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Potentiation of the Anticonflict Effects of Diazepam, but not Pentobarbital and Phenobarbital, by Aminooxyacetic Acid (AOAA)

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McCLOSKEY, T. C., J. F. BESHEARS, N. A. HALAS AND R. L. COMMISSARIS. *Potentiation of the anticonflict effects of diazepam, but not pentobarbital and phenobarbital, by aminooxyacetic acid (AOAA).* PHARMACOL BIOCHEM BEHAV 31(3) 693–698, 1988.—The Conditioned Suppression of Drinking (CSD) paradigm is an "animal model" for anxiety which has been used to study the anticonflict effects of the benzodiazepines. It has been postulated that benzodiazepines produce their effects through interactions with GABA. The present study examined this potential GABA-BZ interaction on CSD behavior. In daily 10-minute sessions, water-deprived rats were trained to drink from a tube which was occasionally electrified (0.5 mA), electrification being signalled by a tone. Within 2–3 weeks control CSD responding had stabilized (16–24 shocks session and 10–14 ml water/session); drug tests were conducted at weekly intervals. As expected, diazepam (0.3–20.0 mg/kg), pentobarbital (0.6–10.0 mg/kg) and phenobarbital (10.0–40.0 mg/kg) alone markedly increased the number of shocks received at doses which did not depress background responding (i.e., water intake). Treatment with the GABA-transaminase inhibitor aminooxyacetic acid (AOAA: 2.5–10.0 mg/kg, 10- or 60-minute pretreatment) alone had no anticonflict effect on CSD behavior. However, pretreatment (60-minute) with 10.0 mg/kg AOAA significantly potentiated the effects of diazepam, as indicated by a significant shift to the left in the diazepam dose-response curve relative to diazepam alone. By contrast, the anticonflict effects of pentobarbital and phenobarbital were unaffected by this AOAA pretreatment. Thus, while increases in GABA transmission alone do not appear to affect CSD behavior, the anticonflict effects of benzodiazepines, but not barbiturates, appear to be potentiated by increases in GABA transmission.

AOAA Diazepam Phenobarbital Pentobarbital Conflict behavior Anxiety
GABA/benzodiazepines GABA/barbiturates

SINCE the earliest reports that benzodiazepine (BZ) and gamma-amino-butyric acid (GABA) receptors are "linked," it has been postulated that the various benzodiazepine actions (anticonvulsant, sedative-hypnotic, muscle relaxant and antianxiety) are mediated via an allosteric GABA-BZ interaction, which ultimately results in an increase in chloride permeability (31). It has been further proposed that barbiturates (BBs) exert actions directly on the chloride ionophore within this GABA-BZ receptor complex. The increase in chloride permeability and the resultant membrane hyperpolarization have been suggested to account for many of the behavioral actions of benzodiazepines and barbiturates [(22,28); see also reviews by (23,31)].

Studies examining the significance of this GABA-BZ receptor interaction in "anxiety" have focussed primarily on conflict behaviors in the rat, where both benzodiazepines and barbiturates have been reported to exert very dramatic anticonflict effects (2, 3, 5, 8–10, 14–19, 21, 34–36).

Studies on the effects of GABAergic agents alone on conflict behavior have failed to strongly support a role for GABA in this behavior. For example, the systemic administration of a number of GABAergic agents [aminooxyacetic acid (AOAA), 4,5,6,7-tetra-hydroisoxazolo (5,4-6) pyridin-3-ol (THIP), muscimol] alone does *not* increase punished responding in conflict behaviors (6, 9, 24, 27, 33). It should be noted, however, that the GABA-T inhibitory sodium valproate has been reported to increase punished responding in conflict paradigms (16, 17, 24, 34).

The results of studies employing the coadministration of various GABAergic agents with benzodiazepines have sup-

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ported the GABA-BZ interaction hypothesis, with the administration of either GABA-T inhibitors or muscimol reported to potentiate the actions of benzodiazepines on conflict behavior (7, 11, 13). To date, the effects of AOAA pretreatment on the anticonflict effects of diazepam have not been examined.

It should also be noted that in no study has the possible *specificity* of the GABA-BZ interaction been explored with the use of a GABA-BB combination. Although benzodiazepines and barbiturates share many of the same pharmacological actions (including a marked increase in punished responding in conflict procedures), the barbiturates are presumed to exert their effects by direct actions at the chloride ionophore [i.e., "downstream" from GABA and/or benzodiazepine receptors; (12, 22, 23, 28, 31)]. Thus, if an alteration in a benzodiazepine response associated with the coadministration of a GABAergic agent is mediated at the benzodiazepine and/or GABA receptor (i.e., before the level of the chloride channel), a similar shift would not be expected with an GABA-BB combination.

