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BRIEF COMMUNICATION

The Possible Role of Benzodiazepine Receptors in Morphine Analgesia

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PALAOĞLU, Ö. AND I. H. AYHAN. *The possible role of benzodiazepine receptors in morphine analgesia.* PHARMACOL BIOCHEM BEHAV **25**(1) 215–217, 1986.—Diazepam within its therapeutic dose range, was shown to have no effect on nociception, but was shown to antagonize the analgesic action of morphine. This antagonism was found to be statistically significant at 0.5 mg/kg diazepam. To elucidate the mechanism of this inhibitory action of diazepam against morphine analgesia, Ro 15-1788, the specific antagonist of benzodiazepine receptors was used. As a result, Ro 15-1788 was found to partially reverse the inhibitory action of diazepam against morphine analgesia. This overall interaction between the supramolecular GABA receptor complex and morphine is discussed.

Analgesia Diazepam Morphine Ro 15-1788 Benzodiazepine receptor

PHARMACOLOGICAL properties ascribed to benzodiazepines have now been shown to be mediated by the stereospecific, high-affinity binding sites located in the central nervous system [7,10]. It has been demonstrated that a newly synthetized imidazobenzodiazepine compound Ro 15-1788 [4] can antagonize all the central effects of benzodiazepines in a competetive manner [1,9].

It has recently been shown that the analgesic activity of morphine and endogenous opioids can be affected by benzodiazepines [3, 6, 13]. The present study is undertaken to elucidate the effect of diazepam on the analgesic effect of morphine. In addition, the role of benzodiazepine receptor antagonist Ro 15-1788 on the interaction of diazepam and morphine has been evaluated.

METHOD

Male albino mice weighing 20–25 g were housed prior to the experiments for three days in a laboratory environment with an ambient light/dark cycle, at a temperature of 18–22°C. Food and water were available ad lib. Each animal was used once. The tail-flick method [2] with a cut-off time of 6 seconds was used to assess the analgesic effect. Control reaction time for each animal was obtained immediately before the administration of drugs and the standard deviation (SD) of the mean control time for each group was calculated. All or none response was used such that the increment of the reaction time of an individual animal greater than 3 SD of the mean control time was accepted as an analgesic response and thus the percent of animals with an analgesic response was found. At least 30 animals were used to determine each dose-response curve and ED_{50} (median effective dose). The ED_{50} values and their 95% confidence intervals were determined by the method of Litchfield-Wilcoxon [5]. The ED_{50} values were considered to be significantly different from the control values if the experimental ED_{50} lay outside the 95% confidence interval of the control ED_{50} and also the control ED_{50} lay outside the 95% confidence interval of the experimental ED_{50}. The Student's *t*-test and paired *t*-test were applied when necessary.

Morphine, diazepam (Hofmann-La Roche) and Ro 15-1788 (Hofmann-La Roche) were injected into mice either alone or in combination with each other. A 30 minute time mark for morphine, 60 minute for diazepam and 20 minute for Ro 15-1788 were chosen for the acute effects and interactions of drugs; all drugs were administered subcutaneously. All of the dose-response curves were determined 30 minutes after morphine injection. It was of interest to detect whether the time dependent profile of the analgesic effect of morphine would change by diazepam and Ro 15-1788 so the effects of drugs on nociception were observed 30, 60, 120 and 180 minutes after morphine administration in all the drug-

