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MODIFICATION OF THE ANTINOCICEPTIVE EFFECT OF MORPHINE BY CENTRALLY ADMINISTERED DIAZEPAM AND MIDAZOLAM

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1 Intracerebroventricular administration of diazepam or midazolam decreased the antinociceptive effect of morphine in rats as measured by the 'tail flick' method.

2 Midazolam, injected into the periaqueductal grey matter (PAG) antagonized the analgesic effect of morphine. The action of midazolam was partially reversed by bicuculline.

3 These findings support the view that the effect of benzodiazepines on morphine antinociception may be mediated through γ-aminobutyric acid receptors.

Introduction

Even though benzodiazepines and morphine are sometimes used clinically in combination, there have been few studies of the analgesic interactions of these two compounds. Weiss (1969) reported that peripherally injected chlordiazepoxide antagonized morphine antinociception, but according to another report, oxazepam and diazepam did not significantly modify the dose-response curves for morphine or methadone (Shannon, Holtzman & Davis, 1976).

Gavish & Snyder (1980) demonstrated that γ-aminobutyric acid (GABA) receptors possess recognition sites for benzodiazepines and recent studies suggest a functional link between GABA receptors and the pharmacological action of benzodiazepines (Costa & Guidotti, 1979). Furthermore, benzodiazepines have been shown to facilitate behavioural responses thought to be mediated by GABA (Waddington, 1978).

Recently attempts have been made to link the antinociceptive effect of morphine with the GABAergic system (Ho, Loh & Way, 1973; 1976; Yoneda, Takashima & Kuriyama, 1976; Biggio, Della Bella, Frigeni & Guidotti, 1977; Mantegazza, Tammiso, Vicentini, Zambotti & Zonta, 1979; Zonta, Zambotti, Vicentini, Tammiso & Mantegazza, 1981). The present study was carried out to investigate whether diazepam and midazolam, injected into the lateral ventricles, affect morphine's antinociceptive effect, as measured in rats by the tail-flick method. In addition, midazolam was microinjected locally into the periaqueductal grey matter (PAG), an area of the CNS highly responsive to morphine (Yaksh, Plant & Rudy, 1977). The effect of midazolam on morphine antinociception was also evaluated in rats pretreated with the GABA antagonist, bicuculline.

Methods

Male Sprague Dawley Charles River rats (130–150 g) were used. Animals were housed at a constant temperature (22–23 °C). The tail-flick assay (D'Amour & Smith, 1941) was used to assess the antinociceptive effect. The reaction time to heat was measured in tenths of a second. The control mean reaction time before drug administration was 37.5 ± 4.2 s (mean ± s.e., $n = 60$). The cut-off time was 8 s. To prevent tissue damage, only one tail-flick response was induced per time point. Comparisons among groups were made by the Dunnett test (Dunnett, 1964).

Under barbiturate anaesthesia (Nembutal 30 mg/kg, i.p.), permanent polyethylene cannulae (PE10) were implanted into both lateral ventricles by the method of Altaffer, De Balbian Werster, Hall, Long & D'Encarnacao (1970). The tail-flick tests were carried out 7 days after implantation.

Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo (1,5 a) (1,4)benzodiazepine maleate), at a dose of 0.5 μg as free base was dissolved in 5 μl 0.9% w/v NaCl solution (saline; final pH = 4.5) and injected into both ventricles of unanaesthetized animals, while diazepam (0.5 μg/5 μl) (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4 benzodiazepine-2-one) was dissolved in 0.1 N HCl and the pH adjusted to 6.5. Control animals received an equal volume of the vehicle solutions at pH 4.5 and 6.5. Morphine hydrochloride (1.25 mg/kg) was injected subcutaneously simultaneously with diazepam or midazolam.