The present studies were designed to examine the possible GABA-BZ and GABA-BB interactions on conflict behavior. To this end, the influence of AOAA pretreatment on the effects of benzodiazepines and barbiturates were determined in the Conditioned Suppression of Drinking (CSD) paradigm (3, 4, 8, 14, 20, 21), a modification of the Geller-Seifter conditioned conflict test (10) and the Vogel acute conflict test (36).

GENERAL METHOD

Animals

Naive female Sprague-Dawley rats (Charles River Farms, Inc., Cambridge, MA) weighing 200–225 grams at the start of the study were housed in groups of five in a climate-controlled room with 12 hour light:12 hour dark cycle (lights on 0700–1900 hours). Animals were given free access to food but restricted access to water. Water was obtained during daily experiment sessions, which are described below in the Procedure section. One group of rats (n=20) was used for the diazepam ± AOAA studies, while a second group (n=20) was used for the barbiturate ± AOAA studies.

Apparatus

Conditioned Suppression testing was conducted in an apparatus similar to that described by Commissaris *et al.* (4) and McCloskey *et al.* (20). Animals were tested in one of two identical experimental chambers. Each chamber was a rectangular box, 30×30×25 cm, consisting of Plexiglas® sides and a stainless steel floor and ceiling. Protruding from one wall was a metal drinking tube, to which a calibrated (±0.5 ml units) length of polyethylene tubing was attached for measuring the volume of water consumed. Programming for the test sessions was controlled by solid state modular programming equipment (Coulbourn Instruments, Co., Lehigh Valley, PA).

Procedure

For the first four sessions, water-restricted animals were placed individually into the Plexiglas® chamber and allowed to consume fluid freely without the shock contingency. The animals were removed after access to the drinking tube for 10 minutes. After one week of nonshock sessions, the tone/shock contingency was initiated. The duration of the tone was 7 seconds. The first two seconds of each tone were used as a warning signal and no shock was delivered. During the last five seconds of the tone, contact between the drinking tube and the floor resulted in completion of the circuit and the delivery of a 0.5 mA shock to the rat. Shocks were delivered by Coulbourn Instruments Shocker (Model No. E13-02). Alternating with the tone periods were 25-second silent periods in which no shock was delivered when there was contact with the drinking tube. The CSD test sessions lasted 10 minutes.

Initially, the shock inhibited all water consumption in the test chamber. After several days, however, animals learned to consume stable volumes of water during the silent periods and made relatively few and very brief contacts with the tube during the tone periods, receiving a consistent number of shocks from day to day.

CSD testing was conducted between 1400–1600 hours Tuesday through Friday. The subjects were allowed free access to water from Friday posttest until Monday a.m. Drug testing was started after three weeks of nondrug performance. Drug testing was conducted on Thursdays and Fridays of each week using a "cross-over" procedure similar to that described by Commissaris *et al.* (5). For determination of the effects of a single agent, the following procedure was employed: on the first day of each drug test (Thursday), half of the animals were administered the drug and the other half were administered the drug vehicle. The following day (Friday), the treatments were reversed. Thus, each animal served as its own control. The order of doses examined was randomized.

For determination of the effects of benzodiazepines or barbiturates following AOAA pretreatment, the following procedure was employed: subjects received AOAA pretreatment on both Thursday and Friday tests, while on the first day of each drug test, half of the animals were administered the drug and the other half were administered the drug vehicle. The following day the treatments were reversed. Thus, the "net effect" of the benzodiazepine or barbiturate could be determined in the presence of AOAA. The number of punished and unpunished licks and the volume of water consumed were recorded after each experimental session.

Drugs

Diazepam, prepared in a 0.5 percent methylcellulose suspension, was obtained from Hoffmann-La Roche Inc., Nutley, NJ. Pentobarbital, phenobarbital and aminooxyacetic acid, dissolved in 0.85 percent saline, were purchased from Sigma Chemical Co., St. Louis, MO. Dose-response data were obtained from each agent alone using a 10-minute pretreatment. AOAA was also tested alone with a 60-minute pretreatment. Dose-response data were obtained from the drug combinations after 60-minute pretreatment with AOAA and 10-minute pretreatment with diazepam, pentobarbital or phenobarbital (i.e., 50 minutes after AOAA administration). All drugs were injected intraperitoneally in a volume of 1.0 ml/kg.

