Drug Development Research 4:83–93 (1984)

Behavioral and Neurochemical Studies on the Anticonflict Actions of Buspirone

B.A. Weissman, J.E. Barrett, L.S. Brady, J.M. Witkin, W.B. Mendelson, S.M. Paul, and P. Skolnick

Laboratory of Bioorganic Chemistry, NIADDK (B.A.W., P.S.), Adult Psychiatry Branch (W.B.M.), and Clinical Neuroscience Branch (S.M.P.), NIMH, National Institutes of Health, Bethesda, Maryland and Department of Psychiatry, Uniformed Services, University of the Health Sciences, Bethesda, Maryland (J.E.B., L.S.B., J.M.W.)

ABSTRACT

Weissman, B.A., J.E. Barrett, L.S. Brady, J.M. Witkin, W.B. Mendelson, S.M. Paul, and P. Skolnick: Behavioral and neurochemical studies on the anticonflict actions of buspirone. Drug Dev. Res. **4**:83–93, 1984.

A series of behavioral and neurochemical studies were performed in order to determine if buspirone (or an active metabolite of this compound) could perturb a component of the γ-aminobutyric (GABA)-benzodiazepine receptor-chloride ionophore complex. In confirmation of previous findings, buspirone was shown to have anticonflict actions in both the rat and monkey. However, in these tests, buspirone was not as efficacious as benzodiazepines in producing an anticonflict action. The benzodiazepine receptor antagonists CGS 8216 and Ro 15-1788 did not reverse the anticonflict actions of buspirone. Small but statistically significant increases in the binding of [^{3}H]diazepam to brain were observed in vivo after doses of buspirone which are active in the "thirsty rat conflict" test. However, a similar change was not observed in the ex vivo binding of [^{3}H]flunitrazepam. These observations suggest that a metabolite of buspirone may perturb some component of the GABA-benzodiazepine receptor-chloride ionophore complex in an indirect fashion. Further work is necessary to determine whether a causal relationship exists between the changes in [^{3}H]diazepam binding observed in vivo and the anticonflict actions of buspirone.

Key words: buspirone, CGS 8216, Ro 15-1788, benzodiazepine receptor

INTRODUCTION

Buspirone (8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]-decane-7,9-dione) has both anticonflict and antiaggressive activity in animals [Riblet et al., 1982] and anxiolytic actions in man [Goldberg and Finnerty, 1979; Rickels et al., 1982]. The molecular mechanisms by which buspirone exerts these pharmacologic actions are unclear. Neurochemical studies have demonstrated that buspirone does not bind to benzodiazepine, GABA, or α_2-adrenergic

Received July 22, 1983; accepted August 31, 1983.

Address reprint requests to Dr. B.A. Weissman, N.I.H., Bldg. 4, Room 212, Bethesda, MD, 20205.

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receptors in vitro [Riblet et al., 1982]. These receptors have been implicated in the mechanism of action of substances which have anticonflict actions in animals and anxiolytic activity in man [Skolnick and Paul, 1983]. Nonetheless, buspirone has been shown to interact with dopamine receptors in vitro [Riblet et al., 1982] and pharmacologically resembles both dopamine agonists and antagonists [Riblet et al., 1982; Garattini et al., 1982]. These observations have led to the proposal that the interaction of buspirone with dopaminergic pathways may account for its anticonflict and anxiolytic actions [Taylor et al., 1982]. However, MJ 13805, a closely related derivative of buspirone, does not appear to affect dopaminergic pathways like buspirone yet has a similar (viz., anticonflict and antiaggressive) pharmacologic profile to buspirone [Temple et al., 1982]. This observation strongly suggests that an action with dopaminergic pathways may *not* be the primary molecular site of action of buspirone.

