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Use of negatively reinforcing electrical brain stimulation to detect conventional and nonconventional anxiolytics as well as an anxiogenic drug

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Abstract

The present study determined whether anxiolytics such as diazepam (DZP), the benzodiazepine (BZD) receptor-selective agonist abecarnil (ABC), or the 5-HT1$_A$ agent buspirone (BUS) would increase the response latency of rats to switch-off electrical brain stimulation (EBS) of the periaqueductal gray (PAG). We also investigated the effects of pentylenetetrazole (PTZ), a purported anxiogenic. Given acutely, DZP (2.5 and 5 mg/kg, ip) and ABC (0.5 and 1 mg/kg, ip) increased response latency. The BZD receptor antagonist flumazenil (10.0 mg/kg, ip) blocked these effects. Increasing the frequency of EBS reversed the effects of DZP and ABC, suggesting that motor disruption did not account for the increase in latency seen with these drugs. Given acutely, BUS (10.0 mg/kg, ip) also increased response latency, which was likely due to motor disruption because it was not reversed by increasing the frequency of EBS. When BUS (2.5 mg/kg, ip) was given every 8 h for 3 days, an increase in latency was also obtained, which was reversible by increasing the frequency of EBS. Finally, PTZ (10 and 20 mg/kg, ip) shortened the latency to respond. These results (1) suggest that DZP, ABC, and chronic BUS attenuate, whereas PTZ potentiates, the negative reinforcing stimulus (NRS) induced by PAG stimulation, and (2) support the hypothesis that the switch-off procedure accurately detects anxiolytic and anxiogenic drugs.

Keywords: Anxiogenic; Anxiolytic; Buspirone; Diazepam; Flumazenil; Negative reinforcement; Pentylenetetrazole; Switch-off response

A negative reinforcing stimulus (NRS) maintains and strengthens a response that removes the stimulus from the environment (Campbell, 1955). Electrical brain stimulation (EBS) of the dorsal part of the periaqueductal gray (PAG) is known to serve as a NRS (Audi and Graeff, 1984; Delgado et al., 1954; Olds and Olds, 1962; Schenberg and Graeff, 1978; Wada et al., 1970). For instance, a laboratory animal stimulated in the PAG will press a lever to terminate the stimulation: this type of behavior is referred to as a "switch-off response" (Delgado et al., 1954; Depoortere et al., 1990a,b; Olds and Olds, 1962; Sandner et al., 1987). This response can be quantified by means of the switch-off latency (SOL): the time elapsed between the onset of stimulation and its termination by a lever-press. An inverse relationship exists between the strength of the stimulation (i.e. the magnitude of the NRS) and the latency, such that the higher the strength (i.e. frequency or intensity) of the EBS, the shorter the latency.

Drugs that enhance gamma-aminobutyric acid (GABA) neurotransmission increase switch-off latencies, whereas drugs that interfere with GABA neurotransmission shorten latencies. For example, the SOL or the thresholds of EBS are increased by benzodiazepine (BZD) anxiolytics, such as chlordiazepoxide (Depoortere et al., 1990a; Gomita et al., 1991; Schenberg and Graeff, 1978) and diazepam (DZP) (Bovier et al., 1982; Gomita et al., 1991; Lloyd et al., 1981). In addition, GABA agonists such as progabide or muscimol, when microinjected into the PAG, also increase switch-off latencies (Audi and Graeff, 1984; Bovier et al., 1982). In contrast, treatment with the BZD inverse agonist FG 7142, which reduces GABAergic transmission and has been suggested to possess anxiogenic properties in humans

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(Corda et al., 1983; Dorow et al., 1983) and laboratory animals (Sanger and Cohen, 1995; Stephens et al., 1987), shortens this latency (Depoortere et al., 1990a).

Abecarnil (ABC) is a beta-carboline derivative that binds only to a subset of BZD sites in the CNS (Stephens et al., 1990, 1993). Perhaps because of this limited binding, ABC is remarkably devoid of the sedative properties associated with most BZDs, yet it has the profile of an anxiolytic in various animal models of anxiety (Griebel et al., 1999; Stephens et al., 1990, 1993). Clinical data generally support the anxiolytic efficacy of ABC (Ballenger et al., 1991; Lydiard et al., 1997; Mumford et al., 1995), though some studies have shown less positive results (Aufdembrinke, 1998; Pollack et al., 1997). One aim of the present study was to determine whether ABC would increase the latency to switch-off EBS applied to the PAG. We included the prototypical BZD anxiolytic DZP as a reference compound. To confirm that the effects of DZP and ABC on latencies to respond are mediated through the BZD receptor, we also determined whether flumazenil (FLU), a BZD receptor antagonist (Hunkeler et al., 1981), would reverse these effects. To further determine the GABA/BZD receptor involvement in the aversive nature of PAG stimulation, we tested if pentylenetetrazole (PTZ), a $GABA_A$ antagonist and a putative anxiogenic drug (Lal and Emmett-Oglesby, 1983; Rodin and Calhoun, 1970), would shorten the latency.

A second aim of the study, was to determine whether buspirone (BUS), a 5-$HT1_A$ receptor partial agonist (Mennini et al., 1986), would increase switch-off latencies. BUS has been shown to provide significant relief of anxiety in humans following chronic but not acute dosing (Jacobson et al., 1985; Rickels et al., 1982). The present study assessed the effects of both acute and chronic regimens of BUS on the SOL.

