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Methyl β-Carboline-3-Carboxylate Enhances Performance in a Multiple-Trial Learning Task in Mice

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RAFFALLI-SEBILLE, M.-J., G. CHAPOUTHIER, P. VENAULT AND R. H. DODD. *Methyl β-carboline-3-carboxylate enhances performance in a multiple-trial learning task in mice.* PHARMACOL BIOCHEM BEHAV **35**(2) 281–284, 1990.—In contrast to diazepam, a benzodiazepine receptor (BZ-R) ligand, which impairs memory processing, methyl β-carboline-3-carboxylate (β-CCM), another BZ-R ligand, administered before a training session, enhances performance in a retention test. This action, however, has only been demonstrated in single trial or single session learning protocols. The present report extends these results to a multiple-trial learning procedure in mice (brightness discrimination in a T-maze with negative reinforcement). The animals were trained for sessions of ten trials per day for six consecutive days. In a first experiment, the sessions during the first three days took place after administration of β-CCM (0.3 mg/kg), diazepam (2.5 mg/kg) or saline. In a second experiment, especially designed to study the effects of β-CCM, during the first three days animals received β-CCM (0.3 mg/kg), Ro 15-1788 (15 mg/kg), β-CCM + Ro 15-1788, vehicles of these drugs or saline. In the first experiment, performance was improved by β-CCM and impaired by diazepam in the first three sessions as well as in the final three. In the second experiment, β-CCM alone, as well as Ro 15-1788 improved performance, and the simultaneous administration of the two drugs suppressed these effects. These results suggest that the performance enhancing effects of β-CCM observed in single trial learning protocols, during the retention test, can already be observed during drug treatment. They confirm that β-CCM has an action on acquisition (learning). As the effects of β-CCM are suppressed by the simultaneous administration of Ro 15-1788, our results could suggest a role for benzodiazepine receptors in learning. This question is discussed.

Methyl β-carboline-3-carboxylate Benzodiazepines Multiple-trial learning Mouse

BENZODIAZEPINES (BZs) are widely used anxiolytics and anticonvulsants. Their potent sedative properties are routinely used in presurgical anesthesia. BZs are also known to induce, in man, a strong anterograde "amnesia" [see review, (10)]. Similar effects have been shown in rodents (17,18). The term "amnesia" has since been used in accord with its clinical use. It does not exclude that BZs seem to have a more specific effect on the acquisition side (learning deficit) of memory processing.

Specific substances which can block the effects of BZs have recently been described (1,7). Some of these substances have intrinsic pharmacological properties which are opposite to those of BZs; they have thus been called "inverse agonists" (2,14). Ro 15-1788, classically described as an antagonist, also presents some inverse agonist properties (9). We have been able to show that an inverse agonist belonging to the β-carboline group, methyl β-carboline-3-carboxylate (β-CCM) enhances learning in three different tasks: habituation to a new environment and passive avoidance in mice, and imprinting in chicks (20). Since all these three tasks are based on a one-trial (or one session) learning paradigm, in the present study we have tried to generalise the effects obtained in a multiple-trial situation.

METHOD

Animals

Subjects were 30 g Swiss male mice (aged 3 months) (Iffa-Credo, France). They were kept 10 per cage in our animal quarters at 25°C with a day-night cycle of 12-12 hr.

Drugs

Methyl β-carboline-3-carboxylate (β-CCM), synthesised by one of us (R.H.D.), was dissolved in 100 μl of 0.1 N HCl, diluted to volume in saline and administered at the dose of 0.3 mg/kg. The basis of the choice of this drug were former results in which performance varied with an inverted-U function with increasing dose and which showed an optimal effect of β-CCM at this dose (19). Diazepam, provided by Hoffmann-La Roche, Paris, was

diluted to volume with saline and administered at the dose of 2.5 mg/kg, also chosen according to former data (19). Previous results of our laboratory had provided evidence that diazepam solvent, kindly provided by Hoffmann-La Roche Laboratories, Basel (reference Ro 05-2807/767 S), including: propylene glycol, sodium benzoate, benzoic acid, ethyl and benzyl alcohols and water ad injectionen, was devoid of any effect in the present learning situation. It is also known to be comparable to saline in other behavioural situations tested by Hoffmann-La Roche (W. Haefely and N. Eigenmann personal communication). Ro 15-1788, also provided by Hoffmann-La Roche, was suspended in saline with a drop of Tween 80 and administered at the dose of 15 mg/kg. In a first preliminary experiment, to compare β-CCM and diazepam, control injections consisted of saline. In a second experiment with β-CCM and Ro 15-1788, control injections consisted of the vehicles of each drug, that is: saline with a drop of Tween 80 for Ro 15-1788 and saline with 100 μl of acid for β-CCM. A control group of saline-treated mice was also used together with the group treated with both vehicles. Drugs or control solutions were administered subcutaneously (SC) in a volume of 0.05 ml/10 g (body weight).