The GABA-benzodiazepine receptor chloride ionophore complex has been implicated in the actions of a number of commonly used anxiolytics [cf. Skolnick and Paul, 1982a,b] as well as in the pathophysiologic generation of anxiety [Ninan et al., 1982; Skolnick and Paul, 1983]. Anxiolytics such as benzodiazepines and triazolopyridazines (e.g., CL 218,872) are currently believed to act via direct occupation of benzodiazepine receptors. Other compounds (e.g., pyrazolopyridines such as SQ 65,396 and barbiturates such as pentobarbital) with anticonflict or anxiolytic actions have been shown to alter the apparent affinity and/or number of benzodiazepine and associated GABA receptors, and may exert their pharmacologic actions by perturbation of this "supramolecular complex." The latter observations, coupled with some evidence that a metabolite of buspirone may be responsible for the pharmacologic actions of this compound [Geller and Hartmann, 1982] suggests that an in vitro examination of the actions of buspirone at benzodiazepine and GABA receptors would not be adequate to rule out an action of buspirone at some component of this supramolecular complex. Benzodiazepine receptor "antagonists" such as Ro 15-1788 [Hunkeler et al., 1981] and CGS 8216 [Bernard et al., 1981] have been shown to block many of the pharmacologic actions of benzodiazepines, including its anticonflict activity. Recently, CGS 8216 has been reported to antagonize the anticonflict actions of pentobarbital at a dose which did not significantly alter the rate of either punished or unpunished responding [Mendelson et al., 1983]. Thus, if a metabolite of buspirone were acting directly at the benzodiazepine receptor, or in an indirect manner similar to pentobarbital, then benzodiazepine receptor antagonists might alter the anticonflict actions of buspirone. We now report the effects of CGS 8216 and Ro 15-1788 on the anticonflict actions of buspirone in both the monkey and rat, and neurochemical studies examining the effects of buspirone on the binding of [^{3}H]benzodiazepines both in vivo and ex vivo.

METHODS

Behavioral Studies

Monkeys

Subjects. Five adult squirrel monkeys (*Saimiri sciureus*) (0.7–0.8 kg) were used. All subjects were reduced to approximately 85% of their unrestricted feeding weights by limited daily access to food. The monkeys were individually housed and had free access to water in a temperature and humidity controlled room. The colony room was artificially illuminated between 0700 and 2100 hr.

Apparatus. During experimental sessions the monkeys were seated in a Plexiglas restraint chair equipped with a response lever mounted on the front wall facing the animal. A depression of the lever exceeding a force of 0.2 N produced the audible click of a relay mounted behind the front panel and was recorded as a response. The chair was also equipped with a food dispenser which could deliver 300 mg Noyes banana-flavored pellets to a receptacle located adjacent to the lever on the front wall. Three pairs of stimulus lamps mounted behind the front panel at approximately eye level were used as visual discriminative stimuli. The

shaved distal portion of the monkey's tail was held in a small stock; two brass electrodes rested on the tail which was coated with EKG sol electrode paste prior to the session. Electric shock presentation consisted of a 200-msec pulse from a 650-V AC 60-Hz transformer delivered through series resistance. Experimental sessions were conducted with the seated monkey placed inside a fan-ventilated, sound-attenuating enclosure supplied with white noise.

Procedures Three monkeys were studied under a procedure in which every 30th lever response produced a food pellet. During one schedule component in which white lights were illuminated, there were no other scheduled consequences (unpunished responding). During a second schedule condition correlated with red lights, every 30th response produced both food and shock (punished responding). Each component lasted 3 min, at the end of which the chamber was darkened for a 30-sec period when no stimuli were presented. The 3-min schedule components of unpunished and punished responding alternated regularly throughout the session, which terminated after each condition occurred five times. This procedure provided exposure to both components of the schedule and insured that drug effects would be examined under both punished and unpunished conditions.

Two additional monkeys were studied under a procedure in which, during the illumination of white lights, the first response after 3 min produced a food pellet and was followed by a 30-sec time-out period (unpunished responding). The schedule then alternated to a condition in which red lights illuminated the chamber and the first response after 3 min also produced food; however, if 30 responses were made during the 3-min interval, shock was delivered (punished responding). Under this procedure food delivery in either component did not occur until the first response after 3 min had elapsed. Thus, in contrast to the other procedures employed, changes in response rate over a wide range would not affect the frequency of food delivery. Shock intensity was adjusted individually for each monkey to produce a marked degree of suppression; the range of intensities used was between 1–7 mA.

Drugs. Drugs were administered on Tuesdays and Fridays, given that performance on the previous day did not vary from that maintained prior to the beginning of the drug series. Buspirone HCl was dissolved in 0.9% NaCl (saline) and administered intragastrically (p.o.) with an infant feeding tube 30 min prior to the experimental session. Ro 15-1788 (1 ml/kg) was suspended in water with a drop of Tween 80 and injected into the calf muscle 5 min prior to the test session. Midazolam HCl was dissolved in saline and injected into the calf muscle immediately prior to the test session. Drug effects are expressed as a percentage of the mean control rate during Thursday's sessions or during sessions in which the vehicle rather than drug was administered. Each compound was usually given on two occasions. Buspirone was supplied by Dr. K. Wheeler (Mead-Johnson Co., Evansville, IN). Benzodiazepines were supplied by Dr. W. Scott (Hoffmann-LaRoche, Nutley, NJ).