Thus, the present study expands the existing literature in several ways. It investigates the pharmacology of anxiogenic and anxiolytic compounds using a frequency–response curve analysis, several doses of each compound tested, agonist/antagonist interaction studies, acute vs. chronic regimens, and a positive control experiment to determine whether the results obtained with anxiolytics are attributable to the nonspecific motor effects of these drugs.

1. Methods

1.1. Animals

Twenty-three male Long–Evans rats (Harlan Sprague–Dawley, Indianapolis, IN) were kept on a 12-h light–dark cycle (light on between 8:00 a.m. and 8:00 p.m.). Weights of the rats were kept at 400 ± 10 g by restricting access to chow pellets (Harlan Teklad Medicine, Madison, WC). For all rats, food was freely available 3 days before and 3 days after surgery. All housing and procedures were in accordance with the guidelines of the Institute of Laboratory Animal Resources, National Research Council (Institute of Laboratory Animal Resources, 1996) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

1.2. Apparatus

Rats were trained and tested in eight standard operant chambers (model E-10-10TC, Coulbourn Instruments, Lehigh Valley, PA) fitted with two levers and a house light. Each chamber was enclosed in a ventilated and sound-attenuated cubicle, and was connected to an IBM PC compatible computer via an interface (LVB, Med Associates, Georgia, VT). All events were recorded and controlled by the "Operant Package for Neurosciences" Software (Spencer and Emmett-Oglesby, 1985).

EBS was delivered by optically isolated pulse neurostimulators (model 2100, A-M Systems, Everett, WA) through a 5-lead spring-shielded cable. This cable was suspended from the ceiling of the operant box and was terminated by a miniature 5-pin male connector. Electrical parameters were continuously monitored for each neurostimulator on an oscilloscope across a 100-kΩ resistor in series with the stimulation circuit.

1.3. Surgery

Rats were injected with atropine (1 mg/kg, sc) followed by an intraperitoneal injection of a mixture of ketamine (100 mg/kg), chlordiazepoxide (20 mg/kg), and nalbuphine (10 mg/kg). After induction of anesthesia, they were placed in a stereotaxic frame in the flat skull position. Half of the rats were implanted with PAG electrodes in the right side, the other half with electrodes in the left side. Stereotaxic coordinates (with respect to lambda) were: AP: 1 mm, DV: 5.5 mm, and ML: 1.7 mm (with a 15° mediolateral angle in order to avoid piercing the sagittal venous sinus). Electrodes were made of two 175-μm stainless steel Teflon-coated threads (A-M Systems), twisted and held together with a cyanoacrylate glue (Superglue, Rawn, Spooner, WI), with a 0.2 to 0.4 mm dorso-ventral intertip distance. Each of the two threads was soldered onto one of the five pins of a miniature female connector. Each rat hence had two stimulation sites on the same side of the PAG. The connector was then embedded in acrylate resin and anchored to the skull by means of four stainless-steel screws, one of which was used as the common anode. Four days after surgery, rats were subjected to an electrode screening test.

1.4. Procedure

1.4.1. Electrode screening test

This test determined which of the two stimulation sites and which intensity would be used for the study. Details of the procedure have been described elsewhere (Depoortere et al., 1990b). The day after the electrode screening test, rats were shaped to switch-off EBS applied to the PAG.

1.4.2. Switch-off shaping

In the initial phase of this experiment, rats were shaped to approach a lever and subsequently to switch-off the EBS (cathodal pulses, 0.1 ms, 50 Hz, intensity adjusted for each rat: see below) by pressing a lever. Both levers were active, i.e. interrupted the EBS when depressed. During the first EBS trials, the intensity was manually adjusted to produce a mild behavioral activation. When the rat was in the vicinity of a lever, the EBS was manually switched off by the experimenter. The rat was then progressively reinforced for closer approaches to a lever. During the later part of this shaping phase, the EBS was manually turned off only when the rat made physical contact with a lever. During these shaping sessions, each EBS was limited to 30 s, and two consecutive EBS's were separated by a 30-s rest period. The total number of EBS's for each shaping session was limited to 30. No more than two shaping sessions were applied per day.

1.4.3. Switch-off training

After subjects learned to switch-off the EBS by pressing a lever, they were trained once daily for several days. The intensity of the EBS was slowly adjusted so that the latency to respond was within the range of 8–12 s for the 50 Hz frequency. After a rat acquired a stable baseline of switch-off responding (average latency not varying by more than 20% between three consecutive training sessions), it was tested at four stimulation frequencies: 30, 40, 50, and 70 Hz. All four frequencies were tested in a single session, in a randomized order. Each frequency was tested in a block of 12 trials, with a 30-s time limit for each stimulation, and a 30-s rest period in between two consecutive stimulations. There was a 2-min rest period between the initiation of a new frequency for testing. The intensity was eventually readjusted for each rat so that the ranges of response latencies for frequencies of 30, 40, 50, and 70 Hz were, respectively: 25 to 30 s, 18 to 22, 8 to 12 s, and 4 to 7 s. If a rat did not press within the 30 s time limit, an arbitrary latency of 30 s was recorded. Once frequency–response curves varied by less than 20% across three successive training sessions, the stimulation intensity was held constant for the rest of the study, and rats were tested with various drugs.

