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Differential Antagonism of Diazepam-Induced Loss of the Righting Response

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WITKIN, J M , J E BARRETT, J M COOK AND P LARSCHEID *Differential antagonism of diazepam-induced loss of the righting response* PHARMACOL BIOCHEM BEHAV 24(4) 963–965, 1986 —Ethyl-β-carboline-3-carboxylate (β-CCE), inosine and Ro 15-1788 are antagonists of several actions of the benzodiazepines These compounds can be differentiated, however, according to their ability to reverse the loss of the righting response induced by diazepam Ro 15-1788 completely reversed effects of diazepam on the righting response of pigeons and squirrel monkeys but was ineffective against comparable effects produced by pentobarbital Pretreatment with Ro 15-1788 protected against diazepam-induced righting loss Neither inosine nor β-CCE reversed diazepam-induced righting loss or acted prophylactically against this effect Since β-CCE has been characterized as an inverse agonist at the benzodiazepine receptor, the absence of antagonism we report would suggest that β-CCE lacks specific pharmacological activity which opposes suppression of the righting response by diazepam Research with these preferentially-acting antagonists may lead to the development of anxiolytics devoid of the sedative-hypnotic properties inherent in the drugs currently in a clinical use

Diazepam Ro 15-1788 Ethyl-β-carboline-3-carboxylate Inosine Antagonism Righting reflex

BENZODIAZEPINES, the most widely used drugs in the treatment of anxiety, suffer from their lack of pharmacological specificity; sedation is a common side-effect of these drugs Compounds have recently been discovered which can antagonize effects of the benzodiazepines and promise to greatly enhance our understanding of the mechanisms underlying anxiety states Ethyl-β-carboline-3-carboxylate (β-CCE), inosine, and Ro 15-1788 [ethyl-8-fluro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo (1,5a) (1,4) benzodiazepine-3-carboxylate], for example, bind to specific benzodiazepine receptors in brain [12, 14, 15] and produce a dose-dependent antagonism of several pharmacological actions of the benzodiazepines [2, 3, 4, 9, 17, 35]. Ro 15-1788 has little intrinsic activity [4] although, as with inosine (e g., [10,17]), Ro 15-1788 has some benzodiazepine-like actions (e g., [7]). In contrast, β-CCE has actions which are opposite those of the benzodiazepines and has been characterized as an inverse agonist [3, 13, 16] We find that these compounds can also be differentiated on the basis of their ability to antagonize effects of diazepam on the righting response.

METHOD

Adult male White Carneaux pigeons *(Columbia livae)*, 460–580 g, and adult male squirrel monkeys *(Saimiri sciureus)*, 550–920 g, served as subjects The righting response was considered absent if, when placed on their backs, pigeons failed to stand on their feet within 5 sec The righting response of the squirrel monkeys was defined as lost if the monkey's upper body was supported by the waist plate of a primate restraint chair, and if when manually raised, the monkey resumed this posture. Diazepam doses were selected on the basis of the minimal values required to produce the behavioral endpoint

Diazepam (40 mg/kg—pigeons; 30 mg/kg—squirrel monkeys) or diazepam vehicle was administered by gavage into the proventriculus of pigeons and was given by IM injection in the squirrel monkeys Successive doses of Ro 15-1788, β-CCE, or inosine were injected (IM) in 1/2 log increments every 10 min after administration of diazepam or vehicle (pigeons 10 min prior, monkeys 20 min prior)

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TABLE 1

EFFECTS OF Ro 15-1788, β-CCE, AND INOSINE ON THE RIGHTING RESPONSE LOSS INDUCED BY DIAZEPAM

Compound	Doses (mg/kg)	Righting Reversal	Ro15-1788 antagonism
Ro 15-1788 (N=4)	0 003–1	0 62 ± 0 16 mg/kg	—
β-CCE (N=6)	0 1–30	none	1 0 mg/kg
β-CCE (N=4) (Sq Monkey)	0 1–10	none	1 0 mg/kg
Inosine (N=4)	1–300	none	none

Successive doses of each compound were administered intramuscularly in $^1/_2$ log increments every 10 min after administration of diazepam (pigeons 40 mg/kg, PO, 10 min prior, squirrel monkeys 30 mg/kg, IM, 20 min prior) The dose producing righting reversal (mean ± S E M) was determined by averaging the intramuscular dose required to produce a return of the righting response across animals An entry of "none" in the table indicates that the righting response was absent continuously from either 10 to 80 min (pigeons) or 15–70 min (squirrel monkeys) after diazepam administration as when diazepam alone was given (N=6) An entry of 1 0 mg/kg indicates that this dose of Ro 15-1788 resulted in a return of the righting response of diazepam treated animals when given 10 min following the final test dose of β-CCE The number of animals tested is given in parentheses

Diazepam (donated by Hoffmann-La Roche) was dissolved to a concentration of 10 mg/ml in a commercially prepared vehicle from Hoffmann-La Roche Inosine (Aldrich Chemical Co) was dissolved in saline up to a concentration of 150 mg/ml Sodium pentobarbital (donated by Abbott Laboratories) and *d*-amphetamine sulfate (donated by Smith, Kline and French Laboratories) were also dissolved in saline Ro 15-1788 (donated by Hoffman-La Roche) and β-CCE (synthesized by P Larscheid and J M Cook) were prepared in sterile water and physiological saline, respectively A fine suspension of β-CCE and Ro 15-1788 was produced with the use of Tween 80 (1 drop/5 ml)

