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Buspirone Effects in an Animal Conflict Procedure: Comparison With Diazepam and Phenobarbital

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McCLOSKEY, T. C., B. K. PAUL AND R. L. COMMISSARIS. *Buspirone effects in an animal conflict procedure: Comparison with diazepam and phenobarbital.* PHARMACOL BIOCHEM BEHAV 27(1) 171–175, 1987.—Buspirone has been introduced as a novel non-benzodiazepine anti-anxiety agent. The Conditioned Suppression of Drinking (CSD) paradigm is an "animal model" for anxiety which provides information on both the relative potency and relative efficacy of anti-conflict agents. The present study compared the anti-conflict effects of buspirone to those of more "classical" anti-anxiety agents, diazepam and phenobarbital. 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 (approximately 15–20 shocks/session and 10–15 ml water/session); drug tests were conducted at weekly intervals. Diazepam and phenobarbital markedly (400–500%) increased the number of shocks received at doses which did not depress background responding (i.e., water intake). A number of agents, most notably morphine and ethanol, did not reliably affect punished responding in the CSD. Administered IP, low doses (0.25–1 mg/kg) of buspirone increased punished responding only slightly (less than 100% increase); higher doses (2, 4 mg/kg) depressed background responding. Administered SC, buspirone (0.125–1.0 mg/kg) had more potent effects on both punished and unpunished responding; again, anti-conflict efficacy was only marginal. These results suggest that buspirone might be less effective than the benzodiazepines in the management of anxiety.

Buspirone Diazepam Phenobarbital Conflict behavior Anxiety

BENZODIAZEPINES are one of the most frequently prescribed agents in medicine today [13]. These agents have anti-convulsant, anti-anxiety and muscle relaxant actions as well as sedative/hypnotic effects [13]. Although they are relatively safe with respect to acute intoxication problems when administered alone, benzodiazepines are not without their toxicities. In combination with other CNS depressant agents, benzodiazepines can be extremely toxic [13]. In addition, benzodiazepines are also addicting and dependence-producing agents [13].

Because of this potential for adverse effects with benzodiazepines, a number of possibly non-sedative and/or non-addicting alternatives to the benzodiazepines for the management of anxiety states have been developed by drug manufacturers. The drug buspirone represents one potential alternative to benzodiazepines for the management of anxiety [6,18].

Buspirone is structurally unrelated to the benzodiazepines [17,24] and does not bind to benzodiazepine receptors [17,18]. The precise mechanism of action of buspirone is unclear at this time, although both serotonergic [5, 6, 17] and dopaminergic [15, 17, 19, 21, 23] mechanisms have been proposed.

The Conditioned Suppression of Drinking (CSD) procedure has been used in the study of anxiety and anti-anxiety agents [2–4, 7, 14]. The CSD is a modification of the Geller-Seifter conditioned conflict test [9–12] and the Vogel acute conflict test [1,22]. This procedure has proven to be quite useful in the study of anti-anxiety agents, largely because the acquisition of a stable baseline behavior for both punished and unpunished responding requires a relatively short (2–3 weeks) period of training. The effects of the drug buspirone in the CSD have not been investigated previously.

The present studies were designed to determine the effects of traditional anti-anxiety agents (diazepam, phenobarbital), agents which are *clinically-ineffective* in the management of anxiety (e.g., ethanol, morphine) and the novel compound buspirone on behavior in the CSD paradigm.

GENERAL METHOD

Animals

Female rats (225–250 grams at the start of the experiment), purchased from Charles River Farms, Inc. (Cambridge, MA), were used in these experiments. The animals 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 ad lib access to food with restricted water. Details of the water restriction are provided below in the Procedure section.

