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Discriminative Stimulus Effects of Presynaptic GABA Agonists in Pentobarbital-Trained Rats

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GRECH, D. M. AND R. L. BALSTER. *Discriminative stimulus effects of presynaptic GABA agonists in pentobarbital-trained rats.* PHARMACOL BIOCHEM BEHAV 47(1) 5–11, 1994.—The discriminative stimulus effects of indirect-acting GABAergic drugs were compared to those of pentobarbital (PB) and midazolam in rats trained to discriminate 5 mg/kg PB from saline under a two-lever fixed-ratio 32 schedule of food reinforcement. PB and midazolam produced dose-dependent substitution for the training dose of PB with response rate reduction only at doses above those producing full substitution. Valproic acid, an antiepileptic drug and GABA transaminase inhibitor, substituted for PB but only at a dose that produced response rate suppression. Vigabatrin, an irreversible GABA transaminase inhibitor, failed to substitute for PB, but did produce a dose-dependent decrease in response rates. The GABA uptake inhibitors, 1-[2-[bis[4-(trifluoromethyl)phenyl]-methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (CI-966) and (*R*(−)-*N*-[4,4-bis(3-methylthien-2-yl)but-3-enyl] nipecotic acid HCl (tiagabine), produced no greater than 40% PB-lever responding. Aminooxyacetic acid (AOAA), which is described as a nonselective presynaptic GABA agonist, yielded a maximum of 43% PB-lever responding. These results indicate that the discriminative stimulus effects of the indirect $GABA_A$ agonists, PB and midazolam, although similar to one another, differ from those of presynaptic GABAergic drugs. Differences in the discriminative stimulus properties of GABA transaminase inhibitors and uptake inhibitors also exist, indicating that not all presynaptic GABA agonists have similar behavioral profiles. These results contribute to a further understanding of the similarities and differences in the behavioral effects of drugs that enhance GABAergic neurotransmission.

Drug discrimination Pentobarbital GABA AOAA Valproic acid Vigabatrin CI-966 Tiagabine Midazolam

GABA is the major inhibitory transmitter in the mammalian CNS. Modulation of GABAergic activity can influence CNS events in a number of ways. A reduction in GABA availability has been implicated in a variety of psychiatric and neurological diseases (30,40) including epilepsy (38) and Huntington's disease. Compounds capable of enhancing GABAergic inhibition via the $GABA_A$ receptor have therapeutic uses as anxiolytics and anticonvulsants (8,12,31). There are a variety of mechanisms by which drugs can enhance GABAergic function. The most extensively studied $GABA_A$ agonists are the benzodiazepines and barbiturates, which function as allosteric modulators of GABA-mediated postsynaptic inhibition. In addition, a variety of compounds exist that act directly or indirectly via the GABA recognition site. These drugs include direct $GABA_A$ agonists such as muscimol and THIP, GABA uptake inhibitors, and GABA transaminase inhibitors. In this study, GABA uptake inhibitors and GABA transaminase inhibitors were studied in rats trained to discriminate pentobarbital (PB) from saline. This study was undertaken to provide a systematic investigation of the barbiturate-like effects of different classes of GABA agonists. Dose–effect curves for PB and midazolam were obtained for purposes of comparison.

1-[2-[bis[4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (CI-966) and (*R*(−)-*N*-[4,4-bis(3-methylthien-2-yl)but-3-enyl] nipecotic acid HCl (tiagabine) were selected as representative GABA uptake inhibitors for this study. Both compounds prevent glial and presynaptic neuronal GABA uptake, thereby increasing extracellular GABA levels (40). These drugs have anticonvulsant effects in various animals models (30,50). CI-966 functions by selectively inhibiting GABA reuptake in neurons and glial cells, having no influence on $GABA_A$ or benzodiazepine receptor binding (18) or that of other neurotransmitters such as

