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Benzodiazepine-Receptor Mediated Inhibition of Isolation-Induced Aggression in Mice

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SKOLNICK, P., G. F. REED AND S. M. PAUL. *Benzodiazepine-receptor mediated inhibition of isolation-induced aggression in mice.* PHARMACOL BIOCHEM BEHAV **23**(1) 17–20, 1985.—The effects of a benzodiazepine receptor agonist (diazepam), antagonist (Ro 15-1788), and an "active" antagonist [inverse agonist] (3-carboethoxy-β-carboline) were examined in an isolation-induced model of aggression. Diazepam (4 mg/kg) and 3-carboethoxy-β-carboline (10 mg/kg), but not Ro 15-1788, significantly inhibited aggressive behavior in this model. Ro 15-1788 (10 mg/kg) reduced the anti-aggressive actions of both diazepam and 3-carboethoxy-β-carboline, while mice treated with a combination of diazepam and 3-carboethoxy-β-carboline had aggression scores increased to values not significantly different from vehicle treated mice. These findings suggest that both diazepam and 3-carboethoxy-β-carboline have anti-aggressive properties in the isolation-induced model of aggression which are mediated through CNS benzodiazepine receptors.

Aggression Diazepam Ro 15-1788 3-Carboethoxy-β-carboline Isolation

SEVERAL lines of evidence suggest that the principal pharmacologic actions of the benzodiazepines (anxiolytic, anticonvulsant, muscle relaxant, hypnotic) are mediated via saturable, high affinity and stereospecific binding sites present in the central nervous system [16, 18, 27]. Furthermore, statistically significant correlations have been reported between the potencies of a series of 1,4-benzodiazepines in displacing [^{3}H]diazepam from these sites and their potencies in inhibiting electroshock-induced fighting in mice ($r=0.75$–0.87; $p<0.005$–<0.001) [15, 18, 19]. However, in another widely used animal model of aggression, the taming of cynomolgus monkeys, either no significant correlation [15,18] or a correlation lower ($r=0.655$; $p<0.01$) [27] than that observed for reducing footshock-induced fighting was reported. The anti-aggressive actions of benzodiazepines have also been extensively studied in an isolation-induced model of aggression in mice (cf. [9,29] for review). Although benzodiazepines have been reported to reduce aggression in this model, correlations between the anti-aggressive potencies of benzodiazepines in this model and their affinities for benzodiazepine receptors have not been reported. Thus, the extent to which CNS benzodiazepine receptors are involved in the anti-aggressive actions of benzodiazepines is unclear.

In the present study, we have examined the effects of a benzodiazepine antagonist (Ro 15-1788) [12] and an "active" benzodiazepine antagonist [inverse agonist] (3-carboethoxy-β-carboline; β-CCE [20]) alone and in combination with diazepam in an isolation-induced model of aggression. We now report a robust anti-aggressive action of both the benzodiazepine, diazepam, and the active benzodiazepine receptor antagonist, β-CCE. Further, the anti-aggressive effects of both diazepam and β-CCE are antagonized by Ro 15-1788. These findings suggest that CNS benzodiazepine receptors are involved in the pharmacologic actions of both diazepam and β-CCE in this model of aggression. β-CCE, which possesses "proconflict" actions in rodents [4] and elicits a syndrome resembling fear or anxiety in primates [3, 13, 20] also inhibits isolation-induced aggression through a specific action at CNS benzodiazepine receptors, suggesting that some forms of aggressive behavior may be inhibited through the production of "fear" or "anxiety."

