Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1487–1489, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Microinjections of Methyl-β-Carboline-3-Carboxylate Into the Dorsal Raphe Nucleus: Behavioural Consequences

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JONES, B. J., I. A. PATERSON AND M. H. T. ROBERTS. *Microinjections of methyl-β-carboline-3-carboxylate into the dorsal raphe nucleus: Behavioural consequences.* PHARMACOL BIOCHEM BEHAV 24(5) 1487–1489, 1986.—Very small quantities (0.01–10 ng) of the inverse benzodiazepine receptor agonist, methyl-β-carboline-3-carboxylate (β-CCM) microinjected into the dorsal raphe nucleus (DRN) of the rat selectively reduced social interaction, an effect consistent with an increase in anxiety. Similarly, intraperitoneally injected β-CCM, within a limited dose range, reduced social interaction without affecting locomotor activity. The benzodiazepine receptor antagonist, RO15-1788 (1 ng) microinjected into the DRN, reversed the suppression of social interaction induced by either intra-raphe or intraperitoneal β-CCM. Histological examination of the β-CCM microinjection sites showed that locations within the DRN were almost invariably associated with decreases in social interaction; microinjections failing to decrease social interaction were located primarily outside the DRN. We conclude that the DRN has a major role in expressing the anxiogenic effect of β-CCM and it may therefore be an important area in the neuronal system controlling anxiety.

Dorsal raphe nucleus Methyl-β-carboline-3-carboxylate Microinjection Social interaction Anxiogenic RO15-1788

SINCE the anxiolytic benzodiazepines were discovered, their interactions with neurotransmitter systems in the brain have been widely investigated. The outstanding observation to emerge from these studies is that benzodiazepines facilitate the inhibitory actions of gamma-aminobutyric acid (GABA) (for review see [9]). This has secondary implications in that GABA in turn influences other neurotransmitter systems. We have been investigating the possibility that benzodiazepines may express their anxiolytic effects by modifying the interactions of GABA with 5-hydroxytryptamine (5-HT) neurones. In this context, we have attempted to study the involvement of the dorsal raphe nucleus (DRN), one of the main sources of the 5-HT projections to the forebrain.

Very small amounts of chlordiazepozide injected into the DRN of rats were shown to release punishment suppressed responding [10]. This observation indicated that the benzodiazepine receptors in the DRN might have an important role in the mediation of anxiety, so we investigated the effect of injecting the benzodiazepine receptor inverse agonist, methyl-β-carboline-3-carboxylate (β-CCM) into the DRN. To assess the potential anxiogenic effect of β-CCM, we used the social interaction test, a procedure that has been shown to be sensitive to anxiogenic drug action [4].

SOCIAL INTERACTION STUDIES

Male Lister hooded or Wistar rats were implanted with guide cannulae; microinjections were made through fine glass tubing [1]. The sites were verified histologically after completion of the experiments. Pairs of rats consisted of one cannulated, treated rat and an untreated partner. This design was adopted to avoid the difficulty of obtaining identical cannula placements in both rats of a pair. Rats were used as their own controls; they were tested twice, once after β-CCM and once after vehicle. Four days were allowed between tests. Social interactions were assessed under low light and the rats were familiarised to the arena.

Very small amounts of β-CCM (0.1–10 ng) injected into the DRN consistently and reproducibly reduced social interaction [5] (Fig. 1). Locomotor activity, as measured by light beam crossings, was very variable and on occasions we have observed a statistically significant reduction. The inconsistency in the locomotor activity results was probably because the contribution of the untreated rat attenuated changes in the treated rat.

When both rats in a pair were injected intraperitoneally with β-CCM, 4 mg/kg, social interaction and locomotor activity were reduced. However, doses of 1 or 2 mg/kg selec-

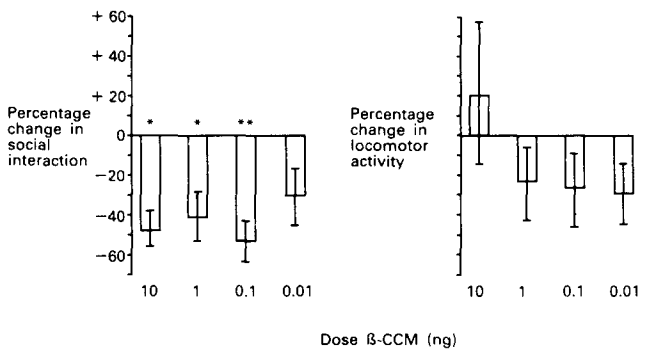

FIG. 1. The effects of β-CCM microinjected into the dorsal raphe nucleus on social interaction in rats. The changes shown are the differences between the measurements taken from the same rats on two occasions—once after β-CCM and once after vehicle treatment. *$p<0.05$; **$p<0.01$.