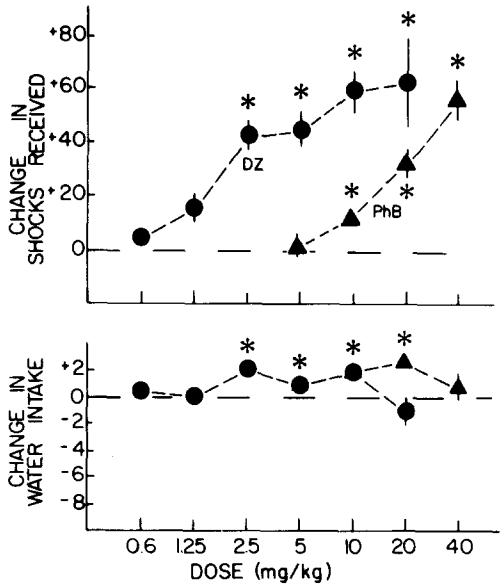

FIG. 1. The effects of diazepam (circles) and phenobarbital (triangles) on behavior in the CSD paradigm. Upper Panel: The increase in the number of shocks following diazepam (0.6–20 mg/kg) or phenobarbital (5–40 mg/kg) administration. Each symbol and vertical bar represents the mean±SEM change in shocks received (Drug-Vehicle) obtained from 20 subjects. Diazepam and phenobarbital increased the number of shocks received in a dose-dependent manner. *$p<0.05$, Student's t-test for paired values. Lower Panel: The change in water intake (unpunished responding) following diazepam or phenobarbital administration. Again, each symbol and vertical bar represents the mean±SEM change in water intake obtained from 20 subjects. Diazepam and phenobarital increased water intake slightly at the lower doses (statistically significant for 5, 10 and 20 mg/kg phenobarbital and 2.5 and 10 mg/kg diazepam). At no doses tested did diazepam or phenobarbital significantly depress water intake. *$p<0.05$, Student's t-test for paired values.

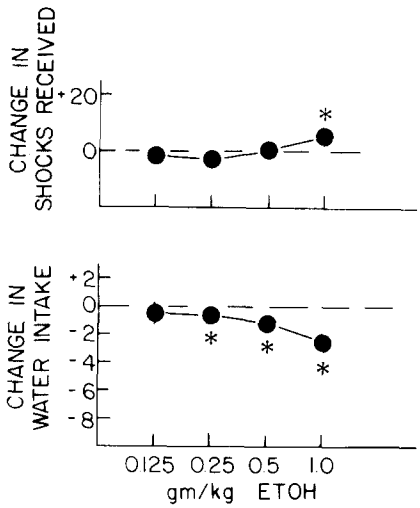

FIG. 2. The effects of ethanol on behavior in the CSD paradigm. See Fig. 1 legend for details. Ethanol did not increase punished responding except at the 1.0 g/kg dose; this dose was also associated with a significant depression of water intake. *$p<0.05$, Student's t-test for paired values.

Apparatus

The CSD apparatus [2] was a rectangular box (30×30×25 cm high) with Plexiglas sides and a metal floor and top. 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 session was controlled by solid state modular programming equipment (Coulbourn Instruments, Co., Lehigh Valley, PA).

Procedure

Conditioned Suppression training and testing were conducted according to the procedure described by Commissaris and Rech [4] and Commissaris *et al.* [3]. For the first few sessions, water-restricted subjects (food provided ad lib) were placed in the experimental chamber and allowed to consume fluid freely without the shock contingency. After five non-shock sessions, the tone/shock contingency was initiated. The 7-second tone periods were presented at regular (22 second ISI) intervals to the subjects. During the latter 5 seconds of these tone periods, contact between the floor and the metal drinking tube completed a circuit which resulted in the delivery of a 0.5 mA shock to the rat. Shocks were delivered by a Coulbourn Instruments Shocker (Model No. E13-02).

Initially, the shock inhibited all fluid consumption in the test chamber. After several days (5–10 test sessions), however, all subjects 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, receiving a consistent number of shocks from day to day. Subjects were tested singly in 10 minute sessions at the same time of day (1200–1400 hours) Monday through Friday, and were allowed free access to water from Friday post-test until Sunday a.m. Five day/week testing was maintained throughout the period of drug testing.

Drugs

Diazepam (NIDA) was prepared in a 0.5 percent methylcellulose suspension. Phenobarbital Na (NIDA), buspirone (courtesy of Dr. R. H. Rech, Michigan State University, East Lansing, MI), ethanol (Sigma Chemical Co., St. Louis, MO), morphine sulfate (NIDA), chlorpromazine HCl (Sigma Chemical Co., St. Louis, MO), *d*-amphetamine sulfate (NIDA), and diphenhydramine HCl (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.85 percent saline. All drugs were injected intraperitoneally (IP) in a volume of 1 ml/kg, except ethanol which was administered in a constant 10% (v/v) solution. All drugs were administered IP 10 minutes before the test session, except *d*-amphetamine which was administered 60 minutes before the test session. In addition to IP administration, the effects of buspirone were also determined following its subcutaneous (SC) administration.