Training Apparatus

The training apparatus used was a 4.5×4.5 cm section T-maze, consisting of a 15 cm long departure alley and of two 10 cm long choice alleys. The walls and ceiling of the maze were of transparent plastic. The floor consisted of a metallic grid which could be electrified. The three alleys could be electrified separately, and the connecting square was electrified with the departure alley. The two choice alleys could be lit by a 25-W electric bulb situated above the ceiling.

Training

Training consisted of 6 successive daily sessions of 10 trials each. Because our population of mice had a slight tendency to prefer the lit alley, the animals were trained to choose the dark alley. For a given trial, a mouse was placed at the entrance of the departure alley. It had 30 seconds to choose between the lit and the dark alley. Past this delay, it was forced to choose by a 50-μA 2-sec electric footshock in the departure alley. If it chose the lit alley, it received other 50-μA 2-sec electric footshocks separated by 2-sec intervals, until it chose the dark alley. If it chose the dark alley, it could escape the maze and, after 30 sec, was placed at the entrance for another trial. Thus, an error was counted only when an animal entered the lit alley, and, therefore, only errors in discrimination were counted as errors. Speed of response was not investigated since this discrimination procedure appeared to be a better measure. Light or darkness were attributed to the left or right arm of the maze according to a Gellerman random sequence.

Administration of Drugs

In the first experiment, 10 min before the first three sessions, the animals were administered β-CCM, diazepam or saline. In the second experiment, again 10 min before each of the first three sessions, the animals received two simultaneous injections of β-CCM and Ro 15-1788, β-CCM and vehicle of Ro 15-1788, Ro 15-1788 and vehicle of β-CCM, both vehicles or one injection of saline.

Statistics

The statistical test used is the one-way analysis of variance.

RESULTS

In the first preliminary experiment, animals received either saline, diazepam or β-CCM. Analysis of variance provides evidence that all groups improved their performance in successive training sessions, as shown by the difference in errors between the first three sessions and the last three [controls, $F(1,38)=25.60$, $p<0.001$; diazepam-treated, $F(1,30)=9.78$, $p<0.01$; β-CCM-treated, $F(1,30)=14.75$, $p<0.001$]. Global analysis of variance shows a significant effect of drugs for the first three days, $F(2,49)=20.11$, $p<0.001$, as well as for the last three without injection, $F(2,49)=20.75$, $p<0.001$. Detailed analysis reveals (Fig. 1) that performance is impaired by the administration of diazepam (increase in the number of errors) both during the sessions under drug treatment [diazepam versus controls, $F(1,34)=14.75$, $p<0.001$] and during the sessions without drug, $F(1,34)=10.50$, $p<0.01$. The opposite effect, that is an improvement in performance (reduction of the number of errors), is obtained with β-CCM, both during the sessions under drug treatment [β-CCM versus controls, $F(1,34)=7.13$, $p<0.05$] and during the sessions without drug, $F(1,34)=11.0$, $p<0.01$.

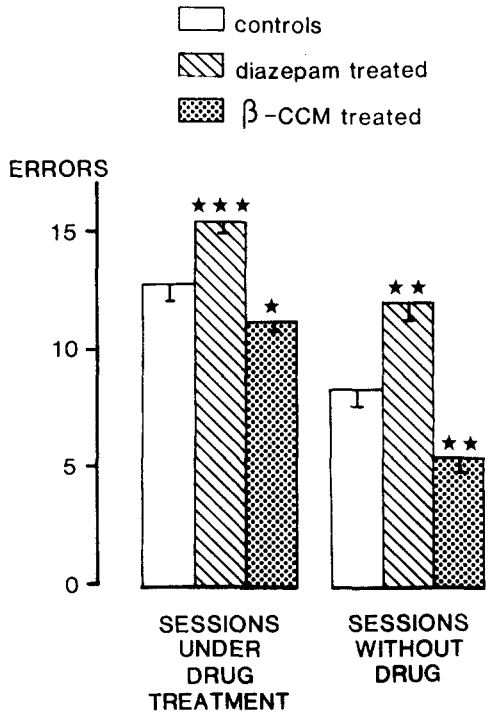

FIG. 1. Effects of diazepam and β-CCM on performance in maze learning. The bars represent the number of errors (mean ± s.e.) of the different groups of animals during the 30 trials of the first three sessions under drug treatment or of the three last sessions without drug. Open bars: controls (N = 20); hatched bars: diazepam-treated (2.5 mg/kg, N = 16); dotted bars: β-CCM-treated (0.3 mg/kg, N = 16). ★$p<0.05$, ★★$p<0.01$, ★★★$p<0.001$ as compared to respective controls.