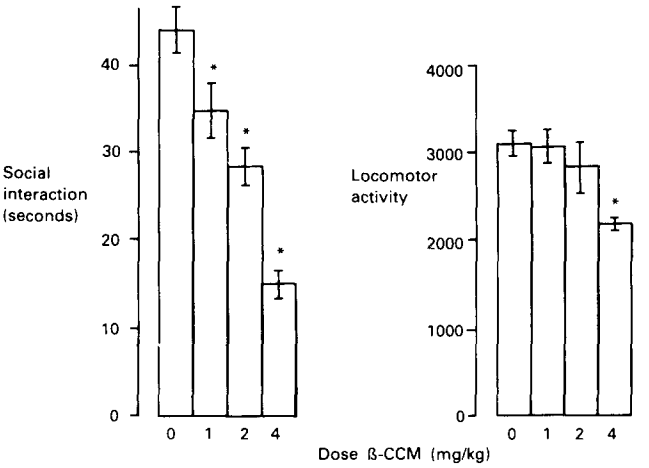

FIG. 2. The effects of β-CCM injected intraperitoneally on social interaction in rats. *$p<0.05$; **$p<0.01$.

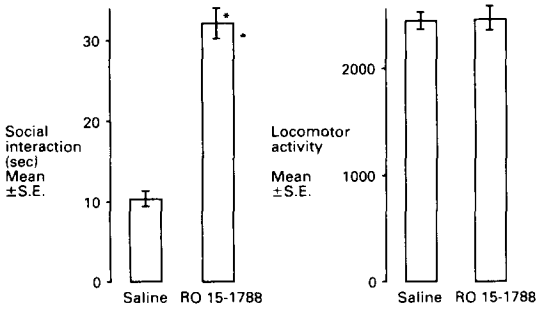

FIG. 3. The effect of RO15-1788 (1 ng) microinjected into the dorsal raphe nucleus on the social interaction of rats treated with β-CCM (4 mg/kg IP). *$p<0.05$.

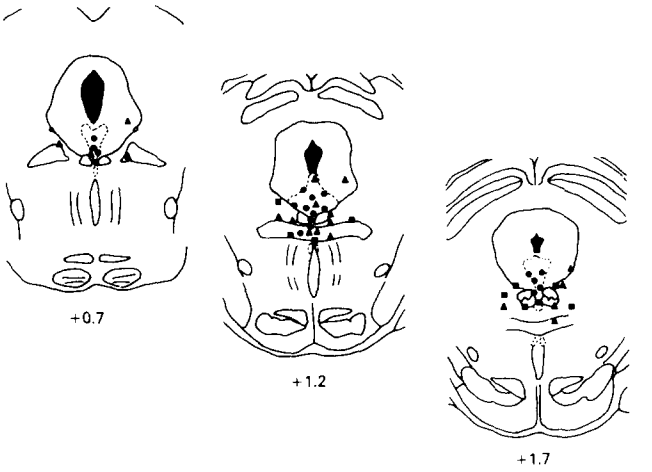

FIG. 4. Sites of microinjections of β-CCM correlated with effects on social interaction. ● Sites where β-CCM reduced social interaction by more than two standard deviations of the differences between the two tests; ▲ sites where β-CCM did not affect social interaction; ■ sites where β-CCM increased social interaction.

tively suppressed social interaction (Fig. 2). Whether the reduction in social interaction was induced by β-CCM injected intraperitoneally (4 mg/kg) or microinjected into the DRN (0.1 ng), it could be reversed by the benzodiazepine receptor antagonist RO15-1788 (1 ng) microinjected into the DRN [6] (Fig. 3). These results suggest that the benzodiazepine receptors in the DRN are important in mediating the effect of β-CCM on social interaction.