1.5. Pharmacological tests

The frequency–response test following drug treatment was conducted at frequencies of 30, 40, 50, and 70 Hz in a single session, except for PTZ, which was tested only at frequencies of 30, 50, and 70 Hz. A single dose of a drug (or vehicle) was tested per day. Drug treatments were spaced 3 days apart to minimize carry-over effects. During this drug-free period, rats received daily training to maintain stable baseline latencies.

1.5.1. Effects of treatment with DZP or ABC and combined effects with FLU

DZP or ABC was injected 30 min prior to each test. To test the combined effects of DZP or ABC with FLU, drug treatments were as follows: rats were first treated 30 min presession with either vehicle or drug (2.5 mg/kg of DZP or 0.5 mg/kg of ABC). This was followed by a 15-min presession injection of either vehicle or FLU. The effects of FLU alone were also assessed.

1.5.2. Effects of treatment with PTZ, and acute or chronic BUS

PTZ was administered 10 min prior to the test. For acute administration, BUS was injected 15 min prior to the test session. For chronic administration, BUS (2.5 mg/kg) was administered thrice (8:00 a.m. and 8:00 p.m.) daily for 3 days, during which rats were not subjected to switch-off sessions. The frequency–response test was carried out 15 min after the injection of BUS on the fourth morning.

1.5.3. Effects of DZP, ABC, acute, and chronic BUS at high frequency of stimulation

To examine whether the effects of drugs on the response latency were influenced by motor disturbance, the effects of DZP (2.5 mg/kg), ABC (0.5 mg/kg), acute BUS (10.0 mg/kg), and chronic BUS (2.5 mg/kg) on the frequency–response function were tested at a higher frequency (100 Hz). We performed this test based on the supposition that anxiolytic drugs produce an effect that is equivalent to reducing the magnitude of the EBS. Thus, increasing the magnitude of the EBS would be expected to reverse the effects of anxiolytics. In contrast, if a drug increased the response latency because of motor disruption effects, then increasing the EBS would not necessarily reverse this effect.

All other details of the procedure were the same as those described above. For practical reasons (latencies at 70 Hz approach floor values) and for ethical considerations (higher frequencies of stimulation generate more potent NRS), rats were not tested at 100 Hz under control (vehicle) conditions.

1.6. Drugs

DZP (Sigma, St. Louis, MO), ABC (a gift of Schering, Berlin), and FLU (a gift of Hoffman-LaRoche, Basel) were suspended in 3% carboxymethylcellulose (CMC); PTZ (Sigma), and BUS (Sigma) were dissolved in 0.9% saline. All drugs were prepared fresh daily and given in a volume of 1 ml/kg (ip). Controls consisted of treatment with the appropriate vehicle. Doses were administered in a randomized order.

1.7. Data analysis

A mean latency was calculated for each frequency by averaging the last 10 latencies from each block of 12 trials. The first 2 latencies for each block of frequencies were discarded because they showed more variability than the remaining 10. Mean vehicle latencies were obtained by averaging latencies across three vehicle sessions collected during each drug treatment. Mean latencies were then sub-

jected to a two-way repeated measures analysis of variance (drug dose × frequency) followed by post hoc tests (Bonferroni adjustment) across all four frequencies or at each frequency (Jerrold, 1984). For the test including an additional