In the second experiment, animals received simultaneous administration of β-CCM + Ro 15-1788, β-CCM + vehicle of Ro 15-1788, Ro 15-1788 + vehicle of β-CCM, both vehicles or saline. As before, all groups improved their performance with training, as shown by the difference in errors between the first three sessions and the last three [controls, $F(1,30)=56.96$, $p<0.001$; vehicles, $F(1,30)=34.08$, $p<0.001$; β-CCM alone, $F(1,30)=53.33$, $p<0.001$; Ro 15-1788 alone, $F(1,30)=62.42$, $p<0.001$; β-CCM + Ro 15-1788, $F(1,30)=54.95$, $p<0.001$] (Fig. 2). Global analysis of variance shows a significant effect of the drugs during the first three days, $F(4,75)=6.53$, $p<0.001$, as well as during the last three days, $F(4,75)=5.70$, $p<0.001$.

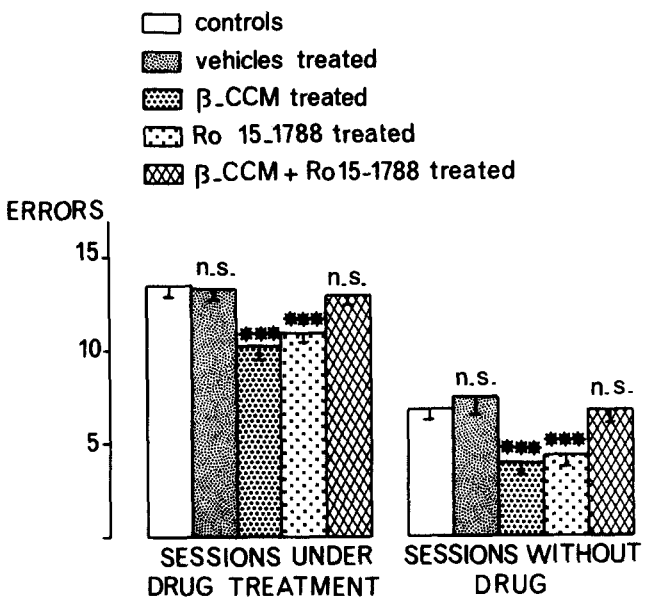

FIG. 2. Reversal of the effects of β-CCM by simultaneous administration of Ro 15-1788. The bars represent the numbers of errors (mean ± s.e.) of the different groups of animals during the 30 trials of the three first sessions under drug treatment and the three last sessions without drug. Open bars: saline-treated controls (N = 16); grey bars: animals treated with both vehicles (N = 16); tightly dotted bars: β-CCM-treated (0.3 mg/kg, N = 16); sparsely dotted bars: Ro 15-1788-treated (15 mg/kg, N = 16); crossed bars: β-CCM (0.3 mg/kg) + Ro 15-1788- (15 mg/kg) treated (N = 16). ***$p<0.001$ as compared to saline respective controls.

Detailed analysis reveals that the vehicle-treated group did not differ from controls. Ro 15-1788 alone, as well as β-CCM alone, improved performance both during the sessions under drug treatment [β-CCM versus controls, $F(1,30) = 13.54$, $p<0.001$; Ro 15-1788 versus controls, $F(1,30) = 10.04$, $p<0.001$] and during the sessions without drug [β-CCM versus controls, $F(1,34) = 11.44$, $p<0.001$; Ro 15-1788 versus controls, $F(1,30) = 7.75$, $p<0.01$]. There is no difference, however, between the β-CCM + Ro 15-1788-treated group as compared to the controls either during the three first sessions or during the last three (without drug). Thus, Ro 15-1788 suppressed the effects of β-CCM administered alone.

DISCUSSION

Previous results of our group (3, 19, 22) have provided evidence that when β-CCM is administered before acquisition, performance is enhanced during a retention test. These results were obtained in a passive avoidance task in mice, in a habituation to a new environment task in mice and in imprinting in chicks. All these tasks involved a single training trial (passive avoidance) or a single training session (habituation, imprinting), the second trial or session only being used for the test of performance. We verified that the performance enhancing effect obtained with pretraining administration of β-CCM could not be obtained when the drug is administered immediately after training or before the retention test. In all these models, diazepam had the direct opposite effect to that of β-CCM.