Rats

Apparatus and procedures. A modification [Mendelson et al., 1983] of the "thirsty rat conflict" test [Vogel, et al., 1971] was used in these studies. Male Sprague-Dawley rats (200 g, Taconic Farms, Germantown, NY) were water deprived for 48 hr prior to testing. The rats were briefly placed in a 10 3/4 × 8 × 8 1/2-chamber (Lafayette Instrument Co., Lafayette, IN) and allowed to locate a drinking spout (this procedure usually required less than 1 min). The rats were then removed from the chamber and administered buspirone (p.o.). In some experiments, rats were injected with CGS 8216 (2.5 mg/kg, i.p.) immediately before administration of buspirone. The animals were then returned to the home cage for 10 min prior to a 3-min trial in the experimental chamber. During the trial, 0.55-mA shocks (1-sec duration) were delivered through the drinking spout after the animal accumulated 3 sec of contact with the tube. Statistical significance was assessed using a one-way analysis of variance (ANOVA) for independent groups. A post hoc comparison of any two groups was then performed using a least significant difference test.

Drugs. CGS 8216 was the gift of Dr. W. Cash (Ciba-Geigy Corp., Ardsley, NY). This compound was suspended in 10% dimethyl sulfoxide (DMSO)-90% saline and administered in a volume of 1 ml/kg (i.p.). Control animals received equivalent volumes of vehicle. Buspirone HCl was dissolved in water and administered (1 mg/ml) orally with a feeding needle.

Neurochemical Studies

Determination of [^{3}H]diazepam binding in vivo. Ten minutes following the administration of either saline (1 ml/kg) or buspirone (2,4, or 10 mg/kg, p.o.), rats were restrained and injected via the lateral tail vein with 50 μCi of [^{3}H]diazepam (sp. act. 85.3 Ci/mmol, New England Nuclear, Boston, MA) diluted to 200 μl with saline. Rats were killed by decapitation 60 sec after injection, the brains rapidly removed, and the binding of [^{3}H]diazepam to hippocampal, cerebellar, and cortical homogenates determined as previously described [Williamson et al., 1978] with the following modification: aliquots of tissue homogenate were incubated at 0°C for 30 min in the presence and absence of flunitrazepam (final concentration, 6 μM). Data is expressed as "% Bound," which is the ratio of the amount of [^{3}H]diazepam specifically bound to the total radioactivity present in an equal volume of homogenate [Williamson et al., 1978].

Determination of [^{3}H]flunitrazepam binding ex vivo. Ten minutes after administration of saline (1 ml/kg) or buspirone (2,5, or 10 mg/kg), animals were killed by decapitation. The brains were immediately removed, then dissected, weighed, and homogenized in 9 vol (1:10) of Tris-HCl buffer (50 mM, pH 7.4) with a Brinkmann Polytron (15 sec, setting 6.5). Fifty microliters of homogenate was added to tubes containing 0.1 ml of [^{3}H]flunitrazepam (sp. act. 72.4 Ci/mmol, New England Nuclear, Boston, MA) (final concentrations, 0.5 and 5 nM) and the appropriate amount of buffer in a total volume of 1 ml. Diazepam (final concentration, 3 μM) was used to determine nonspecific binding. The tissue was incubated for 60 min at 0°C and the reaction terminated by rapid filtration over Whatman GF/B filters washed twice with 5 ml of ice-cold Tris-HCl (50 mM, pH 7.4) buffer. Protein content was determined by the Miller modification [1959] of the Lowry technique [1951].

RESULTS

Behavioral Studies

Effects of buspirone on responding of squirrel monkeys. Average rates of unpunished and punished responding were 2.36 and 0.02 responses per second, respectively. Buspirone (3 and 10 mg/kg) had little effect on unpunished responding. At the highest dose of buspirone used in this study (30 mg/kg), the rate of unpunished responding was reduced to less than 50% that observed in vehicle-treated animals (Fig. 1). A combination of buspirone and Ro 15-1788 (1 mg/kg, a dose which does not alter responding when administered alone) did not alter the effects of buspirone on unpunished responding.

