

DISCRIMINATIVE AND AVERSIVE PROPERTIES OF  $\beta$ -CARBOLINE-3-CARBOXYLIC ACID ETHYL ESTER, A BENZODIAZEPINE RECEPTOR INVERSE AGONIST, IN RHESUS MONKEYS.

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Summary

Rhesus monkeys were trained to discriminate injections of saline from those of  $\beta$ -carboline-3-carboxylic acid ethyl ester ( $\beta$ -CCE), a compound that binds to the benzodiazepine receptor, but often has actions opposite to those of the benzodiazepines. A benzodiazepine agonist midazolam and low doses of a specific benzodiazepine antagonist, Ro 15-1788, reversed the discriminative effects of  $\beta$ -CCE. Higher doses of Ro 15-1788 produced stimulus effects similar to  $\beta$ -CCE. In a separate experiment, monkeys responded to terminate intravenous infusions of  $\beta$ -CCE, but not midazolam. This aversive effect of  $\beta$ -CCE was reversed by Ro 15-1788. The behavioral effects of  $\beta$ -CCE in these non-human primates are consistent with other data that have shown it to act on benzodiazepine receptors, and support the hypothesis that  $\beta$ -CCE can be considered an inverse agonist at this receptor.

The discovery of brain binding sites for the benzodiazepines (1,2) spurred the search for potential antagonists of these anxiolytic drugs. One of the first non-benzodiazepines that was found to bind to benzodiazepine receptors was  $\beta$ -carboline-3-carboxylic acid ethyl ester ( $\beta$ -CCE [3]). It reversed the effects of benzodiazepines (4-7) and, in contrast to the more recently developed benzodiazepine antagonists such as Ro 15-1788 (8), had actions of its own that were in many instances opposite to those of the benzodiazepines. While many benzodiazepines have anti-convulsant activity and attenuate the effects of punishment on behavior (e.g. 9,10,11),  $\beta$ -CCE is a proconvulsant in rodents (12-13), a convulsant in squirrel monkeys (14-15), and it and related drugs have been shown to enhance the effects of punishment (15-16).  $\beta$ -CCE decreased rates of punished responding and increased rates of shock-maintained responding in squirrel monkeys. These effects were opposite to the effects of benzodiazepines and were reversed by administration of Ro 15-1788 which had no effects of its own (15). When given intravenously to rhesus monkeys,  $\beta$ -CCE has been reported to produce apparent anxiety-like effects, including agitation, vocalization and increases in plasma cortisol, epinephrine, norepinephrine, heart rate and blood pressure. These effects

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were prevented by administration of the benzodiazepine receptor antagonist Ro 15-1788 (17) and by diazepam (18).

Studies in man showed that FG 7142, the ethylamide congener of  $\beta$ -CCE, elicited physiological responses similar to those produced by  $\beta$ -CCE in monkeys. In these subjects, plasma levels of cortisol as well as heart rate and blood pressure were increased (19). In addition, the human subjects gave verbal reports of severe anxiety and intense inner tension.

These data, supported by biochemical information (20), have led to the development of a novel classification of drugs that act on the benzodiazepine receptor. The anxiolytic benzodiazepines, with punishment attenuating and general disinhibitory effects, are referred to as benzodiazepine receptor agonists.  $\beta$ -Carbolines that enhance effects of punishment and increase autonomic activity are called inverse agonists at the benzodiazepine receptor, whereas drugs such as Ro 15-1788, with little intrinsic activity but with the capacity to reverse the actions of both the agonists and inverse agonists are termed benzodiazepine receptor antagonists (21-23). The concept that markedly different drugs can produce such a wide spectrum of effects through their interactions at a single receptor is unique in experimental pharmacology and deserves extensive scrutiny in pharmacological and behavioral systems.

We report in this paper the results of studies designed to determine whether the behavioral effects of  $\beta$ -CCE in the rhesus monkey are consistent with the conceptualization of this drug as an inverse agonist at the benzodiazepine receptor.  $\beta$ -CCE was established as a discriminative stimulus in rhesus monkeys, and it was also evaluated for aversive properties by examining its capacity to act as a negative reinforcer in primates. Primates have a distinct advantage over rodents in the evaluation of  $\beta$ -CCE, since rodents metabolize this drug very quickly (23), while primates apparently do not (14).