METHOD

Adult, male NIH mice (20–25 g) (Veterinary Resources Branch, National Institutes of Health, Bethesda, MD) were individually housed in suspended wire cages (20.3×28×16.5 cm) or group housed (20/group) in metal cages (35.6×35.6×17.8 cm). Mice were housed for at least 28 days prior to testing in a 12 hr light/dark cycle (lights on at 0600) with free access to rat chow and water. Pairs of group housed (from a common cage) or isolated mice were placed in a neutral arena (4 L beaker, 14 cm diameter) and observed for ten minutes by two investigators. The beaker was thoroughly rinsed and dried between trials. Aggressive behavior was rated according to a modification of the scale reported

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by Valzelli *et al.* [30]. Pairs of mice which displayed no aggressive behavior during the 10 min observation period were rated "0." Mice which assumed a posture of readiness to fight (arched back, tail rattling) and occasionally attacked (3–10 times/observation period) were rated "25." Pairs which fought 20 or less times during this period were rated "50." Mice were rated "75" if more than 25 episodes of fierce wrestling and biting were observed. A rating of "100" was given if continuous fierce biting and wrestling was observed for most of the observation period, or if the attacks were severe enough for blood to be drawn. An attack (which was often preceded by tail rattling) usually consisted of biting, wrestling, tumbling, and boxing. Attacks were usually brief, broken off abruptly, and rapidly resumed. Mice frequently vocalized during these attacks. If animals were rated "100," attacks usually occurred continuously once the initial encounter was made. The number of attack episodes was independently scored by two observers. The reliability of this rating system is supported by the close agreement in both the number of episodes scored (±10%) and the overall rating score (identical in >95% of the cases) between the two observers.

The sensitivity of group housed and isolated mice to diazepam was also examined in a rotarod test. Both group housed and isolated mice were allowed a one week period in their home cages after testing for aggressive behavior. Immediately prior to diazepam injection, mice were placed on a rotarod apparatus (Rotamex, Columbus Instruments, Columbus, OH) for 1–2 min at a speed of 6 RPM. Mice unable to remain on the rotarod during this pretest were discarded. Thirty min after diazepam treatment, mice were placed on the rotarod and observed for 5 min. Animals that dropped from the rotarod twice during this period were scored as motor impaired. Ten grouped and 10 isolated mice were used at each dose of diazepam.

Drugs were dissolved in 10% diluted Emulphor (50% Emulphor-EL 620-50% ethanol)-90% saline [20] with warming and injected subcutaneously (midline dorsal surface) in a volume of 0.1 ml. Each injection was made at a separate site along the dorsal surface. Vehicle injected mice received 0.1 ml of 10% diluted Emulphor-90% saline. Drugs examined for their anti-aggressive properties were administered 30 min prior to behavioral testing with the exception of Ro 15-1788, which was injected 10 min prior to testing. Ro 15-1788 and diazepam were the gifts of Dr. Peter Sorter, Hoffmann-LaRoche, Nutley, NJ. β-CCE was supplied by Dr. James Cook, Univ. Wisconsin-Milwaukee. Emulphor (EL-620) was donated by GAF Corporation, New York, NY.

Statistical Evaluation

The number of attacks was recorded and aggressivity scores rated as described. Two-sided *t*-tests [26] on mean aggression scores were used to evince differences between drug treatments. The chosen significance level was ≤ 0.05, but because multiple comparisons were made, the Bonferroni criterion [17] (i.e., reject the hypothesis of no difference when $p \leq 0.05$/number of comparisons [17]) was applied to individual comparisons in order to maintain an overall level of significance of $p \leq 0.05$. Rotarod performance was evaluated using a χ^2 test comparing group housed and isolated mice treated with the same dose of diazepam.

RESULTS

Ten pairs of group housed mice were examined in the neutral arena for the presence of aggressive behavior. No signs of aggressive behavior (tail rattling, arched back, biting, boxing, vocalization, or wrestling) were observed in these animals (data not shown). In contrast, pairs of isolated animals displayed a marked degree of aggressive behavior characterized by fierce wrestling, boxing and biting episodes. These behaviors (scores≧50) were observed in 15/16 pairs of vehicle injected (control) animals (Fig. 1A). In confirmation of previous findings [30] diazepam (4 mg/kg) reduced ($p << 0.001$) isolation-induced aggression. No attacks were observed in 8/9 pairs tested (Fig. 1B). β-CCE also inhibited isolation-induced aggression behavior to about the same extent as diazepam ($p << .001$) (Fig. 1C). Mice administered the benzodiazepine antagonist, Ro 15-1788 (10 mg/kg) had mean aggression scores which were not statistically significantly different from vehicle-injected mice, but the distribution of these scores suggests a weak anti-aggressive action (Fig. 1D).

