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Naloxone Blocks the Effect of Diazepam and Meprobamate on Conflict Behaviour in Rats

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DUKA, TH., R. CUMIN, W. HAEFELY AND A. HERZ. *Naloxone blocks the effect of diazepam and meprobamate on conflict behaviour in rats.* PHARMAC. BIOCHEM. BEHAV. 15(1) 115–117, 1981.—The effect of naloxone on the anticonflict action of diazepam was studied in a model involving foot shock-induced suppression of food-rewarded operant behaviour. Both 1 and 10 mg/kg naloxone SC abolished the increase in punished responding produced by diazepam and chlordiazepoxide. Naloxone also blocked the anticonflict effect of meprobamate. These observations are discussed in terms of a possible involvement of endogenous opioid peptides in the anxiolytic effects of tranquillizers.

Antianxiety Diazepam Meprobamate Naloxone Opioid peptides

A number of recent studies deals with interactions between benzodiazepines, opiates and opiate antagonists. For example, some of the behavioural effects of chlordiazepoxide or diazepam were reported to be antagonized by naloxone [1, 9, 14]. Furthermore, Duka *et al.* [4] found that diazepam alters met-enkephalin levels in certain areas of the brain and produces antinociception in rats pretreated with intracerebroventricular bacitracin, an inhibitor of enzymatic met-enkephalin breakdown [17]. Based on these findings benzodiazepines were proposed to stimulate the release of endogenous opioids in vivo [17]. The possible pharmacologic relevance of this interaction remains in need of investigation, in particular in humans. We were interested to see whether the changes induced by benzodiazepines in endogenous opioids contribute to the anxiolytic action of these drugs.

In so-called conflict tests approach-avoidance behaviour is studied in animals in which positive-reinforced behavioural responses are coupled with punishment; foot shock-induced suppression of food-rewarded operant behaviour has been suggested as a model of anxiety (see Sepinwall and Cook [13]). Benzodiazepines are well known to attenuate the response inhibition induced by contingent punishment (see Haefely [8]).

In the present study, we describe the interaction between two benzodiazepines and naloxone, a potent opiate antagonist, in a rat conflict procedure. To test whether a possible effect of naloxone was related to anxiolytic effects of benzodiazepines only, meprobamate, another drug with antianxiety properties, was also used in the present experiments. In contrast to similar studies performed by Billingsley and Kubena [1], we used doses of naloxone that can be considered to interact selectively with opiate systems.

METHOD

The test used was a simplified modification of procedures described by Geller *et al.* [6] and Cook *et al.* [3]. The ability of drugs to enhance food-reinforced lever press responses suppressed by punishment is examined. The animals used were female rats (stock Füllinsdorf Albino SPF, outbred), weighing 200–230 g at the end of the training period.

The test apparatus was an operant behaviour box, 30×25×30 cm, equipped with an electrifiable grid floor, one lever and a pellet chute.

Naive rats (150–160 g) were fasted 24 hr before each session. They were first trained in three 1 hr sessions (each on a different day) to press a lever for 45 mg food pellets on a continuous reinforcement (crf) schedule. In the third session, the rats reached a lever-pressing rate of 150–250 per hour. In the fourth session, each pellet reward was combined with a brief electric foot shock (1.0 mA). Rats confronted for the first time with this kind of conflict situation initially continued pressing for 5–10 food pellets (i.e. emitted a few punished responses) before ceasing pressing. In a fifth session, the rats were allowed to press for pellets in the absence of foot shock (150–250 lever presses per hour). Each of these

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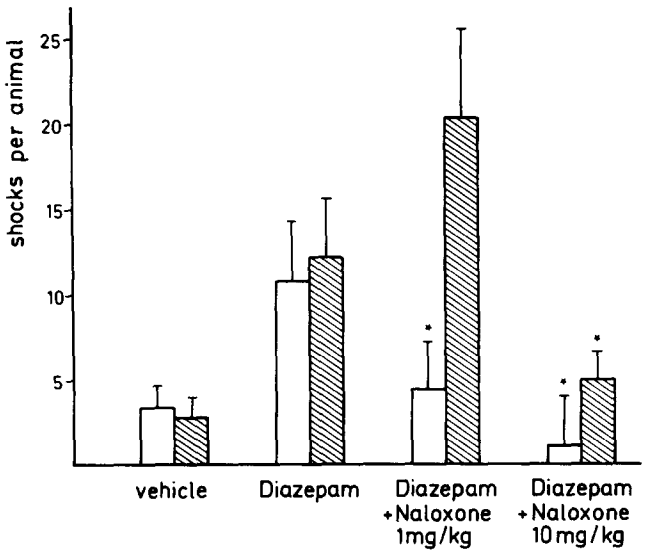