In a present work we extend these data to a multiple-trial situation. The first experiment enables us to show that the opposite effects of β-CCM and diazepam obtained in the above-mentioned tasks during the test session are already observable under drug treatment. One can observe, as before, β-CCM improvement and diazepam impairment of performance when the animals are no longer under treatment (last three sessions), but both this improvement and this impairment can already be observed during the administration of β-CCM or diazepam, respectively (first three sessions). This observation could not be made in the one trial or one session learning of the former studies. Though more work must still be carried out in this area, our data seem to rule out a "state-dependency" interpretation of the effects of β-CCM and diazepam. A "state dependency" hypothesis would require that effects observed under drug treatment would not persist when the drug is no longer administered. On the other hand, previous results of our group have shown that a dose of 1 mg/kg β-CCM had no effect on pain threshold in mice (16). We can thus rule out a possible explanation of the effects of β-CCM in terms of analgesia. Finally, our present data confirm what we have previously suggested (19,20), i.e., both β-CCM and diazepam have an action on the acquisition (learning side) of memory processing rather than on retention (memory) itself.

Since, in the second experiment, the effects of β-CCM on learning are suppressed by administration of Ro 15-1788, the specific antagonist of the BZ receptor, it could be assumed that these effects are mediated by the BZ-R. A similar conclusion was drawn from the results obtained in the one-trial passive avoidance task (20). Indeed, β-carbolines are known to bind with high affinity to the central BZ-R (1,5) and some have been proposed as endogenous ligands for these receptors (1,13). Ro 15-1788 is described as a ligand of BZ-R with high affinity and great specificity, which reaches its sites of action within the CNS very rapidly and prevents and reverses, dose-dependently, all effects that BZ-R agonists and inverse agonists produce via the BZ-R (6). It is, however, important to note that Ro 15-1788, classically used as a "neutral" antagonist of the BZ receptor, has effects of its own. Lal *et al.* have shown a similar effect in another negatively reinforced learning task (9): these authors hypothesize that pretreatment with Ro 15-1788 "may facilitate learning or memory processes by reversing a negative modulatory influence of endogenous diazepam-like ligands for benzodiazepine receptors." In the light of these different data, i.e.:

a) the effects of β-CCM on learning vary with an inverted U-function,

b) the dose of β-CCM used in this study produces an optimal effect,

c) Ro 15-1788 is intrinsically active in the paradigm,

d) simultaneous administration of β-CCM and Ro 15-1788 suppresses the enhancement of performance,

two interpretations may be proposed:

1) According to b), c), and d), a classical interpretation could be that in spite of their intrinsic action, β-CCM and Ro 15-1788 antagonize each other in case of simultaneous administration; however, this would not necessarily require the effects of β-CCM and Ro 15-1788 to be mediated via the same sites within BZ-R;

2) according to a) and c) a new interpretation could be proposed. It could be assumed that Ro 15-1788 enhances (instead of antagonizing) the effects of β-CCM. Thus, combined administrations of β-CCM and Ro 15-1788 could produce the same effect as higher doses of β-CCM alone, i.e., a weaker effect on performance.

Experiments with several doses of β-CCM and several doses of Ro 15-1788 could be assumed to clarify this point. However, when doses of both compounds are high, it is known that Ro 15-1788 has its clear (classical) antagonistic effect (15). Only a synergy between very low doses of β-CCM and of Ro 18-1788 could offer an answer to this question and could be the subject of further work allowing a better understanding of the action of Ro 15-1788.

As far as β-CCM is concerned, the question finally remains as

to how β-CCM exerts its effects on learning. One explanation of our data could be that β-CCM increases the level of arousal during the training session. This explanation would concord with the observation that, in rodents, arousal-enhancing drugs improve learning (11) and that several β-carbolines increase arousal (12). The work by Jensen *et al.* (8) tends to confirm this view in rodents, by showing that cognitive effects of several BZ receptor ligands reflect changes in arousal or vigilance. Duka *et al.* (4) found similar effects in man, where the β-carboline ZK 93 426 improved performances in two cognitive tasks: a "logical reasoning task and a picture difference task which estimated concentration and attention, respectively." This interpretation is, however, difficult in the case of chicks where it has been found that, surprisingly, β-CCM has a sedative effect (21). An explanation for this observation could be that the action of β-carbolines in birds has marked differences with that seen in mammals.

Another possibility would be to link the effects on learning to the anxiogenic effect of β-CCM. However, in mice, where anxiogenic and convulsive effects of β-CCM have been extensively studied, it can be noted that the performance-enhancing effects of β-CCM were not seen in the dose range of the anxiogenic or convulsive effects of this drug, since the optimum dose of the present study (0.3 mg/kg) is much lower than the anxiogenic dose in a conflict model (1 mg/kg) or the convulsive doses (1–10 mg/kg), in the same strain of mice. It might, nevertheless, be assumed that the doses used in the present work, insufficient to produce noticeable effects with such classical techniques as conflict models, are still sufficient to act on an emotional component of performance. This explanation does not contradict an effect on learning and memory processes. It simply assumes that both the improvement of performance in learning and memory tasks and the effects in a conflict situation derive from a common mechanism involving anxiety. Such an hypothesis should be submitted to further experiments in the future.

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