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Ro 15-1788 and β-CCE Selectively Eliminate Diazepam-Induced Feeding in the Rabbit

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MANSBACH, R. S., J. A. STANLEY AND J. E. BARRETT. *Ro 15-1788 and β-CCE selectively eliminate diazepam-induced feeding in the rabbit.* PHARMACOL BIOCHEM BEHAV **20**(5) 763–766, 1984.—Food intake was monitored in three female and one male adult rabbits following the administration of three drugs known to result in feeding increases in other species. The drugs, diazepam (1.0 mg/kg), cyproheptadine (0.03 mg/kg) and chlorpromazine (1.0 mg/kg) all produced large increases in food intake; of these, only the effect of diazepam, a benzodiazepine, was reversed by doses of the benzodiazepine antagonists Ro 15-1788 (0.3 mg/kg) and Ethyl β-carboline-3-carboxylate (β-CCE) (1.0 mg/kg) which, when given alone, did not affect feeding. The results support evidence suggesting that Ro 15-1788 and β-CCE are specific antagonists of the benzodiazepine receptor and of their effects on a wide range of behaviors.

Benzodiazepines Feeding behavior β-Carbolines Ro 15-1788 Chlorpromazine Rabbit Cyproheptadine Eating

IT has been reliably demonstrated that, for a variety of species and experimental conditions, benzodiazepines increase food intake in non-deprived and satiated [11] animals [7, 20, 21, 27]. Polydipsia [8,9] and dose-dependent increases in food-maintained lever pressing following the administration of diazepam [27] have also been noted. Benzodiazepines have potent effects on schedule-controlled responding as well; many of these drugs increase operant responding punished by electric shock [13, 24, 29]. Increases in punished responding are thought to reflect anxiolytic action in these compounds. While the mechanisms underlying these changes in behavior are not completely understood, a number of neurotransmitter systems have been implicated in benzodiazepine action [10, 12, 18, 24].

Since the discovery of specific benzodiazepine receptors in the mammalian central nervous system [17] there has been considerable interest in identifying putative endogenous ligands of the benzodiazepine receptor. Among the several recently developed compounds shown to interact with this receptor, two compounds, Ro 15-1788 and Ethyl β-carboline-3-carboxylate (β-CCE), are well documented as antagonists of several benzodiazepine behavioral and electrophysiological effects. Both have been found to bind to benzodiazepine receptors [6,22] and inhibit ^{3}[H]-flunitrazepam binding in rat brain [15,16]. Ro 15-1788 and β-CCE also reverse many behavioral effects of the benzodiazepines. In an extensive series of experiments, Bonetti *et al.* [5] found that while Ro 15-1788 (up to 300 mg/kg) had little intrinsic effect on spontaneous behavior, it potently antagonized diazepam's effects on spontaneous locomotor activity, punished responding, and seizure protection in rats. Other experiments have shown that Ro 15-1788 and β-CCE block the anticonflict [19,26], seizure protectant [25], and hyperdipsic [8] effects of the benzodiazepines.

The purpose of the present experiment was to determine whether the increases in food intake in rabbits observed following diazepam administration could be selectively eliminated by the benzodiazepine antagonists Ro-15-1788 and β-CCE. In order to demonstrate specificity we used two other compounds, chlorpromazine and cyproheptadine, which have also been shown to increase feeding in animals [3,14]. If the antagonists are capable of reversing the effects of diazepam, but not those of the two non-benzodiazepine orectic drugs, this will add further support to the selectivity of Ro 15-1788 and β-CCE for the benzodiazepine receptor, and provide an additional behavioral index with which to test these and other drug interactions.

METHOD

Subjects

Three Dutch Belted (J94, J97, J98) and one New Zealand White (L02) adult rabbits, weighing between 2.5–5.0 kg were individually housed and maintained on Purina Rabbit Chow and tap water in a temperature- and light-controlled (on 0600–1800) environment. J97 was the only male subject used. Those rabbits which were water-deprived (J97, J98) received a 50 ml tap water ration on nontesting days (Saturday and Sunday) and tap water containing 0.25% saccharin during the session on testing days. The two other rabbits had free access to water in their home cages. Food was continuously

available for all rabbits except for a designated pretreatment time; for J97 and J98 this time varied according to the drug administered, while for J94 and L02 the food was removed 1 hour prior to each session.

Apparatus

The experiment was conducted in 38×38×38 cm Plexiglas cages enclosed in ventilated, sound-attenuating chambers. The cages were lit by two white 7 W bulbs mounted on the front wall. The front panel of one cage was equipped with a liquid dipper (Gerbrands model B-LH) that could deliver 1.0 ml tap water containing 0.25% (w/v) saccharin. A cup containing Purina Rabbit Chow pellets was also accessible throughout the session. The other cage contained both a filled food cup and full water bottle.

Procedure

The water-deprived rabbits received a 4-second access to the saccharin solution once every 3 minutes during the 150-minute session, resulting in a 50 ml ration. The presentation of liquid was controlled by electromechanical equipment in a separate room. The two rabbits not chronically water-restricted were placed in the chamber for a 90-minute session during which no preprogrammed events took place.