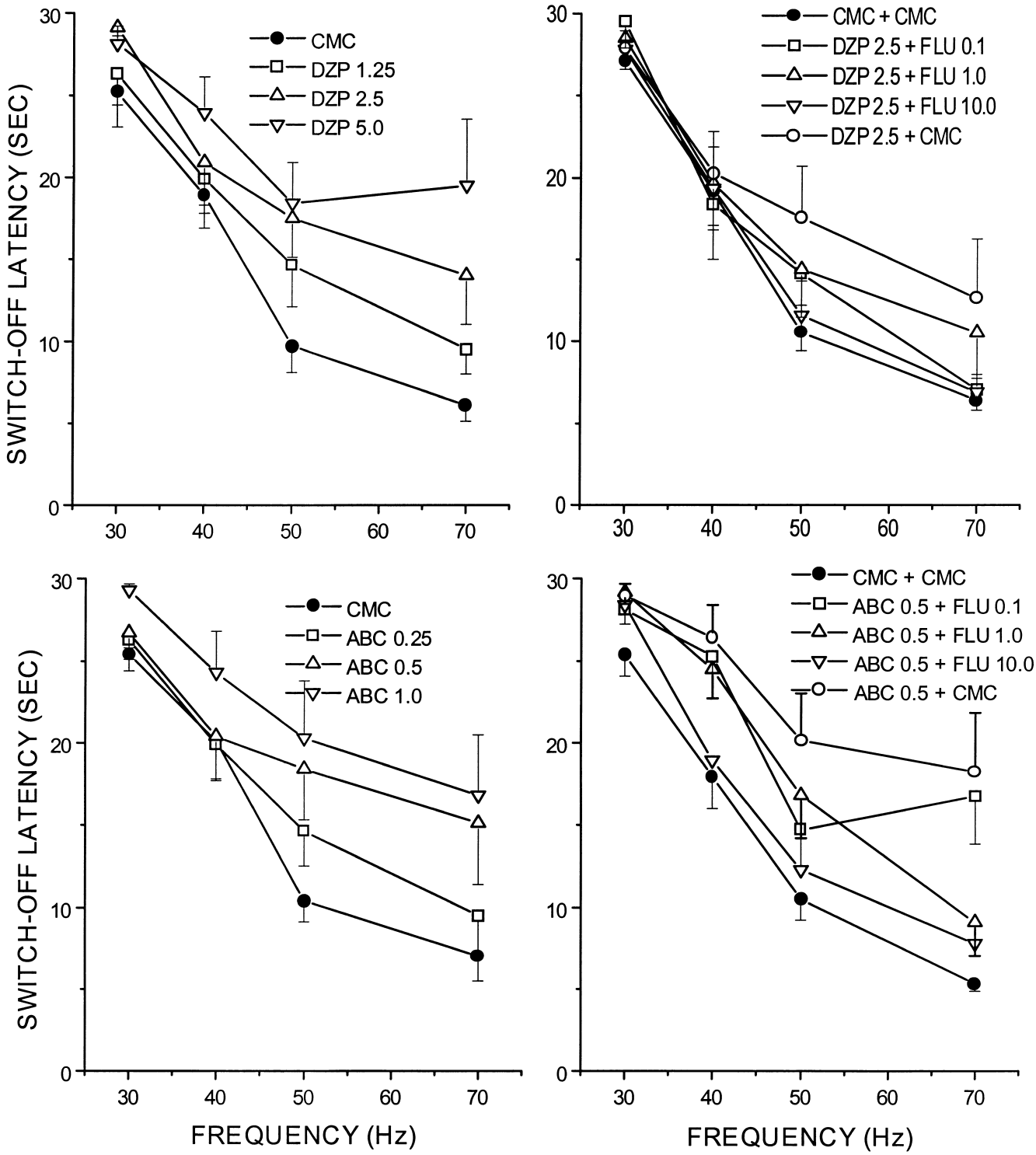

Fig. 1. Effects of DZP (top left panel) or ABC (bottom left panel) alone or in combination with FLU (right panels) on the switch-off response induced by electrical stimulation of the PAG. Abscissa: frequency of electrical stimulation (Hz). Ordinate: SOL (time elapsed between the onset of the stimulation and its offset by a press of a lever, in seconds). Drug treatments (intraperitoneal) are indicated in the panel legends (numbers are mg/kg). CMC: 3% carboxymethylcellulose. $N = 8$ rats per experiment. By post hoc tests, the following comparisons were significantly different: DZP 2.5 ($P < .05$, at 50 and 70 Hz) and 5.0 ($P < .01$, at 50 and 70 Hz) vs. CMC; ABC 1.0 ($P < .05$, at 30, 50, and 70 Hz) and 0.5 ($P = .031$, at 70 Hz) vs. CMC; DZP 2.5 + FLU 10.0 vs. DZP 2.5 + CMC ($P = .012$, at 70 Hz); ABC 0.5 + FLU 10.0 vs. ABC 0.5 + CMC ($P < .05$, at 30, 40, 50, and 70 Hz).

Table 1
Effects of FLU alone on the SOL (seconds ± S.E.M.)

Frequency (Hz)	Vehicle (CMC)	FLU 0.1 mg/kg	FLU 1.0 mg/kg	FLU 10.0 mg/kg
30	24.3 ± 1.3	24.3 ± 2.0	26.9 ± 1.6	24.8 ± 2.1
40	14 ± 1.2	15.9 ± 2.0	15 ± 1.7	15.1 ± 2.7
50	7.7 ± 0.6	9 ± 1.2	9.4 ± 1.0	9.7 ± 1.8
70	5.9 ± 0.7	6.7 ± 1.2	6 ± 1.1	6.1 ± 0.8

The SOL after treatment with FLU alone did not significantly differ from the latency after vehicle treatment at any dose and frequency of stimulation tested. CMC: 3% carboxymethylcellulose. $N = 8$ rats per experiment.

frequency (100 Hz), mean latencies at 70 Hz after a vehicle treatment were compared to mean latencies at 70 and 100 Hz after a drug treatment using a pairwise comparison (Dunnett's *t* test) test. All tests were performed with SYSTAT software.

2. Results

2.1. Baseline switch-off response curve and the effects of DZP, ABC, and combined effects with FLU

There was an inverse relationship between the frequency of stimulation and the SOL to respond (Fig. 1: vehicle curves, filled symbols). Both DZP (Fig. 1: left top panel) and ABC (Fig. 1: left bottom panel) significantly increased these latencies in a dose- [$F(3,112) = 14.5$, $P < .001$ and