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dopamine, norepinephrine, or serotonin (9). In mice, CI-966 potently prevents tonic seizures resulting from low-intensity electroshock and abolishes pentylenetetrazol (PTZ)-induced clonic seizures. CI-966 also interferes with general behavior, producing ataxia, tremors, myoclonus, decreased responsiveness to sensory stimulation, and, at high doses, sedation. Tiagabine, another GABA uptake inhibitor, blocks glial and neuronal uptake with relatively equal affinity, but fails to bind to central GABA receptors and peripheral $GABA_A$/benzodiazepine sites (7). Tiagabine potently inhibits [^{3}H]GABA uptake into rat synaptosomes (IC_{50} = 76 nM), cultured neurons (IC_{50} = 446 nM), and glia (IC_{50} = 182 nM), and may be the most selective GABA uptake inhibitor presently available (33).

Reduction of GABA degradation to semisuccinylaldehyde by inhibition of the enzyme GABA transaminase can lead to a significant increase in GABA concentrations in both neurons and glia (32). Inhibitors of GABA transaminase display sedative, antinociceptive, anticonvulsant, and anxiolytic properties (19,35). Two GABA transaminase inhibitors, vigabatrin and the classic antiseizure drug valproic acid, were selected for testing. Vigabatrin is a systemically active, irreversible GABA transaminase inhibitor that produces sustained increases in nerve terminal and glial GABA levels (24,25,29), with possibly some selectivity for neuronal GABA transaminase (28). Valproic acid was selected for study because it is believed to function as a reversible enzyme inhibitor. Although valproic acid's mechanism of action is unclear (10), administration leads to an increase in nerve terminal GABA levels by exerting an inhibitory effect on the GABA transaminase enzyme (15). One possible mechanism of action may be inhibition of the enzyme semisuccinylaldehyde dehydrogenase (44), resulting in blockade of GABA transamination.

Aminooxyacetic acid (AOAA) was also selected as a test compound. AOAA was first described in the early 1960s and may possibly function as a GABA uptake inhibitor (26). Some studies suggest that AOAA is pharmacologically nonselective, functioning to inhibit both GABA uptake and transaminase.

Drug discrimination procedures using rodents, pigeons, and primates have been useful in studying the central effects of GABAergic drugs [see (37,49) for reviews]; however, most research has been with the barbiturates and benzodiazepines (5,11,23). A few recent reports have described the discriminative stimulus properties of the direct $GABA_A$ agonists muscimol and THIP (1,17). Results of this research provide evidence for a complex profile of commonalities and differences in the discriminative stimulus properties of direct- and indirect-acting $GABA_A$ agonists. Cross generalization usually occurs among the barbiturates and benzodiazepines (4,11, 22,36), although there are some exceptions to this finding (2,3). The direct $GABA_A$ agonists, muscimol and THIP, produce only partial substitution in PB-trained rats (17). In addition, preliminary studies indicate that, at best, partial substitution (40–70%) occurs with midazolam and PB in muscimol- (Grech and Balster, unpublished observations) and THIP-trained rats (1).

Relatively little is known regarding the discriminative stimulus effects of presynaptic GABAergic drugs such as the GABA uptake and GABA transaminase inhibitors. The GABA transaminase inhibitor, valproic acid, the uptake inhibitor, tiagabine, and AOAA were evaluated in benzodiazepine-trained animals (20,33,36). In CGS 9896-trained mice, tiagabine and valproic acid both partially substituted for CGS 9896 (33). When tested in midazolam-trained rats (36), valproic acid produced partial substitution for midazolam. In another study, AOAA failed to substitute for diazepam in diazepam-trained rats (20). No drug discrimination studies have been reported to date with vigabatrin. Thus, it is beginning to be apparent that a different profile of discriminative stimulus effects are produced by direct $GABA_A$ receptor agonists and presynaptic GABA agonists, when they are compared with the postsynaptic modulators of GABA-mediated inhibition; however, additional systematic studies are needed to confirm this.

METHOD

Subjects

Eight male Sprague–Dawley rats (COBS CD) were obtained from Charles River Farms (Wilmington, MA). Subjects weighed approximately 310–370 g and were individually housed in wire mesh cages with water available ad lib. Animals were maintained on a 12 L : 12 D cycle. All training and testing procedures occurred during the light phase. Upon completion of training and testing sessions, rats were fed approximately 10–15 g (Agway Rodent Chow) and returned to their home cages.