#### Methods

Discriminative stimulus: Four rhesus monkeys were trained to discriminate a subcutaneous injection of 1 mg/kg  $\beta$ -CCE from a similar injection of vehicle. During daily experimental sessions, these monkeys were seated in primate restraining chairs which were placed in isolation chambers equipped with two levers, a food receptacle, and a bank of stimulus lights. The monkeys had been trained to make thirty consecutive responses (FR 30) on the rightmost of the two levers and receive a 300 mg Noyes banana-flavored pellet if they had been given 1 mg/kg  $\beta$ -CCE, 30 minutes earlier. If the injection had been of vehicle, responses on the leftmost lever were reinforced on the same schedule. A daily session lasted for 20 minutes or until 50 food pellets had been delivered. During test sessions, 30 consecutive responses on either of the two levers resulted in food delivery..

A separate group of three monkeys was trained to discriminate the effects of 10 mg/kg s.c. methohexital, an ultra short-acting barbiturate. These monkeys were trained and tested using the same equipment as with the  $\beta$ -CCE monkeys, but by a slightly different procedure, described by Bertalmio et al. (24). As shown by Bertalmio et al. (24), there are minimal differences in drug effects with these two procedures. A multiple trials procedure was used; each trial was 15 min in duration, and as many as six trials constituted a daily session. On training days, either an injection of 10 mg/kg methohexital or a sham injection preceded each trial, and the monkey was in a blackout period for the first 10 min of each trial. With the onset of the stimulus light, 100 consecutive responses on the injection-appropriate

lever resulted in delivery of 10 food pellets. Methohexital was given on the penultimate trial of a session, and responding on the methohexital-appropriate lever was reinforced on this and the last trial of a session. From zero to four sham injection trials could precede the methohexital injection on a training day, and six sham injection trials constituted a session on occasion. The criterion for testing was that 90% of each trial's responses had to be made on the injection-appropriate lever on the session before the test session. On test days, 100 consecutive responses on either lever were reinforced. The first trial of a test session was preceded by administration of saline or of a potential antagonist, and subsequent trials were preceded by increasing, cumulative doses of the test drug.

Aversive Effects: Three monkeys were adapted to restraining harnesses and arms that allow them relatively free movement within their individual cages and also permitted the passage and protection of intravenous catheters (25). The monkeys were prepared, under aseptic conditions, using ketamine and pentobarbital anesthesia, with intravenous jugular or femoral catheters. During two daily sessions, one in the morning and one in the afternoon, they were trained first to press a lever in the presence of a stimulus light and receive intravenous infusions of cocaine, then to press the lever and turn off infusions of 7  $\mu$ g/kg/sec histamine. A single response was necessary to terminate the infusion for two of the monkeys, while five responses were required for the third monkey. An unterminated infusion lasted for 15 sec; terminated and unterminated infusions were followed by a five-min light-off period. A maximum of 20 infusions could be delivered during each session. During particular sessions, saline, the hydrochloride salt of  $\beta$ -CCE, or midazolam were infused instead of histamine. When Ro 15-1788 was given as a pretreatment, it was administered subcutaneously, 10 min before the start of the session.

Drugs:  $\beta$ -CCE and Ro 15-1788 were suspended in Emulphor, 95% ethanol, and water in a ratio of 1:1:8. Doses are expressed as the free base. Midazolam maleate was dissolved in water with a few drops of lactic acid added. Sodium methohexital and the hydrochloride salt of  $\beta$ -CCE were dissolved in water. The doses of these drugs are expressed as the salts.

### Results

$\beta$ -CCE produced dose-related increases in selection of the  $\beta$ -CCE-appropriate lever in the monkeys trained to discriminate this drug (Fig. 1A). These same doses of  $\beta$ -CCE did not produce methohexital-appropriate responses in monkeys trained to discriminate methohexital (Fig. 1B). In contrast, the short-acting benzodiazepine midazolam, in doses as high as 1 mg/kg, did not produce  $\beta$ -CCE-appropriate responding in the monkeys trained to discriminate  $\beta$ -CCE (Fig. 1A) but did produce drug-appropriate responding in the monkeys trained to discriminate methohexital (Fig. 1B). Doses of midazolam above 1 mg/kg produced response decrements in the  $\beta$ -CCE-trained monkeys.