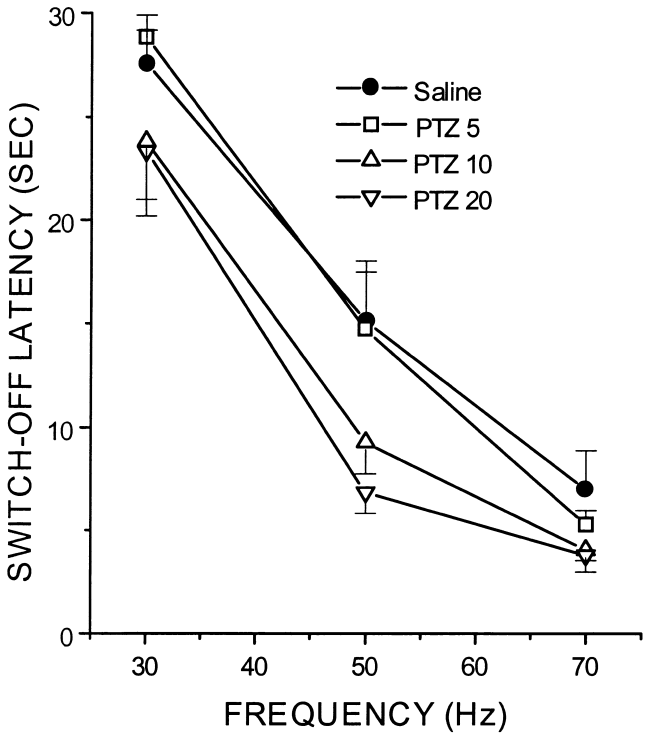

Fig. 2. The effects of PTZ on the switch-off response induced by electrical stimulation of the PAG. Abscissa: frequency of the electrical stimulation (Hz). Ordinate: SOL (seconds). Drug treatments (intraperitoneal) are indicated in the panel legends (numbers are mg/kg). $N = 6$ rats per experiment. By post hoc tests, the following comparisons were significantly different: PTZ 10 ($P = .033$ at 70 Hz) and PTZ 20 ($P < .05$ at 50, 70 Hz) vs. saline.

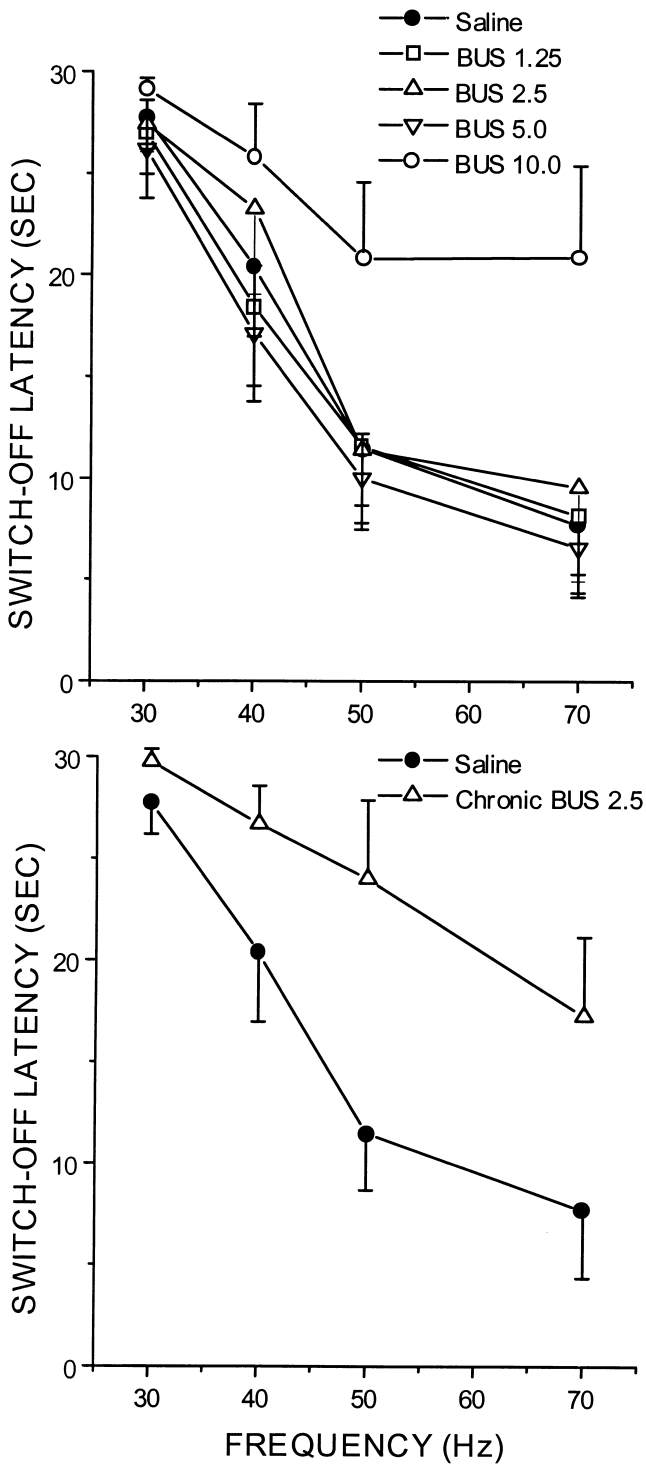

Fig. 3. Effects of acute (top panel) or chronic (bottom panel) BUS on the switch-off response induced by electrical stimulation of the PAG. Abscissa: frequency of the electrical stimulation (Hz). Ordinate: SOL (seconds). Drug treatments (intraperitoneal) are indicated in the panel legends (numbers are mg/kg). $N = 6$ rats per experiment. By post hoc tests, the following comparisons were significantly different: BUS 10.0 ($P < .05$ at 50 and 70 Hz) vs. saline; chronic BUS 2.5 vs. saline ($P < .05$ at 30, 50, and 70 Hz).

