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Behavioral and Neurochemical Studies on the Anticonflict Actions of Buspirone

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ABSTRACT

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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

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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.

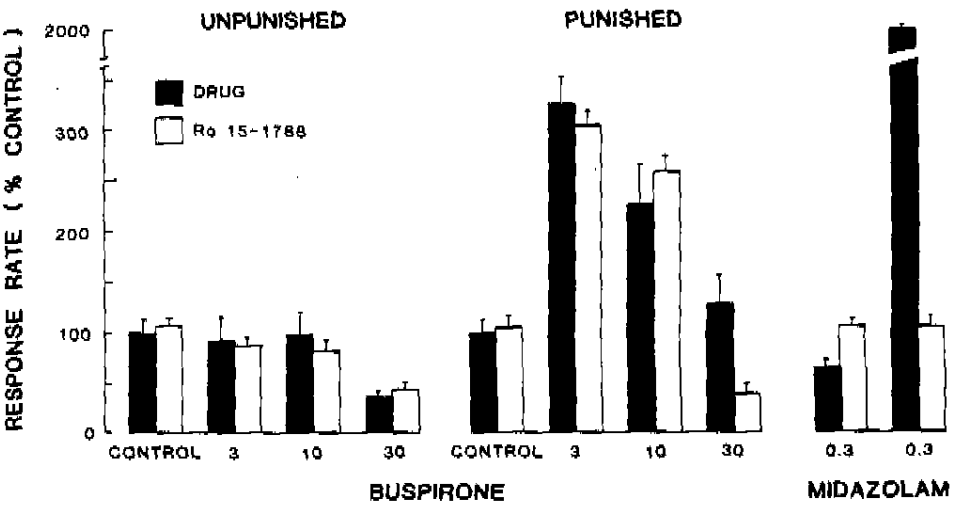

Fig. 1. Effects of buspirone and Ro 15-1788 on "conflict" responding in the squirrel monkey. Control measures represent the average rates of responding under either nondrug or vehicle control conditions. Solid bars, buspirone alone (3–30 mg/kg); open bars, buspirone in combination with Ro 15-1788 (1 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.

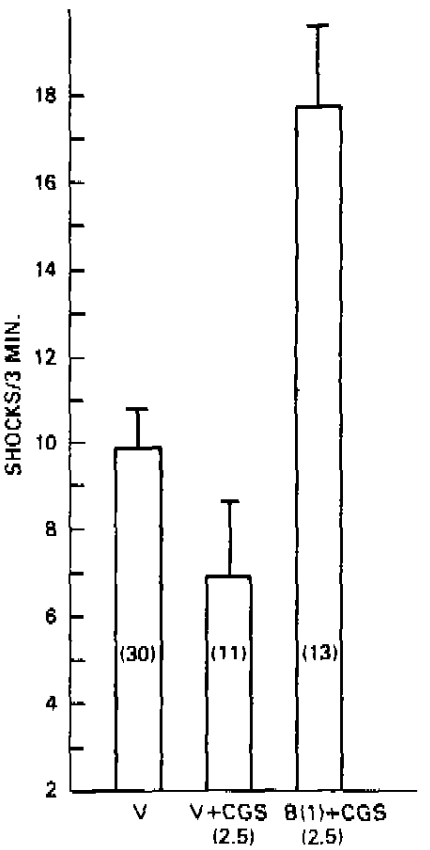

Fig. 3. Effects of CGS 8216 on the anticonflict actions of buspirone: CGS 8216 (2.5 mg/kg) was administered to saline or buspirone treated rats. The number of animals used is in parentheses. V, vehicle; CGS, CGS 8216 (2.5 mg/kg); B, buspirone (1 mg/kg).

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).

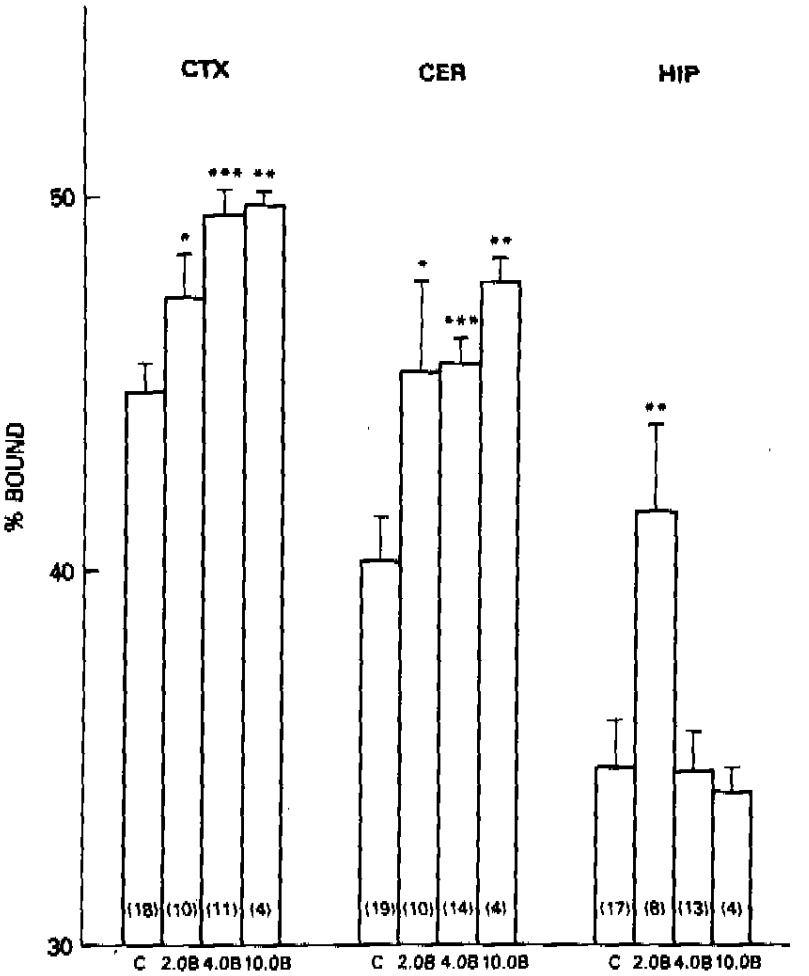

Fig. 5. Effects of buspirone on the binding of [^{3}H]diazepam in vivo: The data represent the mean ± SEM of the percentage of [^{3}H]diazepam specifically bound, as described in Methods. The number of animals used at each dose of buspirone (B) is shown in methods. CTX, cerebral cortex; CER, cerebellum; 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

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