Statistical Analyses

Paired *t*-tests were used to compare the effects of individual doses of the various drugs to their respective vehicle treatments. The effects of AOAA pretreatment on the diazepam, pentobarbital and phenobarbital dose-response curves were assessed by factorial ANOVA (Main Effects: ±AOAA, Drug Dose) with repeated measures (applied over the linear portion of the dose-response curves for the effects

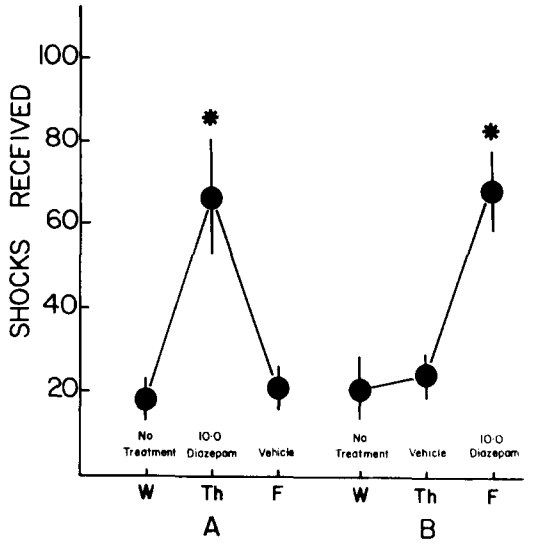

FIG. 1. The day effect of 10.0 mg/kg diazepam on shocks received in the CSD paradigm. Each symbol and vertical bar represents the mean±SEM shocks received obtained from 10 subjects. *$p<0.05$ relative to vehicle controls, t-tests for paired values.

of these agents on punished responding). Post hoc least significant differences (lsd) tests were used to identify individual doses which differed along the ± AOAA factor. In all statistical comparisons, $p<0.05$ was used as the criterion for statistical significance (29).

RESULTS

Experiment 1: Effects of Various Agents Administered Alone on CSD Behavior

Subjects in the present studies consumed an average of 11.2±0.6 (mean±S.E.) ml water per session and accepted an average of 20±3.9 shocks per session in the CSD paradigm. It should be noted that the number of tube contacts during the shock component (16–24 per session) was small when compared to the number of tube contacts during the unpunished component (2000–3000 per session).

Figure 1 illustrates the effects of 10.0 mg/kg diazepam as a function of Test Days within the cross-over design. In this figure are depicted the absolute number of shocks received on the no treatment day (Wednesday), the day 10.0 mg/kg diazepam was administered (Thursday for Group A and Friday for Group B) and the day the vehicle was administered (Friday for Group A and Thursday for Group B). It is clear that there is no difference in punished responding on the vehicle day and the no treatment day, while diazepam administration resulted in a dramatic increase in punished responding. It is also noteworthy that following the administration of a very efficacious dose of diazepam to the subjects in Group A on Thursday, no residual or "carry-over" effect was detected when these subjects were tested on Friday following vehicle injection.

Table 1 illustrates the dose-dependent anticonflict effects of diazepam, pentobarbital and phenobarbital on CSD behavior. Administration of each of these agents alone resulted in a significant dose-dependent increase in shocks received which was accompanied by a significant but not necessarily dose-dependent increase in water intake.

TABLE 1

EFFECTS OF DIAZEPAM, PENTOBARBITAL AND PHENOBARBITAL ON PERFORMANCE IN THE CSD PROCEDURE

Treatment (mg/kg)	Change in Shocks Received	Change in Water Intake
Diazepam		
0.3	7.5 ± 4.1[a]	0.0 ± 0.4[b]
0.6	9.1 ± 2.8*	1.4 ± 0.3*
1.25	15.9 ± 3.8*	1.2 ± 0.4*
2.5	19.9 ± 3.2*	2.2 ± 0.5*
5.0	31.8 ± 5.8*	2.2 ± 0.6*
10.0	44.9 ± 7.2*	2.3 ± 0.7*
20.0	45.6 ± 11.5*	−2.1 ± 0.6*
Pentobarbital		
0.6	4.4 ± 3.0	0.7 ± 0.3*
1.25	12.3 ± 3.8*	2.1 ± 0.5*
2.5	15.9 ± 4.1*	2.1 ± 0.4*
5.0	41.2 ± 5.1*	3.2 ± 0.7*
10.0	59.3 ± 6.5*	1.9 ± 1.6*
Phenobarbital		
10.0	13.3 ± 2.9*	1.6 ± 0.3*
20.0	27.0 ± 2.9*	2.5 ± 0.4*
40.0	50.8 ± 6.1*	1.6 ± 0.4*

[a]Values represent the mean ± SEM (n=20) change in shocks received (Drug − Vehicle) during the punished periods.