Midazolam (0.32 mg/kg, given 10 minutes before the session) completely prevented the discriminative effects of 1 mg/kg  $\beta$ -CCE in two  $\beta$ -CCE-trained monkeys (not shown). Ro 15-1788 (1 mg/kg, given 10 minutes before the session) competitively antagonized the discriminative effects of  $\beta$ -CCE in the  $\beta$ -CCE-trained monkeys (Fig. 1A) and produced a similar shift to the right in the discriminative effects of midazolam in two of the three methohexital-trained monkeys. A less pronounced shift was observed in the third monkey (Fig. 1B).

Ro 15-1788 in doses of 10 and 17.8 mg/kg, given 30 minutes before the

session, produced  $\beta$ -CCE-appropriate responding in each of the three monkeys to whom it was given (Fig. 1A). These doses of Ro 15-1788, given in

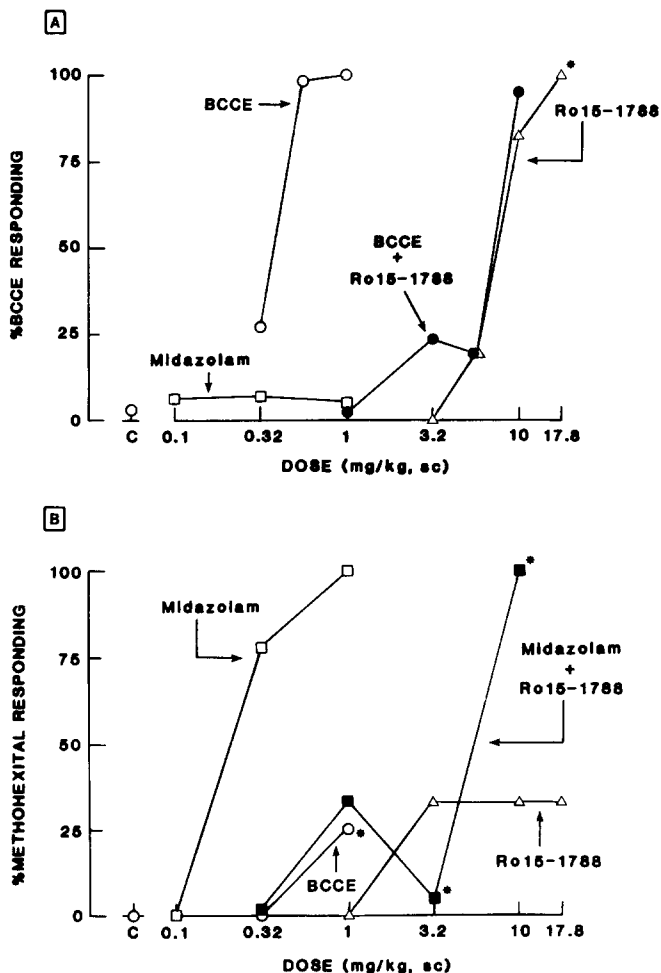


Fig. 1

A. Drug-appropriate responding following subcutaneous administration of  $\beta$ -CCE alone (O, n=4),  $\beta$ -CCE in combination with 1 mg/kg Ro 15-1788 (●, n=2), Ro 15-1788 alone ( $\Delta$ , n=3), and midazolam alone ( $\square$ , n=4) in monkeys trained to discriminate the s.c. administration of 1 mg/kg  $\beta$ -CCE.

B. Dose-effect curves for midazolam ( $\square$ ),  $\beta$ -CCE (O), and Ro 15-1788 ( $\Delta$ ) alone, and midazolam in combination with 1 mg/kg Ro 15-1788 ( $\blacksquare$ ) in three monkeys trained to discriminate the subcutaneous administration of 10 mg/kg methohexital from sham injections. Points at "C" in either panel represent the values after the control injections. The asterisks indicate data obtained in a reduced number of monkeys because animals which showed drug-appropriate responding or response suppression at lower doses of the administered drug were not tested.

cumulative fashion to the methohexital-trained monkeys, produced drug-appropriate responding in one of the three monkeys. This was the same monkey in whom 1.0 mg/kg Ro 15-1788 was less effective as a midazolam antagonist.