Statistical Analyses

The effects of single doses of various drugs on CSD performance were compared to drug vehicle using Student's t-tests for paired values. Dose-response curves for each drug

TABLE 1

EFFECT OF NON-ANXIOLYTIC AGENTS ON PERFORMANCE IN THE CSD PROCEDURE

Treatment (mg/kg)	Change in Shocks Received	Change in Water Intake
Ethanol		
125	−2.0 ± 1.6†	−0.6 ± 0.7‡
250	−2.6 ± 1.8	−0.6 ± 0.3
500	0.4 ± 1.4	−1.2 ± 0.4*
1000	5.8 ± 1.8*	−2.6 ± 0.6*
Morphine		
2.50	0.1 ± 1.5	−0.9 ± 0.4*
3.50	−6.4 ± 3.0*	−2.4 ± 0.4*
5.00	−6.4 ± 2.2*	−5.8 ± 1.0*
7.10	2.3 ± 2.1	−5.9 ± 0.9*
10.00	−8.5 ± 2.9*	−6.6 ± 1.2*
Chlorpromazine		
0.30	1.4 ± 2.1	−0.9 ± 0.6
0.60	−1.5 ± 3.1	0.2 ± 0.4
1.25	−1.0 ± 3.1	−3.0 ± 0.9*
2.50	3.2 ± 3.0	−4.8 ± 0.9*
5.00	−8.4 ± 6.3	−10.6 ± 1.0*
d-Amphetamine		
0.16	−3.1 ± 1.6	−0.5 ± 0.5
0.30	1.0 ± 2.4	−1.5 ± 0.6*
0.60	−5.7 ± 3.5	−5.0 ± 0.7*
1.20	−7.3 ± 2.0*	−6.6 ± 0.9*
Diphenhydramine		
1.25	−5.4 ± 3.4	−0.4 ± 0.4
2.50	−0.3 ± 2.2	0.5 ± 0.7
5.00	4.0 ± 2.8	−0.2 ± 0.5
10.00	1.0 ± 2.6	−1.0 ± 0.6
20.00	0.3 ± 3.2	−0.9 ± 0.5

*$p<0.05$, Student's *t*-test for paired values.

†Values represent the mean ± SEM (n=20) change in shocks received (Drug-Vehicle) during the punished periods.

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

were compared using a factorial ANOVA with repeated measures. In all statistical comparisons, $p<0.05$ was used as the criterion for statistical significance [20].

EXPERIMENT I: WHAT ARE THE EFFECTS OF "TRADITIONAL" ANTI-ANXIETY AGENTS ON CSD BEHAVIOR?

Procedure

Experiment I was designed to determine the effects of two clinically-effective anti-anxiety agents (phenobarbital and diazepam) on behavior in the CSD paradigm. These experiments employed a standard "crossover" design. On Thursday, half of the subjects received vehicle injection while the other half received a dose of the particular drug under investigation. On Friday, this procedure was repeated, except that the treatments were reversed. Thus, each rat served as her own control with respect to drug versus vehicle injection.

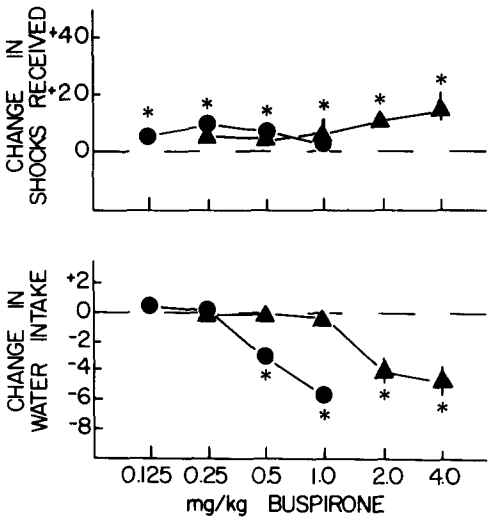

FIG. 3. The effects of buspirone administered intraperitoneally (IP; triangles) and subcutaneously (SC; circles) on CSD behavior. See Fig. 1 legend for details. Buspirone, both IP and SC, produced only a slight increase in punished responding. SC administration of buspirone was more potent in reducing water intake than was IP administration of buspirone. *$p<0.05$, Student's *t*-test for paired values.