$F(3,112) = 12.6$, $P < .001$, for DZP and ABC, respectively] and frequency- [$F(3,112) = 57.5$, $P < .001$ and $F(3,112) = 56.8$, $P < .001$, for DZP and ABC, respectively] related manner. By post hoc comparisons, only SOL at DZP 2.5 mg/kg ($P < .05$, at 50 and 70 Hz) and 5.0 mg/kg ($P < .01$, at 50 and 70 Hz) were significantly different from

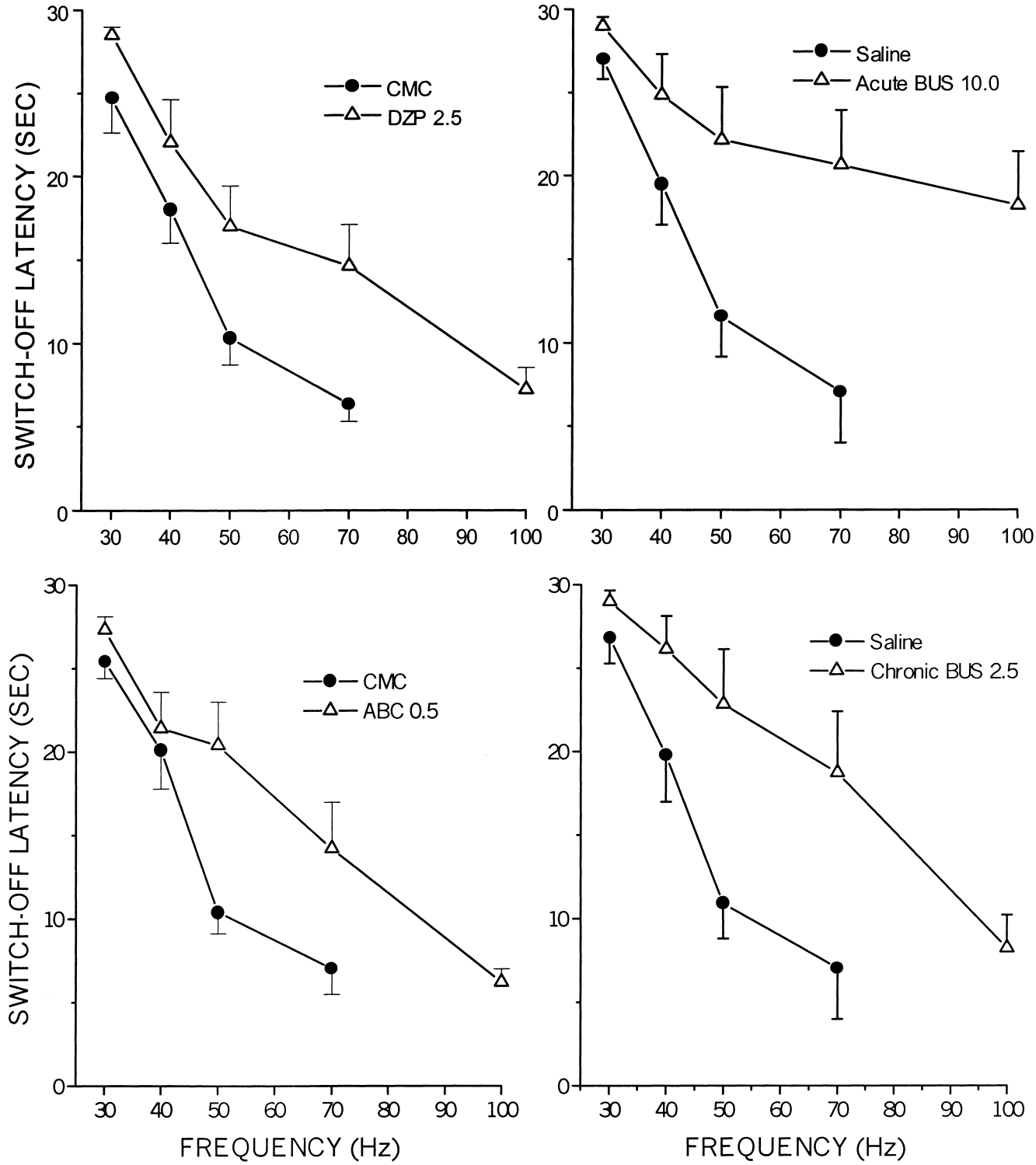

Fig. 4. Effects of DZP (left top panel), ABC (left bottom panel), acute BUS (right top), and chronic BUS (right bottom) on the switch-off response at high frequency (100 Hz), along with the regular four frequencies. Abscissa: frequency of the electrical stimulation (Hz). Ordinate: SOL (seconds). Drug treatments (intraperitoneal) are indicated in the panel legends (numbers are mg/kg). CMC: 3% carboxymethylcellulose. $N = 6$ rats per experiment. The only difference between latencies after drug treatment at 100 Hz and vehicle treatment at 70 Hz was observed for acute BUS 10.0 mg/kg.

control data. For ABC, the SOL at the highest dose (1.0 mg/kg) significantly differed from those of control ($P<.05$, at 30, 50, and 70 Hz). The intermediate dose of ABC (0.5 mg/kg) increased the latency only at 70 Hz ($P=.031$) as compared to control case.