Apparatus

Six two-lever, sound- and light-attenuated operant chambers were used in this study (Coulbourn Instruments, Lehigh Valley, PA). Each chamber was illuminated during sessions by a white houselight. Lever pressing was reinforced with 45-mg food pellets (Dustless Precision Pellets, P.J. Noyes, Lancaster, NH) delivered to a food trough located between the left and right response levers.

Training Procedure

Rats were shaped to lever press for food reinforcement during daily (Monday–Friday) training sessions of 30-min duration. Initially, each response on either lever was reinforced. After responding occurred reliably on both the left and right levers (three to five sessions), discrimination training began. One lever was designated the PB lever and the other the saline lever. On days when 5 mg/kg PB was administered, only responding on the PB lever was reinforced. On days when saline was administered, only responding on the saline lever was reinforced. The response requirement was incremented gradually and independently for each lever to a fixed-ratio value of 32 (FR 32). Responses on the incorrect lever reset the ratio value on the correct lever. Rats received PB or saline injections IP according to a double-alternation schedule (i.e., SAL, SAL, PB, PB, SAL, etc.). Following all injections, rats were returned to their home cages and 15 min later placed in the operant chambers.

Acquisition Testing

Testing the acquisition of the PB/saline discrimination was completed using a series of four 2-min test probes. After reliable responding under the FR 32 schedule of food reinforcement on both the PB and saline lever had been established, test probes were conducted on Tuesdays and Fridays. During the 2-min probes, responding on either the PB or saline lever was reinforced. Following these 2-min probes, responding on only the correct lever was reinforced for the remaining 28 min of the session. Test probes were conducted in a sequence of SAL, PB, SAL, PB, etc., providing individual animals met the following criteria the day before testing: completion of the first FR on the correct lever, greater than 85% correct-lever

responding, and response rates greater than 1.0/s. If subjects failed to meet these criteria, they were provided continued training until qualified. Each subject was required to successfully complete four consecutive probe tests, two saline and two PB, before substitution testing began.

Substitution Testing

Test sessions were 30 min in duration and occurred on Tuesdays and Fridays if the animal had met the criteria listed above throughout the 30-min session on the preceding day. During the test sessions, responding on either lever was reinforced under the FR 32 schedule; responding on one lever reset the FR requirement on the opposite lever.

The following drugs were evaluated: sodium pentobarbital (1–20 mg/kg), valproic acid (10–300 mg/kg), AOAA (0.3–30 mg/kg), CI-966 (0.3–30 mg/kg), tiagabine (3–17.3 mg/kg), vigabatrin (100–1,000 mg/kg), and midazolam (0.1–3 mg/kg). Prior to the assessment of each dose-effect curve, control test sessions with PB (5 mg/kg) and saline were conducted. Midazolam and PB were administered 5 and 15 min, respectively, prior to the start of the test session. Valproic acid, AOAA, CI-966, and tiagabine were given 30 min prior to testing whereas vigabatrin was given 1 h before the test session. All drugs were given IP in a volume of 1 ml/kg except for some vigabatrin doses (300–1,000 mg/kg) that required volume adjustments.

Drugs

Sodium pentobarbital (Henry Schein, Inc., Port Washington, NY) midazolam HCl (Versed, Hoffman-LaRoche, Nutley, NJ), AOAA (Sigma Chemical Co., St. Louis, MO), and vigabatrin (gamma vinyl GABA) (Marion Merrell Dow, Cincinnati, OH) were dissolved in physiological saline. Valproic acid (Sigma) was suspended in a 1% Tween-80/sterile water vehicle. CI-966 (Parke-Davis/Warner Lambert, Ann Arbor, MI) was dissolved in a 10% emulphor (GAF Corp., Wayne, NJ)/sterile water vehicle. Tiagabine (NOVO Nordisk A/S, Denmark) was dissolved in sterile water. Doses refer to the drug forms listed above.