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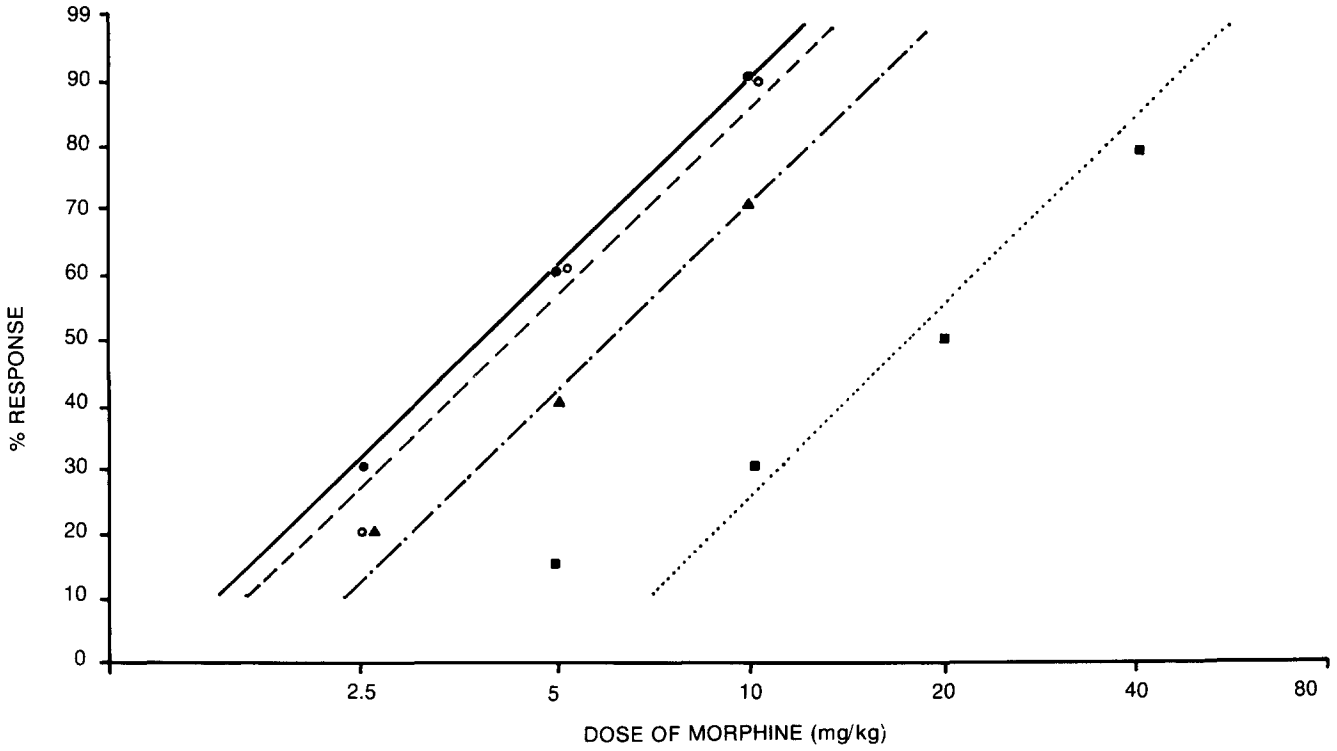

FIG. 1. The effect of different doses of diazepam on the dose-response curve of morphine in mice. Ten mice were used for each dose of drugs. —— Control; — — 0.125 mg/kg Diazepam; —·— 0.250 mg/kg Diazepam; ····· 0.500 mg/kg Diazepam.