FIG. 1. Time course of the effect of naloxone (1 or 10 mg/kg SC) on the anticonflict activity of 1 mg/kg diazepam IP, injected 15 and 30 min, respectively, prior to the test run. The open columns indicate the number of shocks taken during the first half hour of the test and the cross-hatched columns the number of shocks taken during the second half hour. The values are means ± SEM of at least 15 animals per group. The asterisks indicate a statistical significant difference ($p<0.001$) to animals given diazepam alone.

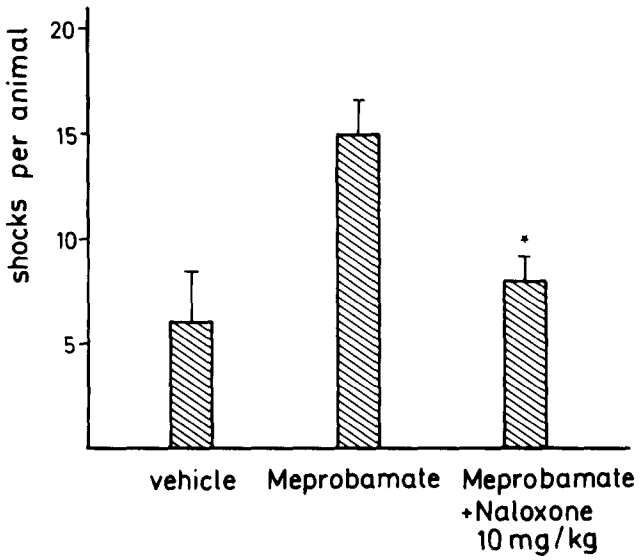

FIG. 2. Effect of 10 mg/kg naloxone SC on the anticonflict effect of 70 mg/kg meprobamate PO. Columns represent the number of shocks taken during the test (mean ± SEM of at least 15 animals per group). The asterisk indicates statistical significant difference ($p<0.01$) to animals given meprobamate alone.

sessions was performed on a different day and successive sessions were separated by an interval of a whole day during which rats were fasted. In order to exclude animals irresponsive to an established anxiolytic drug (this occurs occasionally in a small percentage of any population of rats), 20 mg/kg chlordiazepoxide hydrochloride PO was administered in a sixth session 30 min before a 1 hr conflict run. Only rats increasing their lever pressing rate to 20–50 food pellets (as compared with 5–10 food pellets in the drug-free fourth session) were kept for further testing of agents. About 10% of rats had to be eliminated.

Drug Experiments

For drug experiments, the following fixed sequence of test and control runs combined with a special feeding schedule was maintained.

Day 1 : control run, no foot shock	/ 14 g dry food cubes in the evening
Day 2 : no run,	no food
Day 3 : control run, with foot shock	/ 14 g dry food cubes in the evening
Day 4 : no run,	no food
Day 5 : control run, no foot shock	/ 14 g dry food cubes in the evening
Day 6 : no run,	no food
Day 7 : test run (drug), with foot shock	/ 14 g dry food cubes in the evening

Groups of 10 to 15 rats trained and selected as described were administered different drugs on day 7.

Drugs and doses used were 1 mg/kg diazepam IP, 70 mg/kg meprobamate PO and 1 mg/kg or 10 mg/kg naloxone SC; diazepam was dissolved in NaCl with Tween 80 (10 ml NaCl, 2 drops of Tween 80) in a concentration of 1 mg/2 ml. Diazepam and meprobamate were administered 30 min and naloxone was injected 15 min before the test run.

During the first and second half hour of the test run, the total number of lever pressings for food pellets in combination with brief foot shock (punished responses) was recorded for each rat. The data were analyzed for statistical significance of difference using the Fischer exact probability test.

