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Blockade of 3-Carbomethoxy-β-Carboline Induced Seizures by Diazepam and the Benzodiazepine Antagonists, Ro 15-1788 and CGS 8216

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SCHWERI, M., M. CAIN, J. COOK, S. PAUL AND P. SKOLNICK. *Blockade of 3-carbomethoxy-β-carboline induced seizures by diazepam and the benzodiazepine antagonists, Ro 15-1788 and CGS 8216.* PHARMAC. BIOCHEM. BEHAV. **17**(3) 457–460, 1982.—The benzodiazepine antagonists Ro 15-1788 and CGS 8216 blocked the clonic and tonic convulsions elicited by 3-carbomethoxy-β-carboline (β-CCM). The PD_{50} values for Ro 15-1788, CGS 8216, and diazepam were: 2.0, 0.6, and 2.0 mg/kg, respectively. Neither Ro 15-1788 nor CGS 8216 potentiated the effect of a threshold convulsant dose of β-CCM. Moreover, these benzodiazepine antagonists neither attenuated nor potentiated the tremorigenic actions of another β-carboline, harmaline. Diazepam, however, considerably reduced the tremorigenic actions of this drug.

Ro 15-1788 CGS 8216 3-Carbomethoxy-β-carboline Benzodiazepine antagonists

A NUMBER of C-3 substituted β-carbolines have been reported to bind with high affinities to the benzodiazepine receptor *in vitro* [5], and to antagonize the pharmacologic actions of the benzodiazepine *in vivo* [6, 7, 22, 26]. Two other chemically distinct classes of compounds, the imidazobenzodiazepines (e.g., Ro 15-1788, ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imadazo[1,5a]-[1,4]benzodiazepine-3-carboxylate) and the pyrazoloquinolinones (e.g., CGS 8216, 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one) have also been reported to possess high affinities for benzodiazepine receptors *in vitro* and potently antagonize the pharmacologic actions of benzodiazepines in both experimental animals and man [1, 2, 8, 9, 12, 16]. Nonetheless, initial reports characterizing these benzodiazepine antagonists suggest subtle differences in their pharmacologic actions. For example, both CGS 8216 [1] and 3-hydroxymethyl-β-carboline [6] antagonize the anxiolytic actions of meprobamate in experimental animals while such an action has not been reported for Ro 15-1788 [12]. Furthermore, β-carbolines such as 3-carboethoxy-β-carboline (B-CCE) have a proconvulsant action (i.e., they potentiate the convulsant action of drugs such as pentylenetetrazole) [18], while Ro 15-1788 and CGS 8216 do not appear to be proconvulsants [1,12]. Furthermore, at relatively high doses (35 mg/kg) Ro 15-1788 has been reported [13] to have a partial agonist action in both anxiolytic and anticonvulsant paradigms, while β-carbolines and CGS 8216 exhibit only antagonist actions [1, 15, 25, 26].

Differences in the pharmacologic profiles of these benzodiazepine antagonists, coupled with the recent observation [4] that 3-carbomethoxy-β-carboline (β-CCM) elicts pentylenetetrazole-like seizures in mice, provided an impetus for the present study on the effects of the benzodiazepine antagonists CGS 8216 and Ro 15-1788 on the convulsant properties of β-CCM. The structure of β-CCM is shown in Fig. 1. We now report that both CGS 8216 and Ro 15-1788 antagonize the convulsant actions of β-CCM. Ro 15-1788 is equipotent with diazepam in preventing β-CCM induced seizures, while CGS 8216 is approximately threefold more potent than diazepam. In contrast, neither CGS 8216 nor Ro 15-1788 were able to prevent or facilitate the tremorigenic actions of the β-carboline, harmaline, whose tremorigenic action was readily antagonized by diazepam.

METHOD

Adult, male NIH mice (22–28 g) obtained from the Veterinary Resources Branch, NIH, Bethesda, MD, were used in these studies. Drugs were administered intraperitoneally

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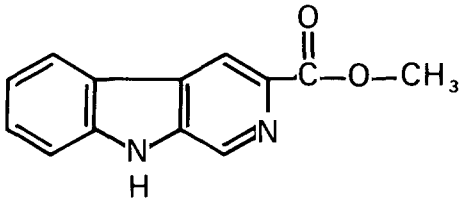

FIG. 1. Structure of 3-carbomethoxy-β-carboline (β-carboline-3-carboxylic acid methyl ester; β-CCM).