Results

Subjects in the present study consumed an average of 11.6±0.5 ml (mean±SEM) of water per session and accepted an average of 16±4.6 (mean±SEM) shocks (contacts with the metal tube) per session in the CSD paradigm. It should be noted that nearly all water intake occurred during the silent or unpunished periods.

The effects of diazepam and phenobarbital in CSD performance are shown in Fig. 1. The upper panel of Fig. 1 illustrates the increase in the number of shocks received by the rats when administered diazepam (0.6–20 mg/kg) versus vehicle and phenobarbital (5–40 mg/kg) versus vehicle. Diazepam produced an increase in punished responding which was dose-related, $F(5,90)=5.07$, $p<0.05$. Phenobarbital administration also produced a significant dose-dependent increase in punished responses, $F(3,54)=28.62$, $p<0.05$. The maximum increase in punished responding with both diazepam and phenobarbital was quite impressive, with subjects accepting 40–50 shocks more than their baseline (nondrug) values.

The lower panel of Fig. 1 illustrates the change in water intake (unpunished responding) produced by diazepam or phenobarbital. Diazepam produced a slight increase in water intake which was dose-related, $F(5,90)=2.43$, $p<0.05$. Phenobarbital administration also produced a slight increase in water intake; this effect was not dose-related, $F(3,54)=2.21$, n.s.

In summary, the clinically-effective anti-anxiety agents diazepam and phenobarbital produced robust and dose-dependent increases in punished reponding at doses which did not depress background behavior (water intake).

EXPERIMENT II: WHAT ARE THE EFFECTS OF AGENTS WHICH ARE *NOT* CLINICALLY-EFFECTIVE IN THE TREATMENT OF ANXIETY?

Procedure

Experiment II was designed to determine the effects of a

number of "negative control" compounds in the CSD paradigm. Subjects were administered various doses of ethanol, morphine or other clinically non-anxiolytic CNS agents (chlorpromazine, *d*-amphetamine, diphenhydramine) over the course of several weeks of testing, using the cross-over procedure described in Experiment I above.

Results

Figure 2 illustrates the effects of ethanol on CSD performance. Ethanol administration did not affect punished responding except at the highest dose (1.0 g/kg); this dose of ethanol was associated with a significant depression of background water intake. Overall, there was a weak, yet statistically significant effect of ethanol dose on punished responding, $F(3,54)=4.84$, $p<0.05$, with only a tendency for a dose-dependent effect of ethanol on water intake, $F(3,54)=2.59$, n.s.

We have also tested numerous additional centrally-acting compounds without anti-anxiety effects in man (morphine, chlorpromazine, *d*-amphetamine, diphenhydramine), and have found that they do not selectively affect punished responding in the CSD. The data from these agents are summarized in Table 1.

In summary, a number of centrally acting agents which are clinically ineffective in the treatment of anxiety did not reliably affect punished responding in the CSD. Thus, the CSD paradigm appears to be selective in screening drugs for anti-anxiety use in man.

EXPERIMENT III: WHAT ARE THE EFFECTS OF BUSPIRONE IN THE CSD?

Procedure

This experiment was designed to determine the effects of buspirone in the CSD paradigm. Subjects were administered various doses of buspirone or its vehicle (saline) over the course of several weeks using the cross-over procedure described in Experiment I above. Buspirone was administered both intraperitoneally (IP) and subcutaneously (SC). The SC route of administration was employed because there is evidence that buspirone exhibits a significant "first-pass" effect via hepatic metabolism when administered by the IP route [8].

Results

Figure 3 illustrates the effects of IP and SC administration of buspirone on CSD performance. Although IP administration of buspirone did increase punished reponding at certain doses, this increase was not dose-dependent, $F(4,72)=1.87$, n.s. Moreover, the magnitude of this buspirone-induced increase in punished responding (approximately 10–15 shocks over baseline) was considerably *less* than that observed with the traditional anti-anxiety agents, diazepam and phenobarbital (see Fig. 1 for comparison). Higher doses administered IP were associated with a decrease in water intake; overall, the effects of IP buspirone administration on water intake were found to be significantly dose-related, $F(4,72)=14.14$, $p<0.05$.