Analysis of Data

For each 30-min test session, response rate and percentage of PB-lever responding were calculated. When the response rate was less than 0.05 responses per second, the lever-selection data for that subject for that dose was not included in the group analysis. Group data for the subjects were presented as the mean + SEM.

RESULTS

Acquisition and Control Tests

Acquisition of the PB/saline discrimination required an average of 58 training sessions (range of 38–77 sessions). Stimulus control by PB and saline injections remained stable throughout the study as indicated by the results of repeated control tests with 5 mg/kg PB and saline, which produced greater than 85% PB-lever responding and less than 10% saline-lever responding, respectively (Figs. 1–4). Response rates after PB and saline were similar, averaging 3.08 and 2.57 responses/s for PB and saline, respectively.

Pentobarbital and Midazolam

Tests with various doses of PB (1–20 mg/kg) produced dose-dependent full substitution for the training dose (5 mg/kg) (Fig. 1). The lowest dose tested, 1 mg/kg PB, failed to produce PB-lever responding, whereas an intermediate dose, 3 mg/kg, resulted in 71% PB-lever responding. Doses of 5 and 10 mg/kg fully substituted for PB, with response rate-decreasing effects at 10 mg/kg. No rate-decreasing effects occurred at PB doses of 5 mg/kg and below, but the 20-mg/kg dose eliminated responding in most subjects. Although the 20-mg/kg dose fully substituted in three rats, the data failed to represent a majority of animals tested and was not included in the dose-effect curve for PB discriminative stimulus effects (Fig. 1, upper panel).

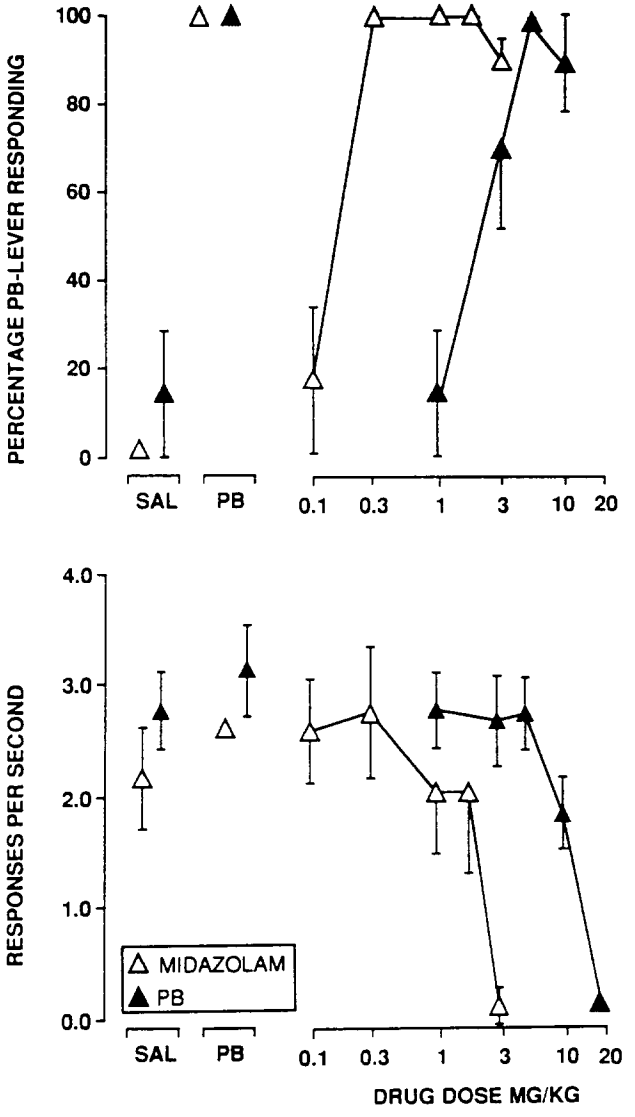

FIG. 1. Dose-response curves for midazolam (△) and pentobarbital (PB) (▲) in rats trained to discriminate 5 mg/kg PB from saline (SAL). Points above SAL and PB represent results of control tests with saline and 5 mg/kg PB before testing midazolam (△) and PB (▲). Percentage PB-lever responding (mean ± SEM) is shown in the upper panel; response rates (mean ± SEM) are shown in the lower panel (n = 8).