Buspirone (3 and 10 mg/kg) produced increases in the rate of punished responding to a maximum of 300% of control (Fig. 1). The increased rate of punished responding was not reduced by Ro 15-1788 (1 mg/kg) (Fig. 1). At a dose of 30 mg/kg, buspirone produced only a slight increase in punished responding, but when combined with Ro 15-1788 (1 mg/kg), the rate of punished responding was significantly reduced compared to the rate observed in vehicle-treated monkeys.

The effects of midazolam (0.3 mg/kg) on punished and unpunished responding are included for comparison. This dose of midazolam slightly decreased the rate of unpunished responding, but resulted in a dramatic (20-fold) increase in the rate of punished responding. Ro 15-1788 (1 mg/kg) in combination with midazolam returned both the rate of unpunished and punished responding to control values (Fig. 1).

Effects of buspirone in the "thirsty rat conflict" test: Buspirone (0.5–5 mg/kg) produced a significant increase in the number of drinking episodes (compared with vehicle-

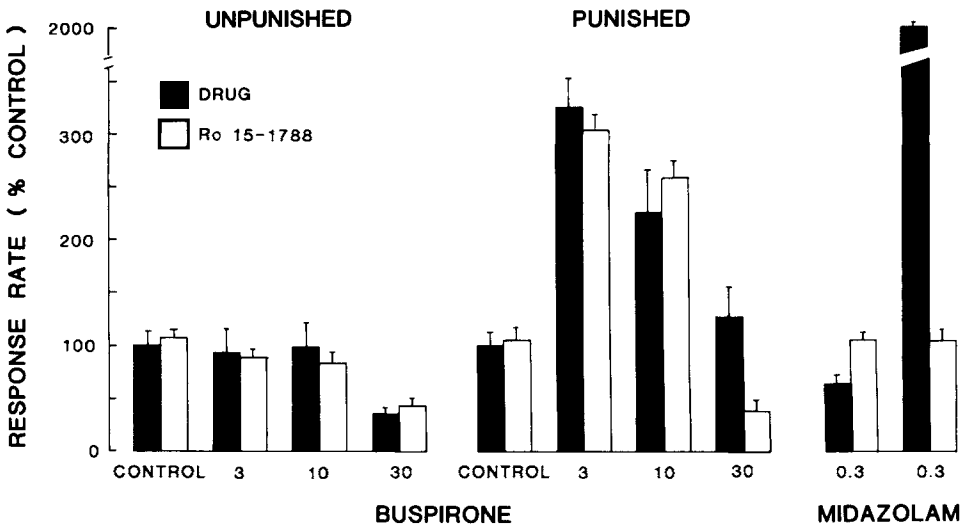

Fig. 1. Effects of buspirone and Ro 15-1788 on "conflict" responding in the squirrel monkey. Control measures represent the average rates of responding under either nondrug or vehicle control conditions. Solid bars, buspirone alone (3–30 mg/kg); open bars, buspirone in combination with Ro 15-1788 (1 mg/kg). The histograms on the right illustrate the effects of midazolam alone (solid bars) and in combination with Ro 15-1788 (open bars). Unpunished responding is shown on the left portion, punished responding on the right portion. Vertical lines above each bar represent 1 SEM.

treated rats) in the punished situation ($P < 0.04$, ANOVA) (Fig. 2). Although CGS 8216 (2.5 mg/kg, i.p.) reduced (29.7%, $P > 0.1$, NS) the number of drinking episodes during the punished situation, it did not significantly reduce the increases in punished responding elicited by buspirone (1 mg/kg) (Fig. 3). However, a combination of CGS 8216 and a higher dose of buspirone (2 mg/kg) reduced punished responding to control levels. Increasing doses of buspirone had a dual effect on performance in the conflict test. In addition to increasing punished responding in this test, doses >2 mg/kg reduced the number of animals approaching the drinking spout. For example, at a dose of 5 mg/kg, only 30% of the animals tested approached the drinking spout (Fig. 4), while at 20 mg/kg, none of the animals tested approached the drinking spout (data not shown). In vehicle treated animals, 100% of the animals approached the drinking spout during the punishment period.