SC administration of buspirone also resulted in a mild increase in punished responding at the 0.125, 0.25 and 0.5 mg/kg doses; this effect was found not to be dose-dependent, $F(3,54)=1.26$, n.s. SC administration of buspirone also depressed water intake at the highest doses; this effect was found to be dose-dependent, $F(3,54)=43.60$, $p<0.05$. Finally, SC buspirone was more potent than IP buspirone, as indicated by the significant shift to the left of the dose-response curve for SC, relative to IP, administration. Route of administration did not alter buspirone's anti-conflict efficacy, however, with neither SC nor IP administration producing as robust an increase in punished responding as did phenobarital or diazepam (see Fig. 1).

GENERAL DISCUSSION

Traditional anti-anxiety agents (diazepam and phenobarbital) produced a dramatic increase in punished responding in the CSD without disrupting background responding. Other benzodiazepines (chlordiazepoxide) and barbiturates (secobarbital, pentobarbital) also produce a similar dose-dependent increase in punished responding (data not shown). This effect of barbiturates and benzodiazepines, in agreement with previous reports in the CSD procedure [3, 4, 7, 14] as well as other conflict procedures [1, 9–12, 16, 22, 23], is consistent with their clinical anti-anxiety efficacy [13].

In contrast to the benzodiazepines and barbiturates, agents which are not effective in the treatment of anxiety in man (morphine, ethanol, chlorpromazine, *d*-amphetamine, diphenhydramine) did not increase responding in the CSD. Thus, the CSD paradigm differentiates true anxiolytics from a number of potential "false positives." The lack of effect in the CSD with the analgesic agent morphine is a particularly interesting finding, since a painful stimulus (shock to the mouth area) is involved in the CSD test.

The observation that agents which are clinically effective in the treatment of anxiety (benzodiazepines and barbiturates) cause a characteristic dose-dependent increase in punished responding, while agents which are clinically ineffective in the treatment of anxiety do not, suggests that the CSD paradigm is a useful model for testing the possible anti-anxiety potential of novel compounds. For this reason, the CSD was used to examine the potential anxiolytic properties of the novel compound buspirone.

In Experiment III it was found that buspirone, whether administered IP or SC, produced a significant increase in punished responding. This finding is consistent with previous reports regarding the effects of buspirone in conflict behavior [5, 6, 10, 16–19, 21, 23]. Higher doses of buspirone resulted in a decrease in background responding. Relative to IP administration, buspirone was more potent when administered SC. This is consistent with the significant "first-pass" effect of this compound [8].

The maximum increase in punished responding produced by buspirone by either route of administration was considerably less than that produced by either phenobarbital or diazepam. In addition to data collected with a pre-treatment interval of 10 minutes, the effect on CSD behavior of buspirone (2 mg/kg) was examined 60 minutes after IP administration. This longer pre-treatment interval did not result in any enhancement of buspirone's anti-conflict effect, with subjects again exhibiting only a modest, yet statistically significant, increase in punished responding (9.2 ± 3.8). This decreased anti-conflict efficacy of buspirone relative to diazepam in rats is consistent with findings in other laboratories employing either the CSD (G. W. Heath and R. H. Rech, personal communication) or the Geller-Seifter conflict test [10,16]. The clinical relevance, if any, of this decreased anti-conflict efficacy of buspirone relative to diazepam and phenobarbital is at present undetermined. It should be noted, however, that the anti-conflict efficacy of buspirone may

vary across species. For example, Witken and Barret [23] have shown that, in pigeons, buspirone is only slightly less efficacious in increasing punished responding than is chlordiazepoxide. Further, Geller and Hartmann [10] have suggested that buspirone and diazepam are equal in efficacy in monkeys. Unfortunately, since a maximal effect was not reported in their study, true efficacy of either diazepam or buspirone could not be ascertained.

In summary, traditional anti-anxiety agents (diazepam, phenobarbital) produced a robust and dose-dependent increase in punished responding in the CSD, while a number of non-anxiolytic agents (negative controls) did not. Buspirone also produced a significant increase in punished responding in the CSD paradigm, and was found to be more potent when administered SC relative to IP. Regardless of route of administration, however, the magnitude of the buspirone-induced anti-conflict effect was considerably less than that observed with either diazepam or phenobarbital. These data suggest that buspirone might be effective in the treatment of anxiety, but perhaps not with the degree of efficacy that is observed with benzodiazepines or barbiturates.

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