FLU alone (0.1, 1.0, or 10.0 mg/kg) did not significantly modify SOL (Table 1) at any dose and frequency tested [$F(3,112)=0.9$, $P=.4$]. There was also no interaction effect between the dose of FLU and frequency.

However, when FLU was treated in combination with DZP (2.5 mg/kg) or ABC (0.5 mg/kg) (Fig. 1: upper right and lower right panels, respectively), FLU (10.0 mg/kg) antagonized the increased latency induced by either DZP at 70 Hz ($P=.012$) or ABC (0.5 mg/kg) at all four frequencies ($P<.05$).

2.2. Effects of PTZ

Treatment with PTZ significantly shortened response latencies [$F(3,60)=15.8$, $P<.001$] (Fig. 2). By post hoc comparisons, PTZ (20 mg/kg) significantly shortened the SOL at 50 Hz ($P=.004$) and 70 Hz ($P=.019$) as compared to control. PTZ (10 mg/kg) shortened SOL only at 70 Hz ($P=.033$). Treatment with the lowest dose of PTZ (5 mg/kg) did not significantly modify the latencies.

2.3. Effects of acute and chronic BUS

When BUS was given acutely, there was a significant effect of treatment [$F(4,100)=13.3$, $P<.001$] and frequency [$F(3,100)=76.7$, $P<.001$] on the SOL (Fig. 3: top panel). By post hoc comparisons, only the 10.0 mg/kg dose significantly increased the SOL as compared to control ($P<.05$, at 50 and 70 Hz). In contrast to acute BUS (2.5 mg/kg), which did not significantly increase SOL, BUS (2.5 mg/kg) given every 8 h for 3 days significantly [$F(1,40)=37.4$, $P<.001$ and $P<.05$, at 30, 50, and 70 Hz] increased the response latency (Fig. 3: bottom panel). There was also a significant effect of frequency on the SOL [$F(3,40)=32.2$, $P<.001$] and a significant interaction between treatment and frequency [$F(3,40)=3.28$, $P=.03$].

2.4. Effects of DZP, ABC, acute BUS, or chronic BUS tested at an additional high frequency of stimulation

When the frequency was augmented to 100 Hz, the increased latencies obtained with DZP (2.5 mg/kg), ABC (0.5 mg/kg), or BUS (2.5 mg/kg given every 8 h for 3 days) were reversed to the baseline latency obtained at 70 Hz under vehicle conditions (Fig. 4). Thus, there were no significant statistical differences between latencies after drug treatment at 100 Hz and vehicle treatment at 70 Hz ($P>.05$). In contrast, the increased latency induced by acute BUS (10.0 mg/kg) remained significantly higher ($P<.001$) at 100 Hz than that obtained at 70 Hz under control conditions (Fig. 4: right top panel).

3. Discussion

The present data support the hypothesis that EBS of the PAG is an aversive anxiety-like event. Thus, response latency was found to vary inversely with stimulation frequency: the stronger the stimulation, the shorter the latency to switch-off the stimulation. Anxiolytics such as DZP, ABC, and chronic BUS increased the latency whereas the anxiogenic PTZ decreased it in a dose-related manner across all frequencies tested.

In previous studies, DZP and chlordiazepoxide have been shown to delay the switch-off response. Thus, rats trained to escape the aversive effects of electrical stimulation of the PAG wait longer to respond when treated with these anxiolytic drugs (Bovier et al., 1982; Depoortere et al., 1990a; Schenberg and Graeff, 1978). This effect is mediated by BZD receptors, because the BZD antagonist, FLU (Hunkeler et al., 1981), blocked the effects of DZP (Lloyd et al., 1981). The present results confirm the previous findings for DZP and DZP + FLU, and they extend those findings to show anxiolytic effects of ABC, an agonist at a subset of BZD receptors (Stephens et al., 1990, 1993), and antagonism of ABC by FLU. At the dose used in the present study, FLU behaved as a pure BZD antagonist. It was inactive by itself, but blocked the effects of DZP and ABC to increase the response latency. These data are in an agreement with other findings that FLU by itself produces no effect in animal models of anxiety but is remarkable for blocking the effects of drugs binding at BZD receptors (Nazar et al., 1997; Rex et al., 1993; Witkin et al., 1996). For instance, when FLU was concurrently administered with ABC or DZP, it blocked the anxiolytic effects of two BZDs on feeding behavior in the open field (Rex et al., 1996). FLU also antagonized the anxiolytic effect of DZP on open arm activity of rats in the elevated plus maze (Rex et al., 1993). Thus, our data with FLU substantiate the hypothesis that the effect of DZP and ABC on the PAG stimulation is in part mediated through the GABA/BZD receptor complex. Furthermore, the absence of effects of FLU alone and of morphine or naloxone (unpublished data), three compounds devoid of efficacy as anxiolytics, further strengthens the pharmacological validity of this switch-off procedure to accurately detect the anxiolytic activity of drugs.