[b]Values represent the mean ± SEM (n=20) change in water intake (in ml).

*$p<0.05$ relative to vehicle control, t-test for paired values.

Figure 2 illustrates the effects of aminooxyacetic acid (AOAA) administration of CSD behavior. AOAA has been shown to increase GABA in the brain by inhibiting the enzyme GABA-transaminase (GABA-T) (1,26). At no dose studied (2.5–10.0 mg/kg IP) did AOAA, administered either 10 minutes (open circles) or 60 minutes (closed circles) prior to testing, result in a statistically significant increase in shocks received (punished responding). In addition to its lack of anticonflict effect, a significant decrease in water intake (unpunished responding) was observed following administration of the highest dose (10.0 mg/kg) of AOAA (both 10-min and 60-min pretreatments).

Experiment 2: Effects of AOAA Pretreatment on the Anticonflict Actions of Benzodiazepines and Barbiturates

The effects of diazepam on punished responding alone or following pretreatment with 10.0 mg/kg AOAA are illustrated in the upper panel of Fig. 3. Factorial (2×5) ANOVA with repeated measures applied to the linear portion (0.3–5 mg/kg) of the dose-response curve (Main Effects: ±AOAA, Diazepam Dose) revealed a significant Main Effect for Diazepam Dose on the change in shocks received, $F(4,75)=15.59$, $p<0.05$. More importantly, AOAA pretreatment significantly potentiated the anticonflict effects of diazepam, $F(1,19)=5.35$, $p<0.05$; there was no interaction between Diazepam Dose and ±AOAA, $F(4,73)=1.81$, n.s.

The effects of diazepam on unpunished responding (water intake) alone and following pretreatment with 10.0 mg/kg AOAA are illustrated in the lower panel of Fig. 3. Factorial

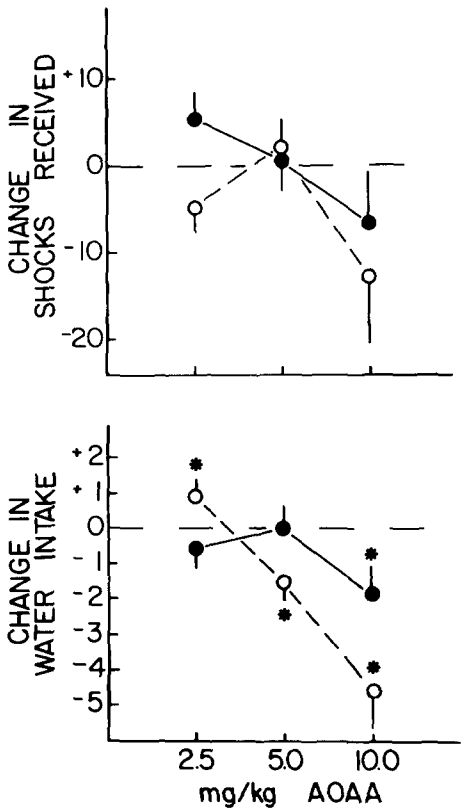

FIG. 2. The effects of AOAA on punished and unpunished behavior in the CSD paradigm. Upper panel: The change in the number of shocks received following administration of AOAA after pretreatment times of ten (open circles) and sixty (closed circles) minutes. Lower panel: The change in water intake (unpunished responding) following administration of AOAA after pretreatment times of ten (open circles) and sixty minutes (closed circles). Each symbol and vertical bar represents the mean±SEM change in shocks received (drug − vehicle) obtained from 20 subjects. *$p<0.05$ relative to vehicle controls, t-test for paired values.