(IP) in a volume of 100 μl. In studies using β-CCM, animals were observed for 15 min following injection for the appearance of clonic and tonic convulsions. In studies using harmaline, animals were observed for 20 min post-injection for the appearance of tremors. Other drugs were administered 15 min prior to either β-CCM or harmaline unless otherwise noted.

β-CCM was dissolved in 0.55 N HCl and diluted sevenfold with phosphate buffered saline, pH 7.2 (PBS). CGS 8216 was dissolved in dimethylsulfoxide and diluted ten-fold with PBS. When used in conjunction with β-CCM, Ro 15-1788 was dissolved in diluted Emulphor (EL-620, GAF Corp., New York, NY, which was diluted 1:1 with 95% ethanol) and this solution diluted tenfold with PBS. When used in conjunction with harmaline, Ro 15-1788 was dissolved in three parts 0.7 N HCl and diluted with seven parts PBS. Harmaline was dissolved in 1 N HCl and diluted twenty-five fold with PBS. Vehicle-injected mice received an equal volume (i.e., 100 μl) of the appropriate vehicle.

β-CCM was synthesized as previously described [6]. Ro 15-1788 was donated by Dr. W. Scott, Hoffmann-LaRoche, Nutley, NJ, and CGS 8216 was the gift of Dr. W. Cash, Ciba-Geigy Corp., Ardsley, NY. Harmaline was obtained from Dr. J. Daly, NIH. All other drugs and solutions were obtained from standard commercial sources.

RESULTS

The dose-response curve for the convulsant action of β-CCM is biphasic (Fig. 2). Clonic and tonic convulsions were routinely observed in 82±2% (Mean±SEM) of mice within 15 minutes of an IP injection of 29±3 mg/kg of drug (17 trials, 5–10 mice per group). This represents a range of 70–100% of mice convulsing following a dose of 10–60 mg β-CCM/kg. Higher doses of β-CCM resulted in a significant reduction in the effectiveness of this compound. A marked strain difference in sensitivity to the convulsant actions of this compound was noted. General Purpose (GP) mice obtained from the NIH Colony proved much less susceptible to the convulsant actions of β-CCM than the NIH strain mice used in these studies (unpublished observations). The factors responsible for this strain difference are currently under investigation.

Ro 15-1788, CGS 8216 and diazepam blocked the convulsant action of β-CCM in a dose-dependent fashion when administered fifteen minutes prior to a CD_{80-100} (the dose of drug needed to elicit convulsions in 80–100% of the mice tested) of β-CCM (Table 1). CGS 8216 was most potent, with a PD_{50} (the dose needed to protect 50% of the mice against β-CCM-induced seizures) of ~0.6 mg/kg. Diazepam and Ro 15-1788 were less potent, with PD_{50} values of ~2 mg/kg. PD_{50} values for diazepam, Ro 15-1788 and CGS 8216 were graphically estimated.