In order to determine whether the behavioral effects of  $\beta$ -CCE can be considered to be those of a benzodiazepine inverse agonist, the capacity of  $\beta$ -CCE and midazolam to maintain escape responding was compared. As shown in Figure 2,  $\beta$ -CCE and histamine maintained escape responding, while saline did not. Midazolam at infusion rates of 2 or 7  $\mu$ g/kg/sec failed to maintain more escape behavior than that maintained by saline in all monkeys (not shown). Ro 15-1788 (1 mg/kg) reduced  $\beta$ -CCE-maintained escape responding but did not greatly affect histamine-maintained escape responding (Fig. 2).

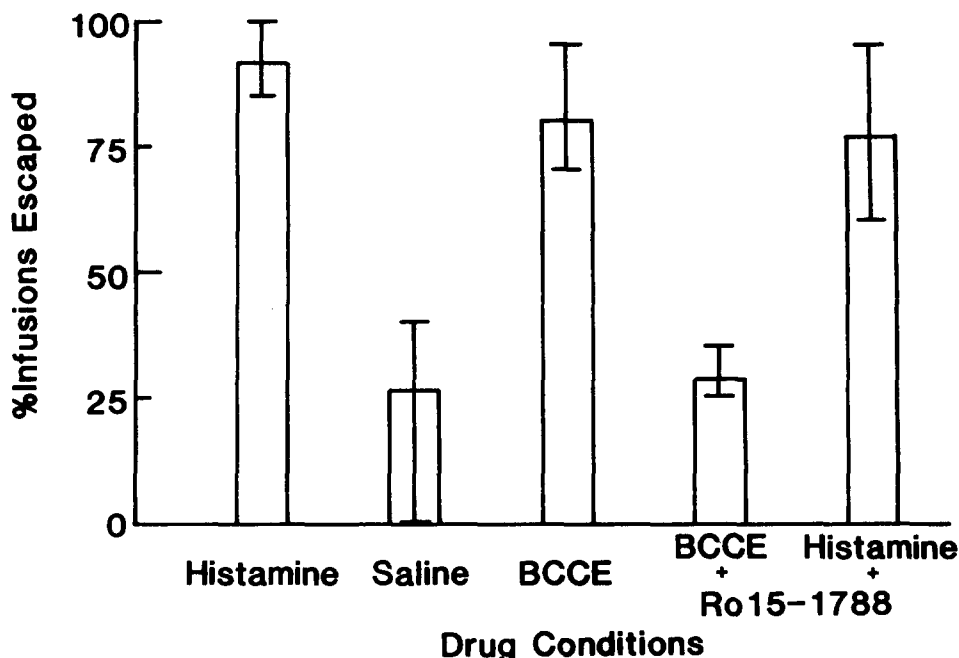


Fig 2

Effects of 7  $\mu$ g/kg/sec histamine, saline, 2  $\mu$ g/kg/sec  $\beta$ -CCE, and these infusion rates of  $\beta$ -CCE or histamine in combination with subcutaneously injected 1 mg/kg Ro 15-1788 on behavior maintained by the termination of intravenous infusions of these drugs. Twenty infusions were initiated during each session and the ordinate indicates the percentage of trials during which the monkey responded and thus escaped from further infusion. The abscissa indicates the drug conditions that were used to maintain responding. The vertical lines at each bar are the range of the percent infusions terminated for that particular drug condition.

### Discussion

Rhesus monkeys learned to discriminate the stimulus effects of the benzodiazepine inverse agonist  $\beta$ -CCE, and the stimulus effects of this drug were dissimilar from those of the benzodiazepine agonist midazolam. Monkeys trained to terminate intravenous infusions of histamine terminated infusions of  $\beta$ -CCE but not midazolam. Therefore,  $\beta$ -CCE, but not midazolam, was a negatively reinforcing stimulus under these experimental conditions. Both the discriminative and negatively reinforcing effects of  $\beta$ -CCE were antagonized by the benzodiazepine antagonist Ro 15-1788, as was the discriminative effect of midazolam. These experiments demonstrate that, using objective behavioral procedures in primates,  $\beta$ -CCE can be shown to act on benzodiazepine receptors to produce effects that are different, and apparently opposite to those of a benzodiazepine agonist, midazolam.