All rabbits were accustomed to the procedure for at least one week before any drugs were administered. Testing took place Monday–Friday with drug injections typically given on Tuesdays and Fridays. On control days the vehicle for the drug administered on the next drug day was injected with a designated pretreatment time identical in length to that for the drug itself. The water-deprived rabbits were deprived of food only for the pretreatment time, while the remaining subjects were always deprived of food and water 1 hour prior to the first injection. Subjects were placed in the cages following the pretreatment time and removed promptly at the end of the session. Food intake was measured by subtracting the final mass of the food and spillage, if any, from the original mass provided.

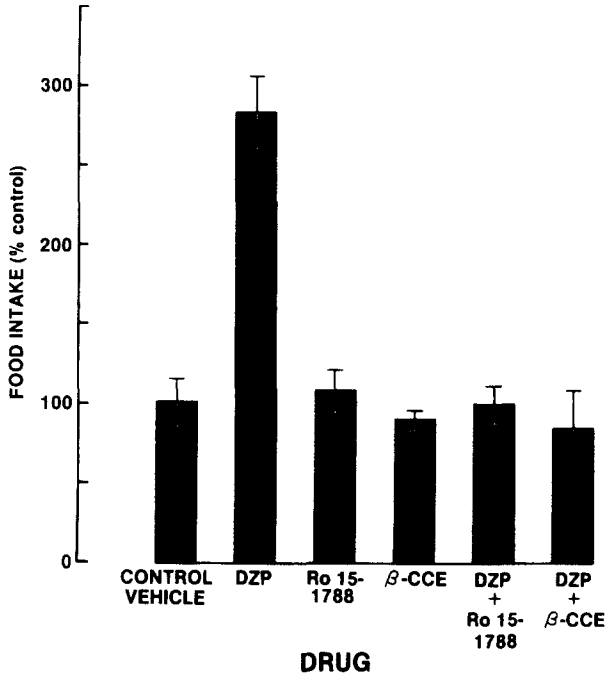

FIG. 1. Drug-induced changes in feeding behavior. Amounts eaten are expressed as percent of control intake±SE. Means for each dose were calculated based on data collected from at least three rabbits, usually multiply determined. Sample sizes range from 5 to 8 for each dose. Control standard errors were determined by averaging the standard errors for each rabbit. The differences in food intake between 1.0 mg/kg diazepam (DZP) and diazepam + 0.3 mg/kg Ro 15-1788 and also between diazepam and diazepam + 1.0 mg/kg β-CCE were statistically significant ($p<0.001$) by the Rank-sum test. Note: one rabbit received 0.3 mg/kg diazepam.

Data Analysis

The effects of drugs on food intake were assessed for each rabbit by comparing the amount consumed following drug administration to that of its vehicle-injected baseline. The data are expressed as percent of control intake. Individual comparisons were performed using the rank-sum test. A confidence level of 0.05 was selected for significance of results.

Drug Procedure

Diazepam (Hoffmann-LaRoche, Nutley NJ) was dissolved in a solution containing one-half saline (0.9%) and one-half commercial diluent (Hoffmann-La Roche, Nutley NJ). Ro 15-1788 and β-CCE were suspended in distilled water and saline, respectively, with 1 drop Tween-80 (Fisher Scientific Co., Fair Lawn NJ) added per 5 ml. Cyproheptadine HCl (Merck, Sharpe and Dohme, Rahway NJ) and chlorpromazine HCl (Smith, Kline and French, Philadelphia PA) were dissolved in saline; 1 drop of lactic acid was added per 5 ml for cyproheptadine. Pretreatment times were as follows: diazepam (15′); Ro 15-1788 (5′); chlorpromazine, β-CCE, and cyproheptadine (0′). Most drug combinations were given in a mixed sequence to at least three rabbits with each rabbit receiving that treatment twice. All injections were administered intramuscularly into the hind limb.

RESULTS

During control sessions (40–50 for each animal) the rabbits developed stable patterns of feeding. Mean food intake for each subject (in grams±SE) was as follows: J94 (7.5±0.44); J97 (10.3±0.46); J98 (9.8±0.32); L02 (3.6±0.64).

Diazepam (1.0 mg/kg) substantially increased feeding in all subjects (283±23 percent of control intake) (Fig. 1). Direct observation of the animals revealed that they were sedated and relaxed throughout the session, and that most of the feeding appeared to occur during the first hour of testing.

Administration of Ro 15-1788 (0.3 mg/kg) and β-CCE (1.0 mg/kg) did not greatly affect the amount of food consumed when given alone (108±13 and 90±6 percent of control, respectively). However, when these drugs were given in combination with diazepam, the increases in food intake were completely eliminated (99±12 and 84±24 percent of control feeding for Ro 15-1788 and β-CCE, respectively) (Fig. 1).