The use of a high frequency stimulation paradigm examined specific anxiolytic vs. nonspecific (motor disturbance) drug-induced effects. We performed this test with an additional frequency of 100 Hz based on the supposition that anxiolytic drugs produce an effect that is equivalent to reducing the magnitude of the EBS. Thus, increasing the magnitude (i.e. frequency) of the EBS would be expected to oppose the effects of anxiolytics, if this effect is specific. The antagonism of the switch-off response that occurred with DZP (2.5 mg/kg) and ABC (0.5 mg/kg) could indeed be overcome by increasing the frequency of PAG stimulation, suggesting that longer latencies are not a product of motor

disruption. Thus, the data are consistent with the hypothesis that DZP (up to doses of 2.5 mg/kg) and ABC (up to doses of 0.5 mg/kg) reduce the aversive quality of electrical stimulation of the PAG, and they do so through interactions with BZD receptors. Note that the greater latency to respond that occurred with 5.0 mg/kg DZP (Fig. 1) may be partly attributable to disrupted motor performance.

The administration of PTZ produced a dose-dependent shortening of response latencies. In previous studies using electrical stimulation of the PAG, shortening of response latencies has been observed with the BZD receptor inverse agonist FG 7142 (Depoortere et al., 1990a). This drug is known to induce anxiety in humans (Corda et al., 1983; Dorow et al., 1983) and has the profile of an anxiogenic in other animal models of anxiety (Sanger and Cohen, 1995; Stephens et al., 1987). Similarly to those findings with FG 7142, PTZ has also been shown to induce anxiety in humans (Rodin and Calhoun, 1970) and to have an anxiogenic profile in laboratory animals (Lal and Emmett-Oglesby, 1983). This anxiogenic profile could be due to an interference with GABA neurotransmission, as PTZ has been reported to antagonize the regulatory sites of the GABA/BZD receptor complex (Simmonds, 1983). Thus, the shortened latencies following PTZ treatment may result from the action of PTZ counteracting the GABA/BZD receptor system, and consequently potentiating the aversive effect of stimulation of the PAG. This result, along with the findings that DZP and ABC with or without FLU further strengthens the hypothesis that GABA/BZD receptors exert an inhibitory action on the aversive effects induced by PAG stimulation (Bovier et al., 1982; Brandao et al., 1982; Di Scala et al., 1984).

Low doses of BUS (1.25 to 5 mg/kg) given acutely had no significant effect on latencies, although at 5 mg/kg there was a tendency for a shortening of the latency. The highest dose tested of BUS (10.0 mg/kg) increased latencies. However, in contrast to what was seen with the two BZD receptor agonists, increasing the frequency of stimulation to 100 Hz did not result in a reversal of the acute BUS-induced increase in latency. These results suggest that the increased latency seen with this high dose of acute BUS may be a consequence of motor disturbance, rather than an effect on the aversive quality of the stimulus. BUS is known to have affinity for 5-$HT1_A$ receptors, where it acts as a partial agonist (Collinson and Dawson, 1997; Gobbi et al., 1991; Griebel et al., 1998), and it is effective as an anxiolytic (Davidson et al., 1999; Lader and Scotto, 1998; Sramek et al., 1999). Interestingly, however, in humans BUS has a different anxiolytic profile than drugs such as DZP. For example, BUS is ineffective as an anxiolytic when given acutely (Sramek et al., 1999). Instead, anxiolytic effects of BUS only become apparent with multiple administration (Jacobson et al., 1985; Petracca et al., 1990; Rickels et al., 1982). Indeed, particularly when used in high doses, BUS has been reported to cause anxiety in humans (Pols et al., 1989). The observation that BUS given acutely did not have the profile of an anxiolytic agent is consistent with the clinical data. In the present study, chronic treatment (t.i.d. over 3 days) with BUS, using a dose (2.5 mg/kg) that by itself had no acute effects, was found to increase the response latency. Moreover, this effect of chronic BUS could be reversed to the baseline level latency seen with 70 Hz, when the frequency of stimulation was increased to 100 Hz. These data suggest that chronic BUS shares with both DZP and ABC the ability to specifically attenuate the aversive effects induced by electrical stimulation of the PAG. This observation on differential effects of chronic and acute BUS is concordant with studies using BUS in animal models of anxiety such as the plus maze (Cole and Rodgers, 1994) and the Vogel conflict procedure (Yamashita et al., 1995).

Does electrical stimulation of the PAG provide a valid animal model of anxiety? Certainly the model has little face validity — anxious patients do not have electrodes stimulating their PAG. On the other hand, this model has excellent predictive validity in terms of identifying anxiolytic and anxiogenic stimuli (Audi and Graeff, 1984; Depoortere et al., 1990a). Our data extend the model to show anxiolytic effects of ABC and BUS, as well as anxiogenic effects of PTZ. Particularly because anxiolytic effects of BUS are not always easily obtained in animals (Collinson and Dawson, 1997; Otter et al., 1997), electrical stimulation of the PAG may be particularly useful for assessing the anxiolytic efficacy of drugs.

In conclusion, using a measure of latency to respond to remove an NRS, our findings suggest that the aversive effects of PAG stimulation covary with stimulation strength. In addition, the latency to respond is increased by nonconventional anxiolytics such as ABC and BUS, and it is shortened by PTZ, an anxiogenic drug. These data are consistent with the hypothesis that electrical stimulation of the PAG provides an animal model of anxiety with high predictive validity for identifying treatments that modulate human anxiety, in particular for pharmacological interventions that aim at modulating $5HT1_A$ neurotransmission. Finally, PAG stimulation at a high frequency appears to dissociate the anxiolytic effects from general motoric impairment, which may be a further beneficial aspect of this model of anxiety.

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