More support for the opposite nature of  $\beta$ -CCE and midazolam comes from data that demonstrate that midazolam has positive reinforcing effects that, while less than those of methohexital (Winger, unpublished observations) or pentobarbital (26), are considerably greater than those of saline.

If this were the extent of the information about the interactions among benzodiazepines,  $\beta$ -carbolines and benzodiazepine antagonists, there would be strong parallels between the benzodiazepine receptor system and the opiate receptor system. In the opiate class, morphine and ethylketocyclazocine (EKC) produce distinctive discriminative stimulus effects in primates (27). Morphine maintains self-administration behavior while EKC does not (28) and EKC may be aversive under some conditions (Woods et al. In press). The effects of morphine and EKC in these behavioral paradigms are reversed by opiate antagonists. In the opiate system, morphine and EKC are both considered to be opiate agonists, but are thought to act on different classes of opiate receptor (29).

This type of classification does not apply to drugs that act on the benzodiazepine receptors however, because of the mutual antagonism of benzodiazepine agonists and inverse agonists such as  $\beta$ -CCE. As described in this report, midazolam was able to block the discriminative effects of  $\beta$ -CCE. Although attempts to reverse the discriminative stimulus effects of midazolam with  $\beta$ -CCE failed because administration of effective antagonistic doses of  $\beta$ -CCE suppressed responding (Winger, unpublished observation), other studies have shown that  $\beta$ -CCE is an effective antagonist of benzodiazepines (4-7). This mutual antagonism suggests that  $\beta$ -CCE and benzodiazepine agonists do not produce distinct effects by acting on different receptors in the same system, but by acting on the same receptor. The fact that midazolam is a positive reinforcer while  $\beta$ -CCE is a negative reinforcer supports the concept of  $\beta$ -CCE as an inverse agonist at this receptor. This is a unique and fascinating aspect of the pharmacology of the benzodiazepine receptor complex.

The data shown here demonstrate another unique feature of the benzodiazepine receptor. The drug Ro 15-1788, regarded as a benzodiazepine receptor antagonist with few effects of its own (8) has been shown, in some species and in some situations, to have actions in common with benzodiazepine agonists (30-32). Less frequently, Ro 15-1788 has been shown to have actions in common with inverse agonists (33). In the experiments shown here, Ro 15-1788 was, at the same dose, an effective surmountable antagonist of both  $\beta$ -CCE and midazolam. At larger doses it produced  $\beta$ -CCE-like stimulus effects in each of the  $\beta$ -CCE-trained monkeys, and methohexital-like stimulus effects in only one of the methohexital-trained monkeys. In this system, Ro 15-1788 appeared to act as a benzodiazepine antagonist, and, at larger doses, as a benzodiazepine inverse agonist.

Nielsen et al. (34) established the discriminative stimulus effects of the convulsant beta-carboline DMCM (6,7-dimethyl-4-ethyl- $\beta$ -carboline-3-carboxylate) in rats. Ro 15-1788 (80 mg/kg) produced 57% responding on the DMCM-appropriate lever. Given as a pretreatment, Ro 15-1788 reduced the capacity of DMCM to produce drug-appropriate responding to about 50% at three doses (20, 40 and 80 mg/kg). These data suggest both antagonist and inverse agonist effects of Ro 15-1788, but, in these experiments, neither of these effects was complete. Other investigators have suggested that the agonist-antagonist profile of Ro 15-1788 may change as a function of the dose of benzodiazepine used to establish a drug discrimination (35). Thus, the discriminative stimulus properties of Ro 15-1788, and its relative effects as an agonist, antagonist or inverse agonist, seem determined by a variety of variables, including dose of Ro 15-1788, as shown in the current study, type and dose of the training drug, perhaps as well as the species and particular paradigm employed.

The experiments described demonstrate that, although both the discriminative and reinforcing stimulus properties of  $\beta$ -CCE are mediated through the benzodiazepine receptor, they are different or in the opposite direction from the discriminative and reinforcing stimulus properties of midazolam. These behavioral studies may be very useful in studying the similarity among substances related to anxiety, both those with exogenous and those with endogenous origins. In addition,  $\beta$ -CCE-induced effects may provide a pharmacological reference for studying environmental or historical factors that modify anxiety.

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