Cyproheptadine (0.03 mg/kg) and chlorpromazine (1.0 mg/kg) produced large increases in food intake (255±61 and 224±13 percent of control, respectively). In marked contrast to diazepam, however, the effects of these drugs on food

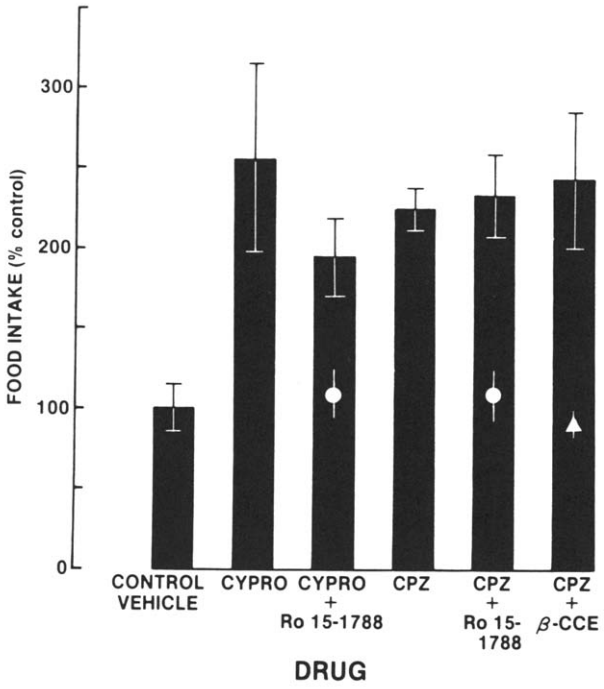

FIG. 2. Drug-induced changes in feeding behavior. Details are the same as for Fig. 1. The differences in food intake between 0.03 mg/kg cyproheptadine (CYPRO) and cyproheptadine + 0.3 mg/kg Ro 15-1788 and also between 1.0 mg/kg chlorpromazine (CPZ) and chlorpromazine + 0.3 mg/kg Ro 15-1788 were not significant as determined by the Rank-sum test. The symbols located within the histograms represent the mean intake±SE for Ro 15-1788 (circles) and β-CCE (triangle). The error bars for chlorpromazine + β-CCE represent the range of two observations. Note: one rabbit received 0.3 mg/kg chlorpromazine.

intake could not be reversed by Ro 15-1788 (Fig. 2). Administration of cyproheptadine in combination with Ro 15-1788 resulted in a mean food intake of 194±24 percent of control. Doses of Ro 15-1788 up to 3 mg/kg (not shown) had no additional effect on feeding; in fact, there was some indication of a potentiation in two rabbits.

Similarly, the effects of chlorpromazine could not be reversed by Ro 15-1788 (233±26 percent of control) or by β-CCE (243 percent of control) (Fig. 2). As with cyproheptadine, higher doses of Ro 15-1788 given with chlorpromazine (not shown) did not cause any additional changes in the amount of food consumed.

DISCUSSION

Ro 15-1788 and β-CCE completely eliminated diazepam-induced feeding in the rabbit. This finding is consistent with reports showing a selective antagonism of benzodiazepine effects using different species and indices of behavior [5, 8, 15, 25]. Accordingly, the effect of the two non-benzodiazepine drugs, cyproheptadine and chlorpromazine, were not antagonized although they produced increases in feeding very similar to those of diazepam. These results strongly suggest a specificity of Ro 15-1788 and β-CCE for the benzodiazepine receptor. The mechanism by which this antagonism occurs is still not certain; however, there is some evidence to suggest that the GABA system may mediate diazepam's effect on feeding. Birk and Noble [4] found that bicuculline, a GABA antagonist, reduced diazepam-induced feeding at a dose which did not reduce food intake below control levels when given alone. The effects of many GABA compounds on food intake are, however, still unknown.

The effect of diazepam on feeding behavior in the rabbit is in accord with earlier studies employing similar drugs in different species [20, 21, 27, 28]. The effects of Ro 15-1788 on rabbit behavior seem similar to those reported for rats, squirrel monkeys, and mice [5]. We observed no unusual effects of β-CCE on feeding behavior at our maximum dose of 3 mg/kg. No signs of seizures or illness were observed for either drug although seizure activity has been observed with β-CCE in squirrel monkeys at 1.0 mg/kg [23]. Our finding that chlorpromazine increases feeding behavior is consistent with an earlier study showing that this drug produces increases in schedule-related eating in the rabbit [2]. Cyproheptadine also produced striking increases in food intake with a dose of 0.03 mg/kg. Doses as low as 0.1 mg/kg were too high to affect food intake, as the rabbits became heavily sedated. Ro 15-1788 did not reverse the increases in food intake induced by cyproheptadine and chlorpromazine. This finding supports earlier reports of a pharmacological specificity of Ro 15-1788 for the benzodiazepine receptor and its relative ineffectiveness at other sites of drug action.

Ro 15-1788 has, however, been shown to reverse the effects of cyproheptadine on schedule-controlled behavior in the squirrel monkey [1]. It is possible that behaviors controlling feeding and operant responding may be under different neuropharmacological influences, or perhaps there may be considerable species differences in the effects of these drugs.

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