RESULTS

As can be seen in Fig. 1, 1 mg/kg diazepam IP increased the number of foot shocks taken ("anticonflict activity") and naloxone at the dose of 10 mg/kg SC completely antagonized this effect of diazepam in both the first and the second half hour of the test run. Naloxone at 1 mg/kg SC was effective only in the first half hour of the test (Fig. 1). Naloxone was also found to antagonize the anticonflict effect of 20 mg/kg chlordiazepoxide PO (data not shown). Meprobamate 70 mg/kg PO produced a significant antianxiety effect which was abolished by naloxone (Fig. 2). Naloxone alone did not significantly alter conflict behaviour of rats.

DISCUSSION

The present study shows that naloxone antagonizes the anticonflict effect of two benzodiazepines, and is in line with recent results of Soubrié *et al.* [14], that naloxone attenuated diazepam-induced release of punished behaviour. The benzodiazepines effect in our study was blocked to a similar degree of both doses of naloxone used (1 and 10 mg/kg SC); the effect of the two doses differed only in its duration (Fig. 1).

In view of the relatively pure opiate antagonistic properties of naloxone [11] and our finding that the anticonflict effect of benzodiazepines was completely blocked by a low

dose of naloxone (1 mg/kg SC), one might assume that this action of benzodiazepines was due to an activation of endogenous opioid systems. Such an assumption would be consistent with data of previous biochemical studies showing that an acute dose of diazepam induces a rapid decrease of enkephalin levels in the striatum, possibly reflecting an increased release of the peptides [5,17]. In similar experiments, naloxone has been found to reverse this diazepam-induced opioid release [4]. In the present study naloxone might have blocked the anticonflict activity of benzodiazepines by reducing their effect on opioid release, by blockade of released opioids, or by both. In experiments similar to ours, Billingsley and Kubena [1] used a high dose of naloxone (60 mg/kg) and obtained the same effect. The high dose of naloxone (which may block GABA receptors) used in their experiments and the similar antagonistic effect of picrotoxin on this benzodiazepine effect led the authors to postulate that naloxone acted by blocking GABA receptors. However, the effectiveness of naloxone at the low dose of 1 mg/kg in the present experiments strongly suggests that interaction with opiate receptors was its predominant mode of action. It was shown in previous experiments that benzodiazepines modulate enkephalin levels indirectly, via a GABAergic mechanism [5] and picrotoxin might, therefore, block GABA-mediated effects of benzodiazepines on opioids.

There are alternative explanations for the interaction of naloxone with benzodiazepines in the conflict test used. The effect of naloxone might be related to changes in food intake and in pain threshold. It has in fact been shown that naloxone itself reduces food intake in rats and guinea pigs [10, 12, 14] and the possibility exists that naloxone-induced inhibition of benzodiazepine-stimulated food intake reflects interaction with consummatory behaviour rather than with anxiety [15]. Effects of benzodiazepines and naloxone on pain threshold can probably be excluded as an explanation for this interaction in the conflict test since the small analgesic effect reported for diazepam has been found not to be antagonized by naloxone [17]. Accordingly, naloxone when given alone in the present study, did not enhance the depressant effect of punishment on lever pressing (data not shown). Another noticeable feature of naloxone's interaction with benzodiazepines appears to be its dependence on actual painful punishment during the conflict period; in fact naloxone failed to modify the disinhibitory effect of diazepam on behavioural responses depressed by conditioned fear in the absence of actual punishment [14].

There are some points which do not easily fit into a hypothetical opioid-mediated anxiolytic action of benzodiazepines. If endogenous opioids were involved in the antianxiety activity of benzodiazepines, one would expect opiates to produce anti-conflict effects. However, morphine was found to be inactive in conflict tests by various authors (see e.g. Sepinwall and Cook [13]) and was inactive also in the present test procedure. Furthermore an explanation is required for the fact that the anticonflict effect of benzodiazepines increases with repeated administration [13] while tolerance developed to the enkephalin reducing effect of diazepam [17].

It is of interest that the anticonflict effect of meprobamate, which possesses antianxiety properties not mediated primarily by GABA [8], was also antagonized by naloxone. This finding suggests that, if endogenous opioid systems contribute to the anxiolytic action, they do so also in the case of non-benzodiazepines.

In conclusion the present results might indicate that endogenous opioids are involved in the antianxiety effects of benzodiazepines as well as of meprobamate. However, since anxiety and antianxiety effects in animals can only be assessed by behavioural responses that are not specific parameters for anxiety, experiments in man are required to corroborate the above hypothesis. An attempt in this direction is the study of Grevert and Goldstein [7] which shows a dose related increase of anxious tension after naloxone.

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