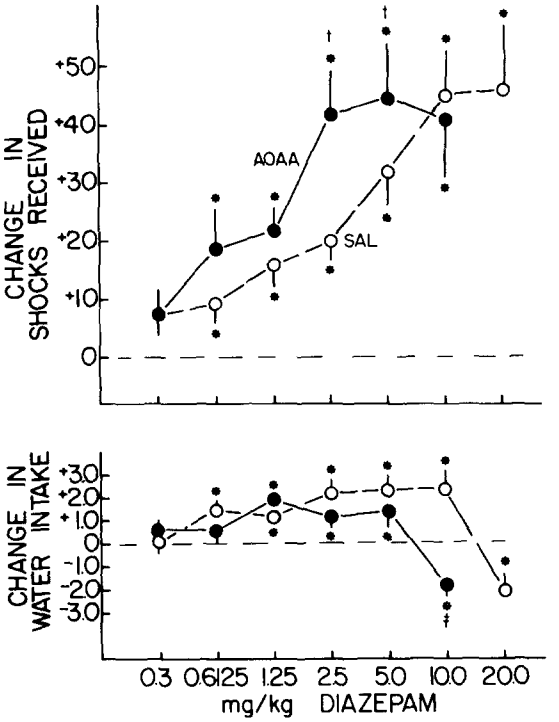

FIG. 3. The effects of AOAA pretreatment on the effects of diazepam in the CSD paradigm. Plotted are the data obtained following administration of diazepam + AOAA vehicle (open circles) and diazepam + 10.0 mg/kg AOAA (filled circles). See Fig. 2 legend for details. *$p<0.05$ relative to vehicle controls, t-test for paired values. †$p<0.05$ diazepam alone significantly different from diazepam + AOAA at that dose, lsd test. ‡$p<0.05$ diazepam alone significantly different from diazepam + AOAA at that dose, t-test for paired values.

ANOVA (0.3–5 mg/kg doses) with repeated measures revealed a small, yet significant Main Effect for Diazepam Dose, $F(4,76)=3.17$, $p<0.05$. There was no Main Effect for ±AOAA, $F(1,19)=2.04$, n.s., nor was there an interaction between Diazepam Dose and ± AOAA, $F(4,73)=2.19$, n.s. Although no effect of AOAA was detected with the factorial ANOVA applied to the 0.3–5 mg/kg dose range, AOAA pretreatment did indeed potentiate the effects of diazepam on water intake, as evidenced by a significant difference between the 10 mg/kg diazepam dose alone and 10 mg/kg diazepam + AOAA on this measure, $t(19)=4.56$, $p<0.05$.

The effects of the combinations of pentobarbital ±10.0 mg/kg AOAA and phenobarbital ±10.0 mg/kg AOAA on punished responding in the CSD are illustrated in the upper panel of Fig. 4. With each barbiturate there was a significant Main Effect for Drug Dose: pentobarbital, $F(4,76)=51.10$, $p<0.05$, and phenobarbital, $F(2,38)=29.53$, $p<0.05$. In contrast to the diazepam-AOAA combination (Fig. 3), there was no Main Effect for ±AOAA with respect to either pentobarbital, $F(1,19)<1.0$, n.s., or phenobarbital, $F(1,19)<1.0$, n.s. Also, in neither case was there an interaction between Drug Dose and ±AOAA: pentobarbital, $F(4,76)=1.09$, n.s., and phenobarbital, $F(2,38)=1.22$, n.s.

The effects of the AOAA-BB combination on water intake in the CSD are indicated in the lower panel of Fig. 4. There was a significant Main Effect for Pentobarbital Dose on Water intake, $F(4,76)=3.64$, $p<0.05$; the Main Effect of Phenobarbital Dose on water intake was not significant, $F(2,38)=2.25$, n.s. There was no Main Effect for ±AOAA on water intake with either barbiturate: pentobarbital, $F(1,19)<1.0$, n.s., and phenobarbital, $F(1,19)<1.0$, n.s. Finally, there was no significant interaction between Drug Dose and ±AOAA with either barbiturate; pentobarbital, $F(4,76)<1.0$, n.s., and phenobarbital, $F(2,38)<1.0$, n.s.

DISCUSSION

There was clearly *no* anticonflict effect produced by administration of AOAA alone. Since AOAA has been shown to increase GABA at these doses and pretreatment times (1,26), it would appear that increasing GABA concentrations alone does not increase punished responding in the CSD paradigm. This finding is consistent with a number of previous findings using AOAA in conflict procedures (6, 9, 24, 27, 33).