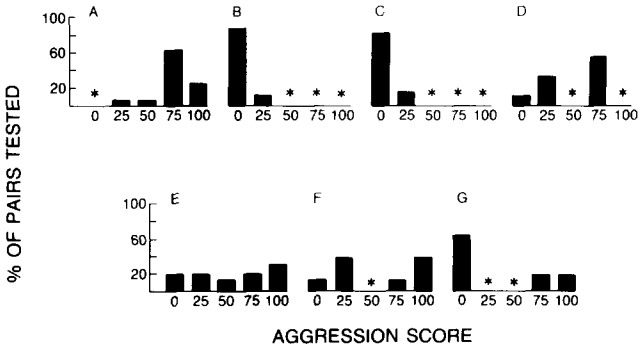

FIG. 1. Effects of benzodiazepine receptor ligands on isolation-induced aggression. Adult male NIH mice were individually housed for at least 28 days prior to testing as described in the Method section. Each pair of mice received the same drug treatment. Panel A: vehicle/vehicle injected mice (16 pairs); Panel B: vehicle/diazepam (4 mg/kg) (9 pairs); Panel C: vehicle/β-CCE (10 mg/kg) (6 pairs); Panel D: vehicle/Ro 15-1788 (10 mg/kg, 10 min prior to testing) (9 pairs); Panel E: Ro 15-1788/diazepam (16 pairs); Panel F: Ro 15-1788/β-CCE (8 pairs); Panel G: β-CCE/diazepam (11 pairs). Statistical significance was determined using a *t*-test. The level of statistical significance ($p \leq 0.05$) was corrected for the seven comparisons to maintain the overall level of significance at $p \leq 0.05$ ($0.05/7 = 0.00714$) (see the Method section): vehicle/vehicle versus vehicle/diazepam or vehicle/β-CCE: $p << 0.001$; vehicle/vehicle versus Ro 15-1788/β-CCE, β-CCE/diazepam, vehicle/Ro 15-1788, Not significant ($p > 0.00714$); vehicle/vehicle versus Ro 15-1788/diazepam, $p = 0.005$; vehicle/diazepam versus Ro 15-1788/diazepam, $p = 0.006$. Symbol: *not seen in any of the pairs tested.

Since Ro 15-1788 can antagonize many of the pharmacologic actions of both benzodiazepines and "active" benzodiazepine antagonists [12, 20, 22], the effects of Ro 15-1788 on the anti-aggressive actions of diazepam and β-CCE were examined. Ro 15-1788 administered 10 min prior to testing markedly attenuated the anti-aggressive actions of both compounds. The combination of Ro 15-1788 and diazepam (Fig. 1E) resulted in aggression scores that were increased to values that were significantly higher than diazepam, though significantly lower than control mice. Aggression scores in mice treated with a combination of Ro 15-1788 and β-CCE were not significantly different from vehicle treated animals (Fig. 1F). Mice treated with both

β-CCE and diazepam had aggression scores which were not significantly different from control mice (Fig. 1G). The gross behavior of animals injected with these agents did not appear to be different from vehicle injected mice. No signs of ataxia, sedation convulsions or other aberrant forms of behavior were seen with this drug combination.