Midazolam produced results similar to those of PB (Fig. 1). A dose of 0.1 mg/kg did not substitute for PB, nor did it

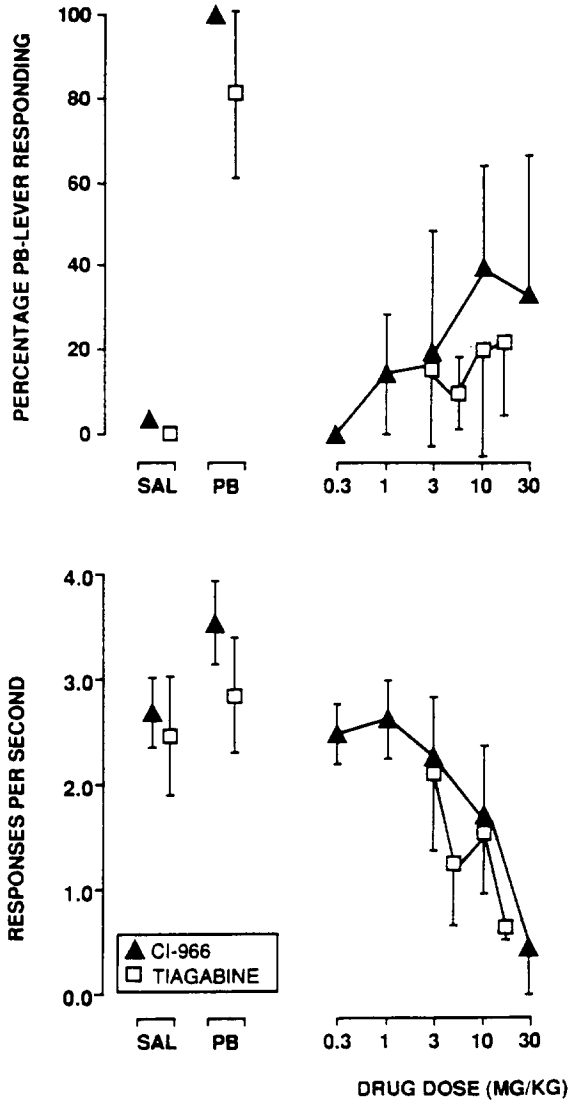

FIG. 2. Dose-response curves for 1-[2-[bis[4-(trifluoromethyl)phenyl]-methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (CI-966) (▲) and (*R*(−)-*N*-[4,4-bis(3-methylthien-2-yl)but-3-enyl] nipecotic acid HCl (tiagabine) (□) in rats trained to discriminate 5 mg/kg pentobarbital (PB) from saline (SAL). Points above SAL and PB represent results of control tests with saline and 5 mg/kg PB before testing with CI-966 (▲) and tiagabine (□). Percentage PB-lever responding (mean ± SEM) is shown in the upper panel; response rates (mean ± SEM) are shown in the lower panel (n = 6).

produce decreases in rates of responding. Doses of 0.3 to 3 mg/kg substituted fully (>89% PB-lever responding) for PB, with response rate decreases relative to the response rate on the corresponding saline control tests only at the 3-mg/kg dose.

GABA Uptake Inhibitors

Tiagabine failed to produce PB-like effects and CI-966 produced, at best, partial substitution for PB (Fig. 2). Maximal levels of approximately 40% PB-lever responding occurred at 10 mg/kg CI-966 and 20% PB-lever responding at 17.3 mg/kg tiagabine. The intermediate level of responding produced by CI-966 reflects two or three animals responding primarily on the PB-associated lever and the remainder responding on the saline lever. There was not an evident pattern in the lever selection of individual subjects (i.e., full substitution with both drugs did not occur in a subset of animals). Dose-dependent decreases in rates of responding occurred following both CI-966 and tiagabine administration.