In previous studies [Mendelson et al., 1983], CGS 8216 (1–5 mg/kg) did not change the percentage of animals that approached the drinking spout. In the present study, the percentage of animals that approached the drinking spout was also not altered by a combination of CGS 8216 and buspirone (1 mg/kg). Nonetheless, a combination of 2 mg/kg of buspirone and CGS 8216 reduced the number of animals that approached the drinking spout (Fig. 4).

Neurochemical Studies

Effects of buspirone on [^{3}H]diazepam binding in vivo. Buspirone (2, 4, and 10 mg/kg) significantly increased the amount of [^{3}H]diazepam bound to cerebral cortex and cerebellum (Fig. 5). However, the in vivo binding of [^{3}H]diazepam was significantly increased (19.8%) in the hippocampus only at a dose of 2 mg/kg. In the cortex and cerebellum, the increases in binding ranged from 7–10% and 12–18.5%, respectively.

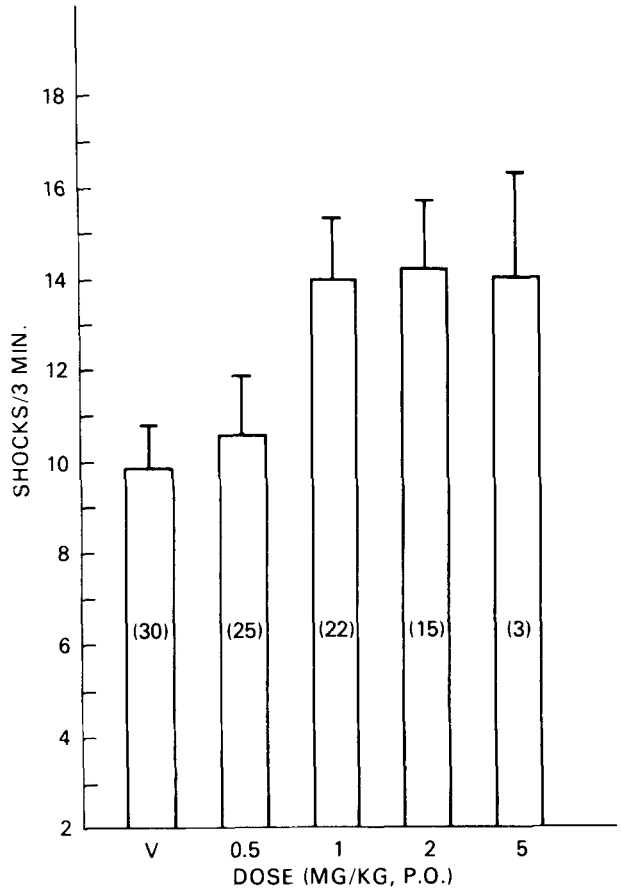

Fig. 2. Effects of buspirone in the "thirsty rat conflict" test. C, saline (1 ml/kg, p.o.); B, buspirone; V, vehicle. Dose administered (in mg/kg) is under the bar. The number of animals at each dose is shown in parentheses. The vertical lines represent 1 SEM. Not every animal tested approached the drinking spout (see Fig. 4).

Effects of buspirone on the binding of [^{3}H]flunitrazepam ex vivo. The binding of [^{3}H]flunitrazepam was examined in rat cerebellum, cortex, and hippocampus after administration of buspirone (2, 5, and 10 mg/kg). Ligand concentrations approximately 0.5- and 5-fold the apparent K_d of flunitrazepam were used in this study. No changes in binding were observed under any of the experimental conditions employed (results not shown).

DISCUSSION

The GABA-benzodiazepine receptor-chloride ionophore complex has been proposed to be the site of action for several chemically disparate classes of compounds that possess some or all of the pharmacologic properties of benzodiazepines [Paul et al., 1981]. A pharmacologic action could result from either direct occupation of the benzodiazepine receptor or an indirect action resulting in a change in either the apparent affinity or number of benzodiazepine and/or GABA receptors. Our studies with buspirone were initiated because in vitro studies have

