, bicuculline, failed to show any effects of this latter compound on the stimulus produced by gidazepam. Bicuculline, administered with the training dose of phenazepam, produced 45.8% of phenazepam-lever responding. The discriminative stimulus properties of phenazepam and gidazepam were not significantly affected by picrotoxin. However, the percent of the rats selecting benzodiazepine levers were decreased compared with control vehicle tests (Table 1). The pentobarbital cue was partially antagonized by picrotoxin under blocking tests in the pentobarbital-trained animals, and only 20% of animals tested made a choice of the lever associated with the barbiturate treatment (Table 1).

DISCUSSION

The data show that phenazepam and gidazepam produce high stimulus control like other established benzodiazepine agonists of the $GABA_A$–benxodiazepine receptor chloride channel complex. Substitution testing revealed that the discriminative effects of phenazepam and gidazepam were similar but not identical. The crossgeneralization tests demonstrated asymmetry of the discriminative effects of phenazepam and gidazepam. Thus, phenazepam completely substituted for gidazepam, while gidazepam produced dose-dependent, but only partial, generalization of the phenazepam discrimination. In term of potency, diazepam was more effective in the substituting for gidazepam than for phenazepam. The finding that gidazepam only partially substituted for the phenazepam stimulus suggests that the stimuli produced by gidazepam and phenazepam have some differences.

Both phenazepam and gidazepam occasioned at least 80% drug-appropriate responding in pentobarbital-trained rats. These results suggest that phenazepam and gidazepam are similar to other benzodiazepine agonists, such as diazepam, chlordiazepoxide, and midazolam, which also share the discriminative effects of barbiturates (3,14,17). However, phenobarbital occasioned only partial, but dose-dependent, drug-appropriate responding in rats trained to discriminate phenazepam or gidazepam from vehicle. The limited cross-substitution between phenazepam and barbiturate is in agreement with data on the generalization profile of lorazepam. Lorazepam has been shown to be the benzodiazepine agonist for which pentobarbital does not substitute in rats and in monkeys (2,3).

Like other agonists of the $GABA_A$–benzodiazepine receptor chloride channel complex, phenazepam and gidazepam failed to show cross-substitution of the nonbenzodiazepine anxiolytic buspirone (9,15). Phenazepam and gidazepam did not produce the buspirone cue in the buspirone-trained rats. Thus, the present data suggest that the discriminative stimuli of phenazepam and gidazepam are similar to those of the other agonists at the $GABA_A$–benzodiazepine receptor complex, and benzodiazepines in particular.

The data from antagonism tests revealed the differences in the $GABA_A$–benzodiazepine receptor chloride channel com-

TABLE 1

ANTAGONISM AND GENERALIZATION TEST RESULTS IN RATS TRAINED TO DISCRIMINATE PHENAZEPAM, GIDAZEPAM, OR PENTOBARBITAL FROM VEHICLE

Treatment	Dose mg/kg	Mean % Drug Lever Responses (SEM)	% Rats Selecting Drug Lever	*n/N*
		Phenazepam–Trained rats		
Vehicle	—	7.2 (1.8)	0	12/12
Phenazepam	2	87.2 (4.3)	100	12/12
Pretreatment	5	78.1 (10.3)	80	10/12
Flumazenil	15	3.8 (1.8)	0	12/12
Pretreatment				
Bicuculline	1	45.8 (14.0)	20	10/12
Pretreatment				
Picrotixin	2	75.7 (10.3)	67	8/12
Calcium				
Valproate	200	26.6 (8.0)	0	12/12
		Gidazepam–Trained rats		
Vehicle	—	6.2 (0.9)	0	6/6
Gidazepam	10	94.0 (1.9)	100	6/6
Pretreatment	5	73.6 (6.6)	80	6/6
Flumazenil	15	17.1 (5.2)	0	6/6
Pretreatment				
Bicuculline	1	86.0 (5.0)	20	6/6
Pretreatment				
Picrotixin	2	77.6 (10.0)	67	5/6
Calcium				
Valproate	200	60.7 (5.2)	0	6/6
		Pentobarbital–Trained rats		
Vehicle	—	4.8 (1.3)	0	12/12
Pentobarb.	10	86.2 (2.5)	100	12/12
Pretreatment	15	78.1 (10.3)	78	9/12
Flumazenil				
Pretreatment				
Bicuculline	1	80.3 (3.2)	60	11/12
Pretreatment				
Picrotixin	2	57.6 (6.8)	20	9/12

n/N indicates the number of rats completing at least 10 responses on a single lever vs. the number of rats tested. Test results obtained in test sessions on one lever or 3 min without reinforcement in both cases.

plex between phenazepam and gidazepam as well as between the benzodiazepines tested and pentobarbiral. Stimulus effects of phenazepam and gidazepam weakly depended on the functional activities of the picrotoxin-binding site of the $GABA_A$ complex. This finding is consistent with the data showing that picrotoxin in subconvulsive dosages failed to block the discriminative effects of diazepam (19). The discriminative cues of pentobarbital was significantly antagonized by picrotoxin. There is evidence that pentobarbital's discriminative effects were inhibited by bemegride, which was similar to picrotoxin in its pharmacological properties (10,11,13,14).

The fact that flumazenil dose dependently and completely antagonized the phenazepam- and gidazepam-discriminative cues suggested that discriminative effects of these compounds were mainly mediated via the BZ binding site of the $GABA_A$–benzodiazepine receptor complex. Similar results were reported in other studies on the discriminative stimulus properties of benzodiazepines (2,4,12,16,20,21). However, bicuculline, at 1 mg/kg, partially antagonized the stimulus properties of phenazepam but did not effect the gidazepam discrimination. Bicuculline has previously been reported not to block the discriminative properties of diazepam, and diazepam did not generalize to the selective $GABA_A$ receptor agonists muscimol or THIP (8).

Molecular biological studies have shown that receptor binding profiles of gidazepam is distinct from that of phenazepam and diazepam in values of the Hill's constant, which was <1 for phenazepam and diazepam and >1 for gidazepam (18). Gidazepam may have an additional site for membrane interactions that is distinct from the benzodiazepine binding site, but which results in functional modulation of the benzodiazepine receptor (18). The stimulus complex of phenazepam and gidazepam seems to be mediated via different $GABA_A$–benzodiazepine receptor-type subunits.

Taken together, the present results suggest that the discriminative stimuli of phenazepam and gidazepam are similar to those of other benzodiazepine agonists. However, the phenazepam cue is more selective than that of gidazepam. These data correspond to the differences in receptor mechanisms and clinical profiles of these drugs.

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