GABA Transaminase Inhibitors

Valproic acid and vigabatrin produced distinctly different dose-response curves (Fig. 3). Valproic acid dose dependently substituted for PB. Low to intermediate doses produced no

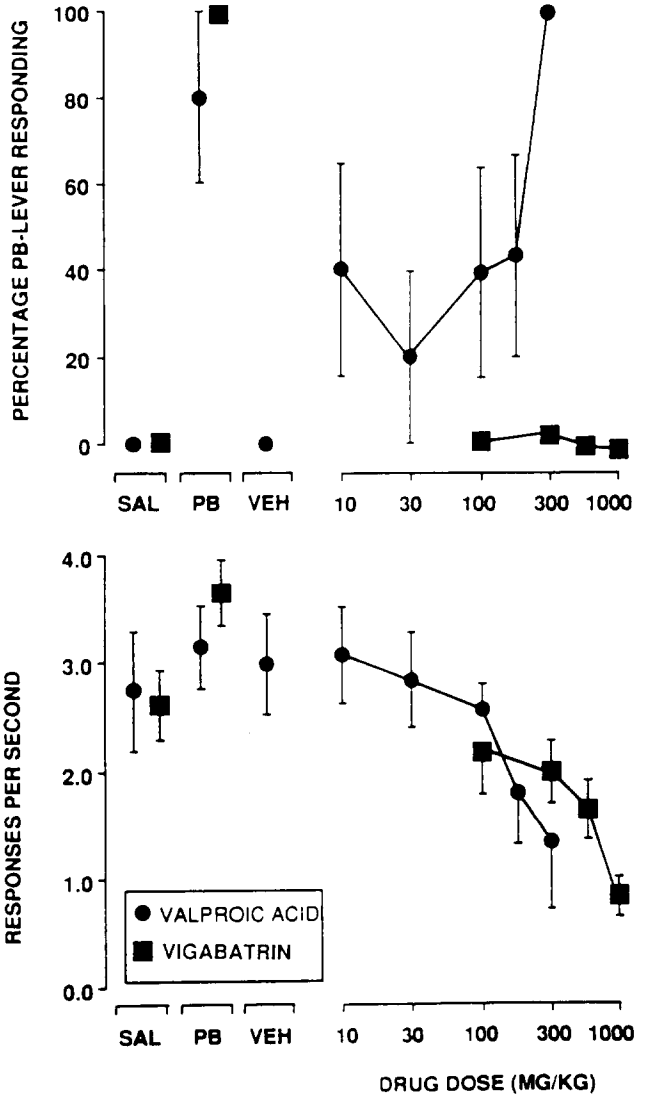

FIG. 3. Dose-effect curves for valproic acid (●) and vigabatrin (■) in rats trained to discriminate 5 mg/kg pentobarbital (PB) from saline (SAL). The points above SAL and PB represent results of control tests with saline and PB before testing with valproic acid (●) and vigabatrin (■). Percentage of PB-lever responding (mean ± SEM) is shown in the upper panel; response rates (mean ± SEM) are shown in the lower panel (n = 5 or 6).