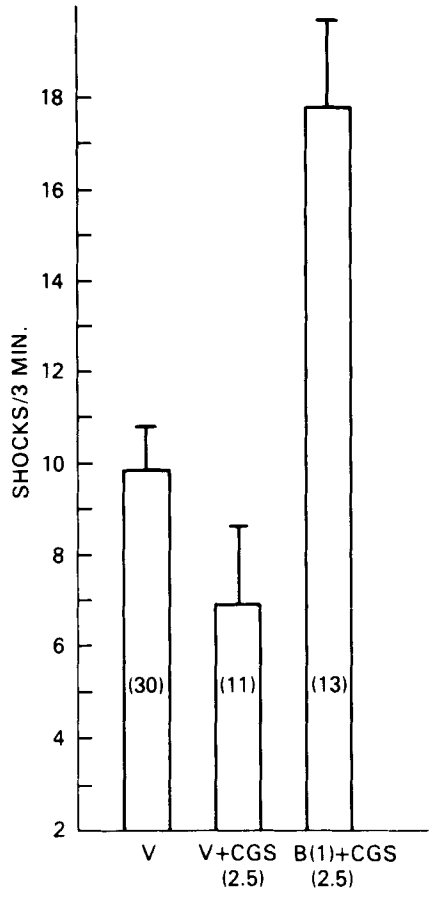

Fig. 3. Effects of CGS 8216 on the anticonflict actions of buspirone: CGS 8216 (2.5 mg/kg) was administered to saline or buspirone treated rats. The number of animals used is in parentheses. V, vehicle; CGS, CGS 8216 (2.5 mg/kg); B, buspirone (1 mg/kg).

shown this compound not to bind to receptors that have been typically associated with the anxiolytic action of drugs. However, in vitro studies do not completely rule out the possibility of an indirect action of the parent compound or a direct (or indirect) action of a metabolite to alter some component of this system. Therefore, we examined the effects of the benzodiazepine receptor antagonists CGS 8216 and Ro 15-1788 on buspirone-stimulated increases in conflict responding in monkeys and rats as well as the effects of buspirone on [^{3}H]benzodiazepine binding both in vivo and ex vivo.

Buspirone had little effect on the rate of unpunished responding in squirrel monkeys between 3–10 mg/kg. At higher doses (30 mg/kg), buspirone inhibited unpunished responding. A similar phenomenon was observed in the rat, since higher doses of buspirone (>2 mg/kg) reduced the number of rats approaching the drinking spout (Fig. 4). Nonetheless, in confirmation of earlier work [Riblet et al., 1982; Geller and Hartmann, 1982] buspirone significantly increased "conflict" responding in both species (Figs. 1,2). In the "thirsty rat conflict" test, buspirone was at least as potent but significantly less efficacious than other commonly used antianxiety agents (e.g., pentobarbital, diazepam) [Mendelson et al., 1983]. In the squirrel monkey, buspirone is neither as potent nor as efficacious as benzodiazepines (Fig. 1).

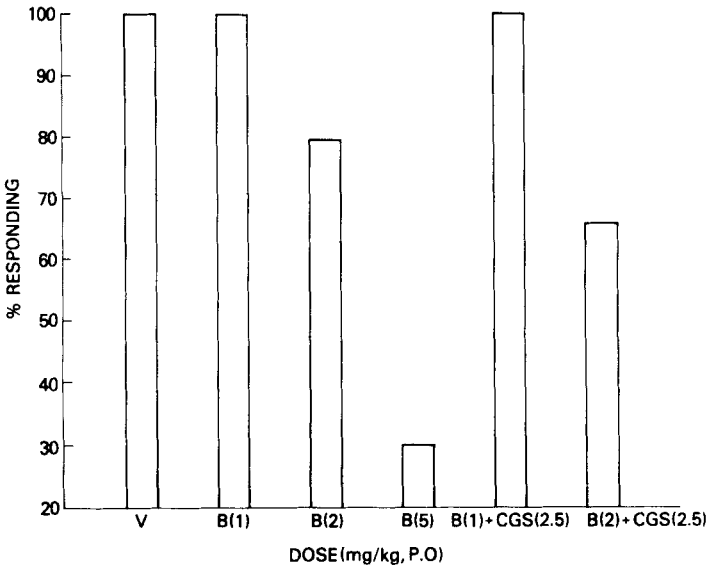

Fig. 4. Effects of buspirone on the percentage of rats approaching the drinking spout in a "thirsty rat conflict" test: V, saline; B, buspirone; CGS, CGS 8216. Numbers represent the dose of drug administered in mg/kg. All animals administered saline approached the drinking spout (100% responding). Using doses of buspirone > 10 mg/kg, none of the animals tested approached the drinking spout (data not shown).