Rotarod performance in both group-housed and isolated mice was impaired in a dose-dependent manner by diazepam (Fig. 2). The dose of diazepam used to inhibit isolation-induced aggression (4 mg/kg) was without effect on the rotarod performance of group housed animals, while a dose of 6 mg/kg, which is 50% higher than that required to completely reduce isolation-induced aggression, elicited only a marginal impairment of rotarod performance in isolated mice (Fig. 2). As previously reported [10,29], isolated mice required significantly higher doses of diazepam ($p<0.01$, χ^2) to produce the same degree of rotarod impairment as seen in group housed mice (Fig. 2).

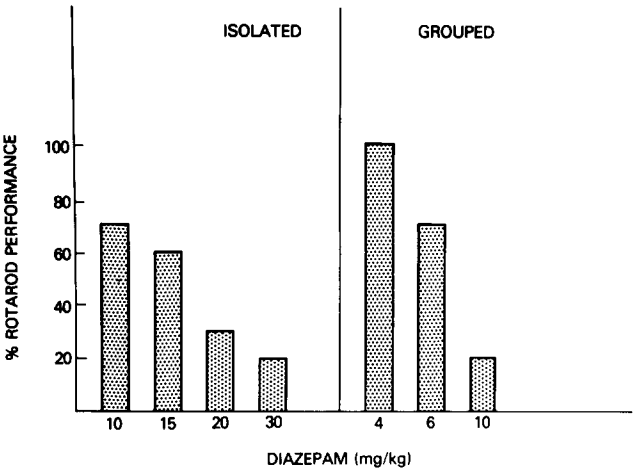

FIG. 2. Impairment of rotarod performance by diazepam: effects of isolation. Mice were tested for their abilities to remain on a rotarod apparatus as described in the Method section. Diazepam (0.1 ml) was administered subcutaneously 30 min prior to testing. Ten animals per group were used for each dose of diazepam. Ordinate: rotarod performance expressed as the percent of animals which remained on the rotarod during the test period. At a dose of 10 mg/kg, a significantly greater proportion ($p<0.01$, χ^2 analysis) of isolated animals remained on the rotarod apparatus compared with group-housed mice. These animals had previously been examined for their aggressive behavior as described in the Method section one week prior to rotarod testing. After examination of aggressive behavior, both group housed mice and isolated animals were returned to their home cages for this one week washout period.

DISCUSSION

Benzodiazepines have been reported to reduce feelings of hostility and aggression in man [2,7] and effectively inhibit aggressive behavior in several animal models [28,29]. Although several lines of evidence link CNS benzodiazepine receptors to the principal pharmacologic actions of the benzodiazepines, the role of these sites in the taming or anti-aggressive actions of benzodiazepines is unclear. Significant correlations have been reported between the affinities of a series of benzodiazepines to displace [^{3}H]diazepam and inhibition of footshock-induced fighting in mice [15, 18, 19], although a much weaker [27] (or no) [15,18] correlation has been reported for the ability of benzodiazepines to tame cynomolgus monkeys, another frequently used measure of anti-aggressive action. Thus, the involvement of benzodiazepine receptors in the anti-aggressive actions of benzodiazepines could depend on such factors as the species and the test used to determine anti-aggressive action. High affinity ligands of the benzodiazepine receptor which have little or no intrinsic pharmacologic action but antagonize the effects of both benzodiazepines and "active" benzodiazepine antagonists (e.g., β-carbolines) provide a pharmacologic tool for determining if a particular pharmacologic effect is benzodiazepine receptor-mediated. The present studies were designed to determine whether the anti-aggressive actions of benzodiazepines are receptor mediated by using the benzodiazepine antagonist, Ro 15-1788. The data clearly demonstrate a reduction in the anti-aggressive actions of diazepam by Ro 15-1788 (Fig. 1). When administered alone under these conditions, Ro 15-1788 tended to reduce aggressive behavior, although this reduction was not statistically significant (Fig. 1C). Benzodiazepine-like actions of Ro 15-1788 have been noted in several pharmacologic paradigms [1, 5, 14, 21], particularly when high doses are used. Nonetheless, at least one group has reported intrinsic actions of Ro 15-1788 which are opposite to those produced by the benzodiazepines in a test of social interaction in rats [11]. The dose of Ro 15-1788 used in the present study is relatively high, especially when the time interval (10 min) between injection and behavioral testing is considered. Thus, a partial reversal of the anti-aggressive action of diazepam could be interpreted as a reversal confounded by a weak partial agonist (diazepam-like) action of Ro 15-1788. Nonetheless, the marked attenuation of the anti-aggressive actions of diazepam by Ro 15-1788 provides evidence that a large component of the anti-aggressive action of diazepam is mediated via benzodiazepine receptors.