RESULTS

The loss of the righting response induced by diazepam was completely reversed by Ro 15-1788 (Table 1) Comparable pentobarbital-induced righting loss (30 0 mg/kg, IM) in pigeons (N=4) was not reversed by Ro 15-1788 (up to 3 mg/kg) Reversal of diazepam-induced suppression of the righting response by Ro 15-1788 was not the result of any general central nervous system stimulant actions of Ro 15-1788 since *d*-amphetamine (0 1–3 mg/kg, IM) did not reverse this effect of diazepam in pigeons (N=4) In addition, Ro 15-1788 (1 mg/kg) completely prevented the righting loss when administered prior to diazepam (Table 2)

In contrast to the effects of Ro 15-1788, neither β-CCE nor inosine were able to reverse the righting response loss produced by diazepam (Table 1) or to prevent this action of diazepam by pretreatment (Table 2) In the squirrel monkey, β-CCE produced convulsions at doses as low as 0 3 mg/kg Despite this potent pharmacological action, β-CCE was unable, even transiently, to reverse the suppression of the righting response induced by diazepam when given at doses up to 33 times higher than those producing convulsions (Table 1) However, diazepam completely prevented β-CCE-induced convulsions Administration of Ro 15-1788 to these animals after the β-CCE experiment resulted in an immediate and enduring antagonism (Table 1)

TABLE 2

EFFECTS OF PRETREATMENT WITH Ro 15-1788, β-CCE, OR INOSINE ON DIAZEPAM-INDUCED RIGHTING LOSS IN PIGEONS

Compound	Dose	Righting Loss
Ro 15-1788 (N=4)	1	none
β-CCE (N=6)	1–10	10–80+ min
Inosine (N=4)	100	10–80+ min

Compounds were administered 10 min prior to diazepam (40 mg/kg, PO) The righting response was evaluated every 10 min Righting loss lasting from 10–80+ min after diazepam (40 mg/kg, PO) administration was no different from diazepam when given alone (N=6) The number of animals in each experiment is shown in parentheses

DISCUSSION

Of the compounds previously reported to act as benzodiazepine antagonists, only the imidazo-benzodiazepine, Ro 15-1788, was active against the hypnotic effects of diazepam Inosine and β-CCE were ineffective against diazepam-induced righting loss but were administered at dose levels that had demonstrable pharmacological action Studies on schedule-controlled behavior of pigeons and squirrel monkeys have demonstrated that intramuscular inosine and β-CCE produced marked behavioral actions that are sensitive to Ro 15-1788 [1,17] and that reverse behavioral effects of benzodiazepines [3] Thus, the pharmacological activities and pharmacokinetics of inosine or β-CCE were apparently not inadequate to ensure antagonism [14], antagonism was absent despite the potent benzodiazepine receptor-mediated actions of these drugs

Inosine, a purinergic compound is a potential endogenous regulator of the benzodiazepine receptor (cf [15]) In the present study, inosine did not act as a benzodiazepine antagonist as previously reported [6, 9, 10] and, in fact, may have produced actions which were not sensitive to Ro 15-1788 (Table 1) The pharmacology of inosine is complex and further experimentation is required to elucidate its possible involvement in benzodiazepine action

The contrasting actions of Ro 15-1788 and β-CCE are striking and enigmatic β-CCE, like Ro 15-1788, has been shown to act as a benzodiazepine antagonist in most preparations that have been investigated including neurophysiological and behavioral systems [3, 16, 18] Moreover, effects of β-CCE can be reversed by Ro 15-1788 [1,13] Two possibilities currently appear feasible as explanations for the differences in the activities of Ro 15-1788 and β-CCE reported here The first possibility is based on the probable existence of multiple benzodiazepine receptor subtypes While Ro 15-1788 binds uniformly to so-called BZ_1 and BZ_2 receptors, β-CCE binds preferentially to the BZ_1 receptor (cf [8,19]) The BZ_1 receptor has been suggested to underly the anxiolytic apart from the sedative-hypnotic actions of the benzodiazepines [5,8] The difference in affinity for the ben-

zodiazepine receptor subtypes or recognition sites may be related to the differential activities of Ro 15-1788 and β-CCE observed here

An alternative explanation rests on the differences in the pharmacological activities of Ro 15-1788 and β-CCE. Given in doses many times higher than required for benzodiazepine antagonism, Ro 15-1788 is essentially devoid of intrinsic activity [4] although recent reports have detected some weak benzodiazepine-like effects [7] In contrast, β-CCE has been described as an inverse agonist [3,13]. β-CCE and related β-carbolines produce an array of actions which are opposite those of the benzodiazepines [3, 11, 13, 16] (e.g., proconvulsant effects, anxiogenic symptoms) If, as proposed, β-CCE produces its benzodiazepine antagonist effects by producing opposing actions, then the present results suggest that β-CCE lacks pharmacological activity opposite that of the suppression of righting studied here

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