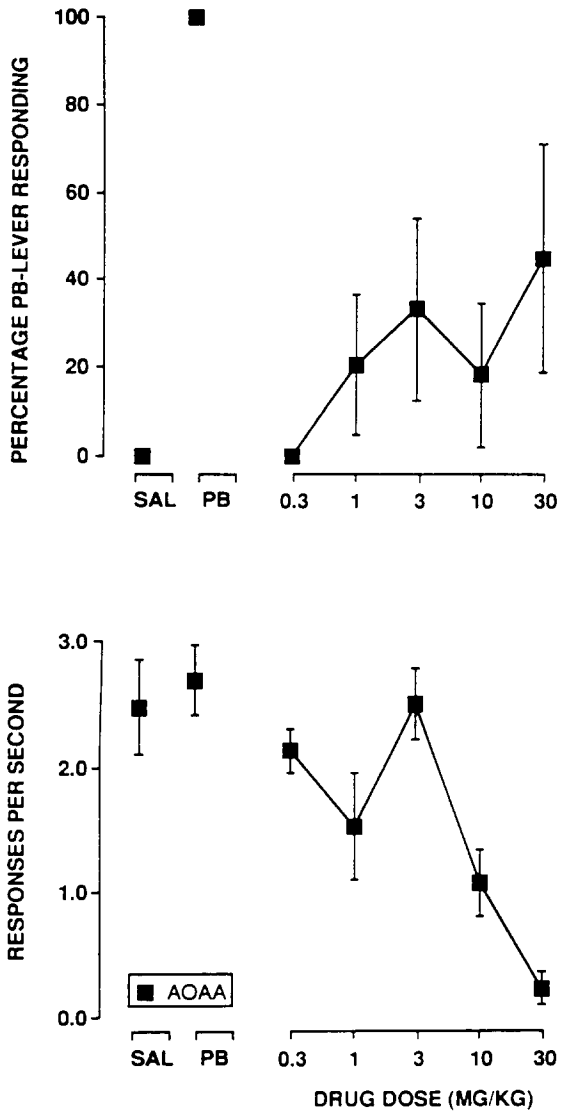

FIG. 4. Dose-response curves for aminooxyacetic acid (AOAA) in rats trained to discriminate 5 mg/kg pentobarbital (PB) from saline (SAL). Points above SAL and PB represent results of control tests with saline and PB prior to testing AOAA. Percentage PB-lever responding (mean ± SEM) is shown in the upper panel; response rates (mean ± SEM) are shown in the lower panel (n = 5).

greater than 43% PB-lever responding and the highest dose tested, 300 mg/kg, produced full substitution in all subjects that responded at this dose. The 300-mg/kg dose of valproic acid also decreased rates of responding by approximately 50% of control values. Vigabatrin, on the other hand, failed to produce PB-lever responding at all doses tested (100–1,000 mg/kg) in all subjects. Vigabatrin did produce dose-dependent decreases in rates of responding, with the 1,000-mg/kg dose suppressing rates by greater than 65% of the corresponding saline control rates.

AOAA

Figure 4 shows the dose-response curve for AOAA. Low doses of AOAA (0.3–1.0 mg/kg) produced primarily saline-lever responding, with mean values of less than 20% PB-lever responding. A dose of 3 mg/kg, although not influencing response rates, produced 38% PB-lever responding. The 30-mg/kg dose yielded a mean of 45% PB-lever responding, representative of two subjects responding on the saline lever and two on the drug-associated lever. Data for an additional two subjects were not included in the overall percentage of PB-lever responding due to the low response rates (<0.05 responses/s). In general, AOAA produced a dose-dependent decrease in response rates, with the dose producing maximal PB-lever responding (30 mg/kg) also substantially lowering response rates.

DISCUSSION

The discriminative stimulus effects of presynaptic GABA agonists are dissimilar to those of PB or midazolam. In PB-trained rats, PB and midazolam dose dependently generalized fully from PB. Administration of the presynaptic GABA agonists, CI-966 and AOAA, at best produced intermediate levels of PB-lever responding. Tiagabine and vigabatrin completely failed to substitute for PB. The GABA transaminase inhibitor, valproic acid, substituted for PB but only in association with marked rate-decreasing effects.

Our results with PB and midazolam are consistent with a number of reports showing cross generalization between the barbiturates and benzodiazepines (4,11,13,22,36). Because both barbiturates and benzodiazepines act postsynaptically to enhance GABAergic neurotransmission, cross generalization between these classes can be taken as evidence that GABAergic mechanisms are involved in the transduction of their discriminative stimulus effects. On the other hand, there are some conditions where benzodiazepines and barbiturates do not completely substitute for one another, or for ethanol (1,2,41,42). This result suggests that other neurotransmitter systems may be involved or that the specific cellular mechanisms required for GABAergic enhancement are responsible for determining the discriminative stimulus effects of GABA agonists. The results in PB-trained rats show that not all classes of GABA agonists produce similar discriminative stimulus effects. Although the partial substitution obtained with CI-966 and AOAA in this study, and with the direct $GABA_A$ agonists muscimol and THIP as reported earlier (17), could be used to further support a role for GABA in mediating PB discrimination, it should be pointed out that similar or greater levels of substitution for PB are obtained with NMDA antagonists (45,46). Thus, it is clear that GABAergic activation is neither necessary nor sufficient for producing partial PB-like discriminative stimulus effects in rodents.