The finding that β-CCE is as efficacious as diazepam in reducing isolation-induced aggression was surprising in view of the observations that β-CCE and other "inverse agonists" can elicit pharmacological actions best described as opposite to the benzodiazepines [25], although many different psychopharmacologic agents have been shown to inhibit this behavior [30]. The mechanism for this reduction in aggressive behavior is unclear, but several explanations are possible. β-CCE (10 mg/kg, IV) has been shown to elicit electroencephalographic seizures in rats [24]. However, β-carboline esters are rapidly degraded by rodent plasma *in vitro* [23], which makes it unlikely that brain concentrations of β-CCE sufficient to elicit seizures would be achieved 30 min after subcutaneous administration. β-CCE injected mice appeared as active as control mice during the 30 min interval prior to behavioral testing (unpublished observation). Although rotarod performance was not examined after β-CCE treatment, de Cavalho *et al.* [6] reported that 1 mg/kg (IV) of 3-carbomethoxy-β-carboline (the methyl analog of β-CCE) did not impair rotarod performance in mice 10 min prior to testing. β-CCE has also been found to have a "proconflict" action in two rodent behavior models predictive of anxiolytic action [4,11]. This "proconflict" action in rodents has been interpreted as an anxiogenic action of β-CCE, which is supported by observations in Rhesus monkeys [3, 13, 20] demonstrating a syndrome reminiscent of fear or anxiety in humans. A closely related β-carboline, FG 7142, has been reported to cause anxiety in human volunteers [8]. The proconflict effects of β-CCE in rodents, and the anxiety-like

symptoms in primates can be antagonized by either a benzodiazepine or Ro 15-1788. Although the inhibition of isolation-induced aggression by β-CCE cannot be directly attributed to an anxiogenic action, a reversal by Ro 15-1788 suggests that the anti-aggressive action of this compound is mediated by benzodiazepine receptors.

In confirmation of previous findings [10,29], isolated mice were less sensitive to diazepam than group housed mice in a test of rotarod performance (Fig. 2). The doses of diazepam used in this study which markedly reduced aggressive behavior in isolated mice (4 mg/kg) did not impair rotarod performance even in group housed mice. This finding supports the suggestion that the anti-aggressive properties of benzodiazepines may be distinct from their muscle relaxant and sedative actions [29], although this hypothesis has been disputed [7].

The present findings implicate benzodiazepine receptors as mediators of the anti-aggressive actions of benzodiazepines in an isolation-induced model of aggression. Furthermore, an anti-aggressive action of an "anxiogenic" benzodiazepine antagonist (β-CCE) has also been documented. Although the anti-aggressive action of β-CCE appears to be receptor-mediated, it is not known whether the anxiogenic action or some other property of this compound is responsible for its anti-aggressive action. Furthermore, the role of other neurotransmitter systems in the anti-aggressive actions of diazepam (and β-CCE) are unknown. However, prolonged isolation has been shown to affect several putative neurotransmitters such as norepinephrine and dopamine [9, 28, 29], as well as benzodiazepine receptors [10]. Nonetheless, benzodiazepine receptor ligands such as Ro 15-1788 and β-CCE may provide important tools for studying a variety of aggressive behaviors.

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