The effects of benzodiazepines and barbiturates in conflict procedures are well documented in the literature (2, 3, 5, 8–10, 14–19, 21, 34–36). The data in the present study com-

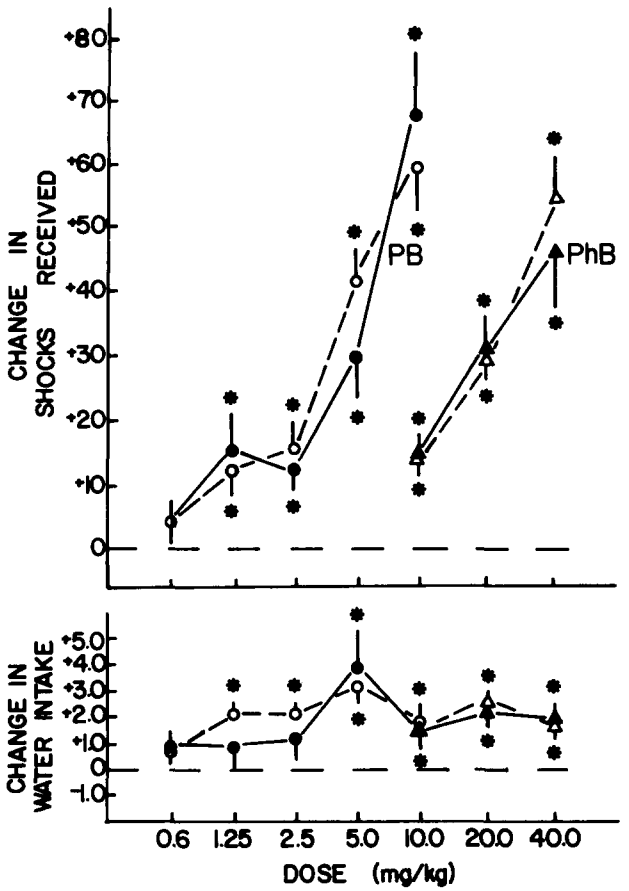

FIG. 4. The effects of AOAA pretreatment on the effects of pentobarbital and phenobarbital in the CSD paradigm. Plotted are the effects of pentobarbital + AOAA vehicle (open circles), pentobarbital + 10.0 mg/kg AOAA (filled circles), phenobarbital + AOAA vehicle (open triangles) and phenobarbital + 10.0 mg/kg AOAA (filled triangles). See Fig. 2 legend for details. $*p<0.05$ relative to vehicle controls, t-test for paired values.

plement the data from previous studies in that it also shows the classic dose-dependent increase in shocks received. It should be noted that a purely "dypsogenic" agent would increase the number of shocks received only by 10–20% of control; as in the present study, efficacious anticonflict agents such as benzodiazepines and barbiturates increase the number of shocks received by 300–500% of control (3, 5, 14, 20).

In contrast to the lack of anticonflict effects of AOAA when administered alone, there was a statistically significant *potentiation* of the effects of diazepam on punished CSD behavior when diazepam was coadministered with 10.0 mg/kg AOAA. This is the first report demonstrating potentiation of the anticonflict effects of diazepam following AOAA pretreatment, and is consistent with the findings of other investigators studying GABA-BZ interactions on conflict behavior using other GABAergic agents (7, 11, 12). It should be noted, however, that other investigators have found that GABAergic agents show *no* potentiation of the benzodiazepines (25, 30, 32, 33). At this time, a clear explanation for these apparently contradictory findings cannot be given.

If the potentiation by AOAA observed in the present study is specific for an interaction at GABA and/or benzodiazepine receptors, then AOAA would not affect the anticonflict actions of the barbiturates. Alternatively, if AOAA potentiated the anticonflict effects of diazepam by actions at the level of the chloride ionophore, then AOAA would be expected to also potentiate the anticonflict actions of pentobarbital and phenobarbital. AOAA did not potentiate the anticonflict effects of either of the barbiturates, indicating that AOAA potentiates the anticonflict effects of diazepam through a mechanism at GABA and/or benzodiazepine receptor(s) (i.e., "upstream" from the chloride channel).

In summary, diazepam, pentobarbital and phenobarbital administered alone resulted in statistically significant, dose-dependent increases in punished responding. In contrast, administration of a GABA-T inhibitor, AOAA, alone clearly showed no anticonflict activity in the CSD procedure. Finally, AOAA pretreatment potentiated the anticonflict effects of diazepam, but not pentobarbital and phenobarbital, illustrating the specificity of the AOAA-induced potentiation for diazepam. Thus, while increases in GABA transmission alone do not appear to affect CSD behavior, the anticonflict effects of the benzodiazepines, but not the barbiturates, appear to be modulated by GABA. The selective role of GABA in modulating the anticonflict effects of the benzodiazepine, but not the barbiturates, is consistent with the proposed "series circuit" postulated for the actions of both benzodiazepines and barbiturates within the GABA-BZ receptor complex.

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