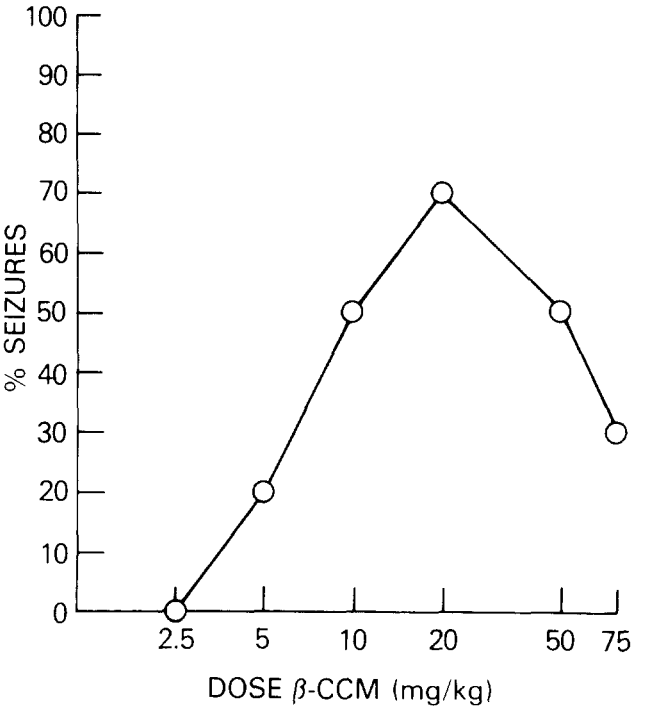

FIG. 2. Dose-response curve for the convulsant activity of β-CCM. Mice were injected IP with various doses of β-CCM. The number of mice undergoing clonic-tonic seizures within fifteen minutes of injection was recorded. Ten mice were examined at each dose level. This experiment was repeated a second time with similar results.

Since a biphasic dose-response curve was obtained with β-CCM, a second series of experiments were conducted analogous to those described above, but using a submaximum dose of β-CCM. These experiments were performed to determine if Ro 15-1788 and CGS 8216 antagonize the actions of β-CCM by shifting the dose response curve past the optimum dose of β-CCM. Neither CGS 8216 (0.5–2.0 mg/kg) nor Ro 15-1788 (0.5–2.5 mg/kg) had any effect on a subconvulsant (5 mg/kg) dose of β-CCM (Table 2).

In agreement with a previous report [14], diazepam attenuated the tremorigenic actions of harmaline. Pretreatment of mice with 2 mg/kg of diazepam (IP) ten minutes prior to administration of harmaline (30 mg/kg) significantly reduced the severity of tremors (n=5). In contrast, neither CGS 8216 nor Ro 15-1788 elicited a similar attenuation (Table 3). Furthermore, these compounds did not induce tremors in mice administered a submaximum dose (4–5 mg/kg, IP) of harmaline.

DISCUSSION

Three chemically distinct classes of compounds (i.e., imidazobenzodiazepines, β-carbolines, and pyrazoloquinolinones) have been reported to possess high affinities for benzodiazepine receptors *in vitro* and antagonize the pharmacologic actions of the benzodiazepines *in vivo*. Nonetheless, subtle pharmacologic and neurochemical distinctions have been reported among these classes of com-

TABLE 1

BLOCKADE OF β-CCM-INDUCED CONVULSIONS BY RO 15-1788, CGS 8216, AND DIAZEPAM

Ro 15-1788 (mg/kg)	0.1	0.45	1.25	2.5	5	10	20	35
% Protection	0 (10)	12 (10)	38 (10)	38 (10)	85 (7)*	100 (10)*	79 (10)*	100 (10)*
CGS 8216 (mg/kg)	0.1	0.5	1	5	10			
% Protection	20 (10)	30 (10)	90 (10)*	100 (10)*	100 (10)*			
Diazepam (mg/kg)	0.5	1	2	3	5			
% Protection	25 (10)	25 (10)	50 (10)	62.5 (10)	100 (10)*			

A $C.D._{50-100}$ of β-CCM was used. See Method section for experimental procedure. Numbers in parentheses denote the number of mice in each group. Control groups contained 5–10 mice. % Protection = [(fraction seizing after β-CCM)-(fraction seizing after treatment with drug and β-CCM)] × 100/(fraction seizing after β-CCM).

*Significantly different from group pretreated with β-CCM only ($p<0.01$) using a test for significance in a 2 × 2 contingency table containing small frequencies [20].

TABLE 2

EFFECTS OF RO 15-1788 AND CGS 8216 ON A SUBMAXIMUM DOSE OF β-CCM

Drug	Fraction of Animals Seizing Dose (mg/kg)				
	0	0.5	1.0	2.0	2.5
Ro 15-1788	0/5	0/10	1/10	—	0/10
CGS 8216	2/15	0/10	0/10	0/10	—

A dose of 5 mg/kg β-CCM was used. See Method section for experimental procedure. There were no significant differences between control and treated mice using a test for significance in a 2 × 2 contingency table containing small frequencies [20].