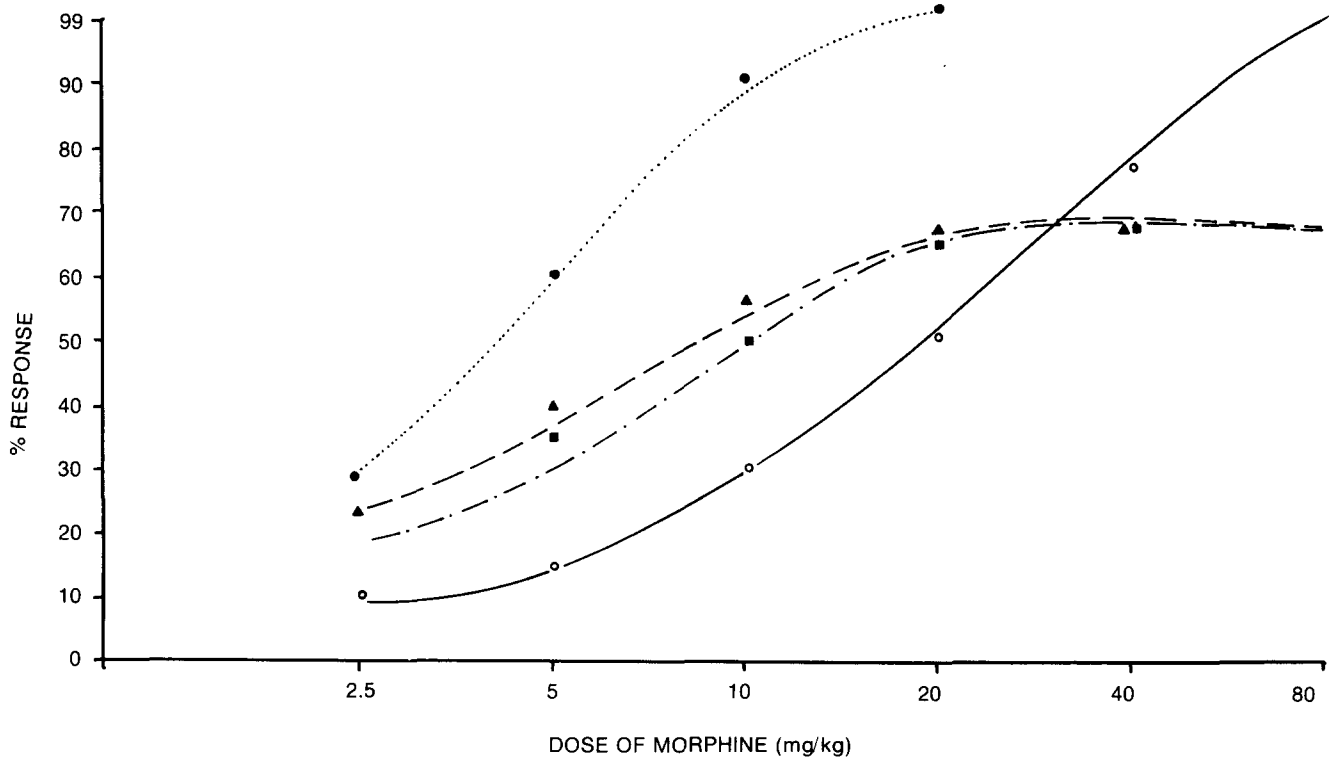

FIG. 2. The effect of Ro 15-1788 on the diazepam induced shift of the dose-response curve of morphine. Ten mice were used for each dose of each drug. ····· Morphine; —— Morphine + 0.5 mg/kg Diazepam (C); —·— C + 10 mg/kg Ro 15-1788; — — C + 20 mg/kg Ro 15-1788.

treated groups. Morphine was dissolved in distilled water; diazepam and Ro 15-1788 were used in suspension with water and Tween 80.

RESULTS

The analgesic ED_{50} (95% confidence intervals) of morphine was found to be 3.86 (2.41–6.19). Diazepam (0.125–1.0 mg/kg) alone did not produce any significant changes compared with its own control reaction time. The effect of diazepam on nociception in excess of 1.0 mg/kg could not be evaluated because of the profound behavioral effects of diazepam. When morphine and diazepam were injected simultaneously, diazepam induced a decrease in morphine analgesia. This effect of diazepam on morphine analgesia is shown in Fig. 1. Diazepam shifted the dose-response curve of morphine to the right in a dose-dependent manner. This antagonistic effect of diazepam on morphine analgesia was found to be significant at a 0.5 mg/kg dose. The analgesic ED_{50} (95% confidence interval) of morphine changed from 3.86 (2.41–6.19) to 17.76 (10.43–30.23). When 1.0 mg/kg diazepam was administered, morphine had no analgesic effect up to a dose of 40 mg/kg.

Ro 15-1788 (10–20 mg/kg) was not only found to have no effect on nociception, but the ED_{50} (95% confidence interval) of morphine was not affected as well. On the other hand, Ro 15-1788 (10–20 mg/kg) significantly antagonized the inhibitory action of 0.5 mg/kg diazepam against morphine analgesia (Fig. 2). Although the dose-response curves shifted to the left, maximum response could not be obtained. Twenty mg/kg Ro 15-1788 shifted the ED_{50} (95% confidence intervals) of morphine—0.5 mg/kg diazepam interaction from 17.76 (10.43–30.23) to 8.68 (3.87–19.73).

The time-dependent profile of the analgesic effect of morphine was found to be unchanged for the interactions of drugs with morphine throughout a 180 minute follow up.

DISCUSSION

Although there is some controversy regarding the effects of benzodiazepines on nociception [3,11], today it is generally accepted that these compounds exert an analgesic-like activity [12]. This is confirmed with the present study as, in its therapeutic dose range, diazepam had no effect on nociception. At a dose greater than 1.0 mg/kg, the effect on nociception could not be evaluated because of the profound sedative and myorelaxant effects of benzodiazepines.

Since benzodiazepines are widely used as therapeutic agents in combination with narcotic analgesics in anaesthesia premedication, it was of interest to evaluate the nature of their interaction. Though there seems to be a controversy for the action of different benzodiazepine compounds against morphine analgesia, previous evidence is equivocal for the effects of diazepam [3, 6, 13], showing an attenuation of the analgesic action of morphine that is in agreement with the results of our study. Our study reveals that diazepam antagonizes morphine analgesia in its therapeutic dose range.

As is known, benzodiazepines are accepted to exert their central effects by their interaction with specific receptors which are considered to be part of the supramolecular GABA receptor complex [8]. Although Ro 15-1788, the specific receptor antagonist of benzodiazepine receptors is said to be a competetive antagonist for all the central effects of benzodiazepines [1,9], as a relatively unexpected result in the present study, Ro 15-1788 was shown to be a partial antagonist of the inhibitory effect of diazepam on the analgesic action of morphine. In agreement with this finding, bicuculine, a specific receptor antagonist of GABA receptors which is also a unit of the supramolecular GABA receptor complex [8], was also found to partially antagonize the inhibitory effect of diazepam against morphine analgesia [6].

In conclusion, the mechanism of the inhibitory action of diazepam on morphine analgesia appears to depend partially on the allosteric interaction between the units of the supramolecular benzodiazepine-GABA receptor complex but has to be further elucidated.

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