CORRELATION OF INJECTION SITES WITH THE EFFECT OF β-CCM ON SOCIAL INTERACTION

The intra-raphe microinjection sites relating to the above results were verified histologically to be within the DRN. Figure 4 summarises the data from a series of experiments including those injection sites that were outside the DRN. Decreases in social interaction were clearly associated with locations within the DRN. Microinjections of β-CCM that did not decrease social interaction significantly were primarily outside the DRN. The observation that some microinjections of β-CCM outside the DRN increased social interaction is as yet unexplained, but deserves further investigation.

EXPLORATORY ACTIVITY STUDIES

To support the social interaction experiments, we examined the behaviour of single rats in a novel environment after administration of β-CCM. The apparatus was a rectangular acrylic cage with holes in the floor and three banks of infra red beams and photocells to measure rearing, ambulation and head-dipping.

A dose of β-CCM (0.1 ng) injected into the DRN that reduced social interaction had little effect on exploratory activity in single rats. Only rearing was significantly reduced. Intraperitoneally injected β-CCM reduced all three

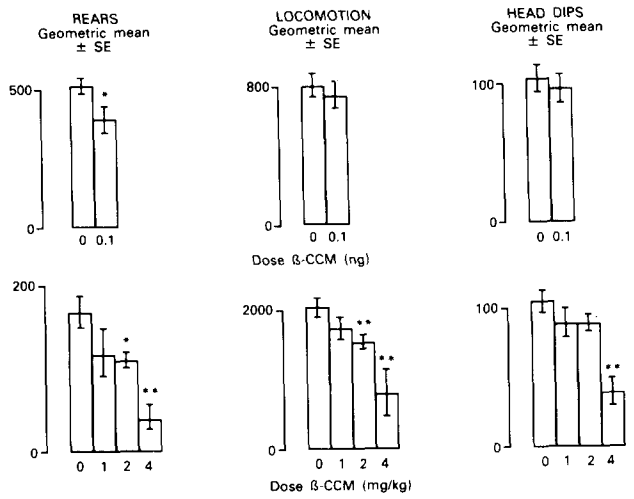

FIG. 5. The effect of β-CCM on exploratory activity in single rats. Upper panel: β-CCM was injected into the dorsal raphe nucleus. Lower panel: β-CCM was injected intraperitoneally. $^{*}p<0.05$; $^{**}p<0.01$.

parameters but tended to have a more pronounced effect on rearing (Fig. 5). At the highest dose (4 mg/kg), all parameters were markedly reduced. Nevertheless, the effects of this dose were completely abolished by pretreatment with RO15-1788, 1 mg/kg IP (result not shown).

DISCUSSION

The observation that βCCM, administered intraperitoneally or microinjected into the DRN, reduced social interaction in rats is consistent with the view that β-CCM is anxiogenic. In support of this view, β-CCM has been shown to have a proconflict effect [3,8]. Nevertheless, the evidence that β-CCM increases anxiety is circumstantial and remains open to criticism. For instance, β-CCM is a convulsant [2,7] and in these experiments it tended to reduce locomotor activity, so its effect on social interaction could be a secondary phenomenon. This is unlikely, however, because social interaction was clearly the most sensitive parameter.

There was little doubt that the effects of β-CCM that we measured were mediated through the GABA-benzodiazepine receptor complex; the benzodiazepine receptor antagonist RO15-1788 clearly reversed these effects. Thus, β-CCM was acting specifically at the receptor complex that is known to be associated with anxiolytic drug action.

Microinjections of β-CCM into the DRN were as effective as intraperitoneally injected β-CCM in reducing social interaction, indicating that the DRN is a major area for mediating the anxiogenic effect of β-CCM. The extreme sensitivity of the DRN also supports this theory. Furthermore, the histological evidence showed quite clearly that the effect was localised to the DRN. Finally, the observation that the benzodiazepine receptor antagonist, RO15-1788, microinjected into the DRN, reversed the reduction in social interaction induced by intraperitoneally injected β-CCM is convincing evidence for the importance of the DRN.

In conclusion, these investigations show that the benzodiazepine receptors in the DRN play a major role in the mediation of the anxiogenic effects of β-CCM. The DRN may therefore be an integral part of the neuronal system involved in anxiety. If this is so, the involvement of 5-HT pathways in the expression of the anxiolytic effects of benzodiazepines clearly deserves re-examination

ACKNOWLEDGEMENT

We would like to thank Mr. G. A. Higgins for performing the exploratory activity experiments.

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