TABLE 3

EFFECT OF PRETREATMENT WITH RO 15-1788 AND CGS 8216 ON TREMORIGENIC ACTIVITY OF HARMALINE

Drug	Drug Pretreatment (mg/kg)	Dose Harmaline (mg/kg)	Fraction with Tremors
Ro 15-1788	0	30	8/8
	5	30	5/5
	10	30	5/5
	20	30	5/5
	35	30	5/5
Ro 15-1788	0	4	2/13
	5	4	0/5
	20	4	0/5
	35	4	0/5
CGS 8215	0	30	13/13
	0.1	30	5/5
	1	30	5/5
	10	30	5/5
CGS 8216	0	5	0/8
	0	10	5/5
	0.1	5	0/5
	1	5	0/5
	10	5	1/10

See Method section for experimental procedure. Animals treated with Ro 15-1788 or CGS 8216 did not differ statistically from their respective controls within a given dose level of harmaline, using a test for significance in a 2 × 2 contingency table containing small frequencies [20].

pounds (generally termed "benzodiazepine antagonists") which may ultimately prove important in determining their molecular mechanisms of action.

In addition to the pharmacologic differences among benzodiazepine antagonists outlined in the Introduction, neurochemical differences have also been noted. For example, while Ro 15-1788 and β-carbolines competitively inhibit [^{3}H] benzodiazepine binding to benzodiazepine receptors [5, 6, 12], CGS 8216 exhibits a mixed-type inhibition of [^{3}H] flunitrazepam binding [8]. Furthermore, the maximum number of binding sites (B_{max}) obtained with [^{3}H] Ro 15-1788 is identical to that obtained with [^{3}H] clonazepam [16], while the B_{max} obtained with [^{3}H] CGS 8216 and [^{3}H] β-carbolines is significantly lower than those obtained with [^{3}H] benzodiazepines in some brain areas [3, 8, 23]. Finally, the apparent affinity of benzodiazepine-like compounds ("agonists") is increased in the presence of agents such as GABA and barbiturates, while the affinity of antagonists is unchanged [11, 17, 24] or, under certain circumstances, reduced [4]. Whether these neurochemical differences are responsible for the differing pharmacologic profiles is still unknown. Recently, it has been suggested [4] that the decrease in apparent affinity of [^{3}H] β-CCM in the presence of high concentrations of GABA may be related to the convulsant actions of this compound. Neither the ethyl nor propyl esters of β-carboline-3-carboxylic acid have been reported to possess a convulsant action, and the apparent affinity of these compounds is not markedly altered in the presence of GABA [10,19].

Thus, it appears that there may be at least three relevant groups of ligands which interact with the benzodiazepine receptor: (1) agonists (i.e., compounds with actions mimicking diazepam), (2) antagonists which do not appear to possess intrinsic pharmacologic activity, such as Ro 15-1788 and CGS 8216 (see, however, [13]), and (3) antagonists which have pharmacologic actions opposite those of benzodiazepines when administered alone (e.g., β-CCM and 3-hydroxymethyl-β-carboline) [4,15]. The data reported here

suggests that compounds with no intrinsic pharmacologic activity not only antagonize the actions of agonists (e.g., diazepam, zopiclone) [12], but also antagonize the convulsant actions of β-CCM, and are at least as potent as diazepam in this regard. Furthermore, antagonists such as Ro 15-1788 and CGS 8216 do not potentiate the convulsant action of submaximum doses of β-CCM (Table 2).

In contrast, when tested against the tremorigen harmaline, an entirely different pharmacologic profile of both Ro 15-1788 and CGS 8216 was obtained, despite the chemical similarity between harmaline and β-CCM. Neither blockade nor enhancement of the tremorigenic action of harmaline was afforded by either compound, while diazepam proved to be an effective antagonist of this action (Table 3) [14]. It has been previously suggested that the tremorigenic actions of harmaline are due to its interaction with the benzodiazepine receptor [21]. This hypothesis was based on the observation that harmaline will occupy a small percentage of benzodiazepine receptors at tremorigenic doses, and the efficacy of diazepam in attenuating these tremors. While the present findings do not disprove this hypothesis, they do suggest that the nature of this interaction is fundamentally different from that associated with β-CCM, which binds with a much higher affinity to brain benzodiazepine receptors.

Despite the demonstration that both Ro 15-1788 and CGS 8216 antagonize the pharmacologic actions of benzodiazepines, we have observed that these compounds also disrupt the convulsant actions of β-CCM, and are at least as potent as diazepam in this regard. Further investigations are necessary to clarify the neurochemical correlates of what appears to be a pharmacologically anomalous interaction among benzodiazepine antagonists.

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