Although the GABA uptake inhibitors and GABA transaminase inhibitors have been shown to enhance GABAergic neurotransmission in vitro, the evidence that their in vivo effects results from these actions is less extensive. The evidence for a GABAergic basis to their pharmacological actions resides primarily in their anticonvulsant effects. Although the presynaptic agonists inhibit kindled seizures (21,39) and protect against PTZ-, strychnine-, picrotoxin-, bicuculline-, and electrically induced seizures (16,33,35,43,50), their profiles of effects in these models differ from those of barbiturates and benzodiazepines. Drug discrimination studies with indirect and presynaptic GABA agonists have failed to clarify whether enhancement of GABA neurotransmission alone underlies their behavioral effects.

There have been few other studies of the discriminative stimulus properties of GABA uptake inhibitors and GABA

transaminase inhibitors. AOAA failed to generalize from diazepam in diazepam-trained rats (20); however, it did potentiate the discriminative stimulus effects of phenobarbital and diazepam in rats (34). Tiagabine did not substitute for PTZ in PTZ-trained rats, nor did it antagonize the PTZ discriminative stimulus (33). This finding suggested that the subjective effects of tiagabine differ considerably from those of the benzodiazepines although both classes share common anticonvulsant effects. In CGS 9896-trained animals, tiagabine partially substituted for CGS 9896, suggesting inhibition of GABA uptake results in non-benzodiazepine-like effects similar to those of benzodiazepine partial agonists. NO-274, a racemate form of tiagabine, was evaluated in diazepam-trained mice, producing no greater than 50% drug-lever responding (33). CI-966 produced intermediate levels of PTZ-lever responding when administered to PTZ-trained rats; however, like tiagabine, CI-966 failed to antagonize the PTZ discriminative stimulus (14). These previous drug discrimination studies of the GABA uptake inhibitors reveal differences between their effects and those of the benzodiazepines and barbiturates.

Valproic acid produced dose-dependent increases in PB-lever responding, although it only substituted for PB at a dose that suppressed responding in all subjects. Valproic acid did not substitute for PB in PB-trained pigeons (22), and produced only 50% midazolam-lever responding in midazolam-trained rats (36). Valproic acid dose dependently antagonized the discriminative stimulus effects of PTZ (27); however, it was also able to produce intermediate levels of flumazenil-lever responding in flumazenil-trained rats (48). Substitution tests with valproic acid in rats trained to discriminate the NMDA competitive antagonist, NPC 12626, resulted in a lack of substitution (6). Although valproic acid differed from PB and midazolam in the present study in producing PB-lever responding only at doses that also decreased response rates, the greater substitution obtained with it, combined with other data showing antagonism of PTZ discrimination (27), suggests that it may have discriminative stimulus effects more similar to those of the barbiturates and benzodiazepines than do the GABA uptake inhibitors.

In PB-trained rats, the GABA uptake inhibitors CI-966 and tiagabine, the GABA transaminase inhibitor vigabatrin, and the nonspecific GABA agonist AOAA produced discriminative stimulus effects that differed from those of the postsynaptic GABA agonists PB and midazolam. Valproic acid fully substituted for PB, but differed considerably from the full substitution produced by PB and midazolam in its accompanying rate-decreasing effects. In addition to providing evidence for differences between the in vivo pharmacological effects of classes of $GABA_A$ agonists, these results support the further use of drug discrimination procedures to distinguish among these subclasses of GABAergic drugs.

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