Neither CGS 8216 (2.5 mg/kg) nor Ro 15-1788 (1 mg/kg) significantly altered nonpunished responding in rats and monkeys, respectively (Figs. 1,2). This dose of Ro 15-1788 was sufficient to antagonize the anticonflict actions of a benzodiazepine in the squirrel monkey (Fig. 1), while the dose of CGS 8216 used in rat has been shown to antagonize the anxiolytic actions of benzodiazepines and barbiturates [Bernard et al., 1981; Mendelson et al., 1983]. Despite the inability of these benzodiazepine receptor antagonists to block the anticonflict actions of relatively low doses of buspirone, a qualitatively similar reduction of the ability of animals to respond in a conflict test was observed in rats and monkeys with a combination of either benzodiazepine receptor antagonist and a larger dose of buspirone (2 and 30 mg/kg in the rat and monkey, respectively). In the monkey, this combination produced a significant decrease in the rate of both punished and unpunished responding, while in the rat, the percentage of animals approaching the drinking spout was lower than when buspirone (or CGS 8216) alone was administered. Neither the rats nor monkeys appeared sedated, and both responded in a "normal" fashion to external stimuli (e.g., handling). Geller and Hartmann [1982] also observed that rats administered buspirone (5 mg/kg) stopped responding in a conflict test involving lever pressing. Direct observation of these rats revealed no ataxia, which is usually seen when benzodiazepine-treated animals stop lever pressing in this test. Since this phenomenon was observed at doses of buspirone higher than those needed to produce a maximum (or near maximum) effect in the conflict tests used, it must be assumed that a complex interaction occurs with higher doses of buspirone and a benzodiazepine receptor antagonist, rather than an antagonism of the anticonflict actions of buspirone. The reduction in the number of rats which approach a drinking spout with a combination of buspirone (2 mg/kg) and CGS 8216 would support this hypothesis. Under conditions which caused a reduction of the percentage of rats approaching the drinking spout, visual observation did not reveal a

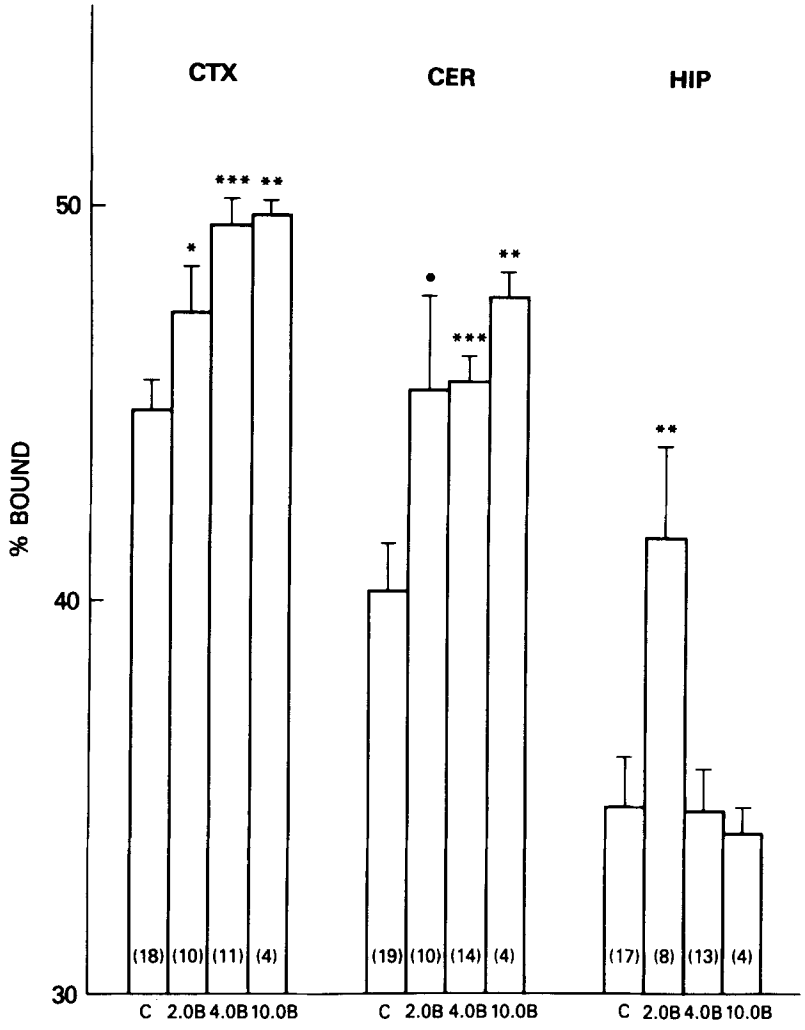

Fig. 5. Effects of buspirone on the binding of [^{3}H]diazepam in vivo: The data represent the mean ± SEM of the percentage of [^{3}H]diazepam specifically bound, as described in Methods. The number of animals used at each dose of buspirone (B) is shown in methods. CTX, cerebral cortex; CER, cerebellum; HIP, hippocampus. $^{+}P < 0.05$ vs. saline-treated rats (C); $^{**}P < 0.01$ vs. saline-treated rats; $^{***}P < 0.001$ vs. saline-treated rats.

sedation or ataxia. Animals responded in an apparent "normal" fashion to handling and tactile stimulation.

Small but statistically significant increases in [^{3}H]diazepam binding in vivo were observed in the cerebella, hippocampi, and cortices of rats pretreated with doses of buspirone which were active in the conflict test. The increases were also present in both the cerebellum and cortex at doses observed to dramatically reduce the number of animals which will approach a drinking spout under these conditions. It is not known if the changes in [^{3}H]diazepam binding in vivo are related to the anticonflict action of buspirone. Such changes have been observed with a number of compounds which have anxiolytic or anticonflict actions (see Mennini and

Garattini [1982] for review). Nonetheless, the dramatic increase in the number of animals which do not approach the drinking spout after higher doses of buspirone may not be related to the increases in [^{3}H]diazepam binding, since increases in binding were observed at doses below those which reduce responding.

Garattini et al. [1982] have also observed an increase in [^{3}H]diazepam binding after buspirone. However, this effect was not observed when [^{3}H]flunitrazepam was used as a radioligand. In contrast, Oakley and Jones [1983] reported increases of 200–300% in [^{3}H]flunitrazepam binding (in vivo) after buspirone (20–80 mg/kg). These doses of buspirone are substantially higher than those reported to cause a cessation of both punished and unpunished responding [Fig. 4 and Geller and Hartmann, 1982]. At the present time, it is difficult to reconcile both the remarkable increases in in vivo binding and the efficacy of buspirone at these high doses with our findings and those of other investigators [Geller and Hartmann, 1982; Garattini et al., 1982].

Since differences were observed in the binding of [^{3}H]diazepam in vivo, we attempted to study this phenomenon ex vivo to examine the kinetic nature of these changes. Comparable doses of buspirone led to no significant difference in [^{3}H]benzodiazepine binding ex vivo. However, it should be noted that using the ex vivo technique requires a tissue dilution of approximately 200-fold. Thus, any active compounds would be diluted by the same amount. Therefore, the failure to find a comparable change in [^{3}H]benzodiazepine binding ex vivo may be due to technical limitations of the method rather than the lack of effect of buspirone (or an active metabolite).

We have observed that benzodiazepine receptor antagonists do not block the anticonflict actions of buspirone. This observation implies that the pharmacologic actions of buspirone do not result from the direct occupation of benzodiazepine receptors by the drug or an active metabolite of buspirone. The failure of CGS 8216 to block the anticonflict actions of buspirone also suggests that the compound may not act in a pentobarbital like fashion [Mendelson et al., 1983]. However, Patel et al. [1983] have reported that 20 mg/kg of CGS 8216 did not antagonize the anticonflict actions of either SQ 65,396 or pentobarbital. This observation suggests that antagonism of the anticonflict actions of a compound which does not act via direct occupation of the benzodiazepine receptor may be very dependent on the behavioral paradigms employed. Furthermore, several 1,5-benzodiazepines such as tofizopam also cause increases in benzodiazepine binding in vitro and in vivo (see Mennini and Garattini [1982] for review), and it is not known if the anticonflict actions of such compounds can be antagonized by benzodiazepine receptor antagonists. The finding by these and other laboratories [Garattini et al., 1982; Oakley and Jones, 1983] that buspirone can increase benzodiazepine binding in vivo suggests an effect of buspirone on the GABA-benzodiazepine receptor-chloride ionophore complex, which has been previously associated with the antianxiety actions of many chemically dissimilar compounds. Whether there is a causal relation between the effects observed and the antianxiety and anticonflict actions of buspirone is currently under investigation.

ACKNOWLEDGMENTS

The authors thank Mr. Michael Jackson for excellent technical assistance and Ms. Cindy Owen for statistical evaluation of the data.

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