Animals in the PAG group had guide cannulae stereotaxically implanted. The coordinates were chosen according to the stereotaxic atlas of König &

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Klippel (1963): A 2.4, L 0.2 and H 6.0 (mm from the external layer of the skull). The tail flick test was carried out three days after implantation. The cannula was connected to a 10 μl Hamilton syringe by a length of plastic tubing. Midazolam (300 ng as free base) was injected in saline in a total volume of 0.5 μl, while bicuculline (6 μg/0.5 μl) was dissolved in 0.1 N HCl and the pH adjusted to 4.5. The injections were made over a period of 40 s using an infusion pump. The same parameters were used for injection of saline at pH 4.5 as a control. During the infusion period the animals were completely unrestrained, moving freely in the cage. Immediately following midazolam injection, rats were treated subcutaneously with 2 mg/kg morphine (0 time). Bicuculline was injected 10 min before midazolam. Diazepam and midazolam were kindly supplied by Hoffman-La Roche, Basle.

Results

Effects of diazepam and midazolam, intracerebroventricularly (i.c.v.) injected, on the antinociceptive effects of morphine

When diazepam or midazolam were injected simultaneously with morphine (1.25 mg/kg, s.c.) both at doses of 0.5 μg/ventricle, they induced significant decreases in morphine antinociception (Figure 1a, b). Diazepam without morphine produced a slight increase in the tail-flick latencies of borderline statistical significance, while midazolam alone did not alter the baseline threshold.

Effects on morphine's antinociceptive effect of midazolam alone and of bicuculline plus midazolam injected into the PAG

Injection of midazolam directly into the PAG (300 ng/0.5 μl) of the rat significantly decreased the antinociceptive effect of morphine (2 mg/kg, s.c.). Bicuculline pretreatment (6 μg/0.5 μl; 10 min before midazolam injection) partially reversed the midazolam effect on morphine antinociception (Figure 2). Neither midazolam nor bicuculline alone altered the tail-flick reaction from that of controls injected with saline at pH 4.5.

Discussion

Benzodiazepines have been shown both to enhance and to reduce the analgesic and stimulant effects of narcotic analgesics, depending upon the particular benzodiazepine compound, the analgesic end point and the species (Gupta & Gaitonde, 1964; Gluckman, 1965; Weiss, 1969; Randall, Scheckel & Pool, 1970; Fennessy & Sawynok, 1973; Pierson, 1974).

The present data indicate that diazepam and midazolam, when injected i.c.v., reduced the antinociceptive effects of morphine, as measured by the tail-flick test. In addition our results show that midazolam antagonized morphine analgesia, when microinjected into the PAG. Most interestingly, the GABA antagonist bicuculline, injected into the PAG was found to antagonize partially the effect of midazolam on morphine antinociception, which suggests that there is a GABA link in the action of

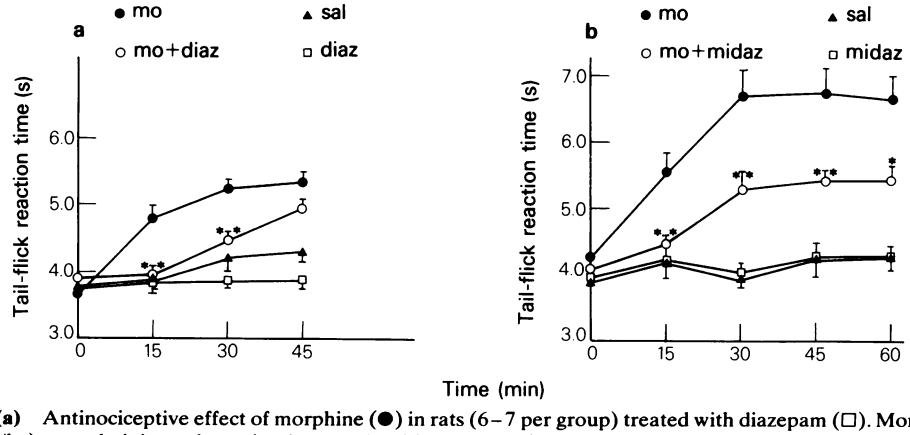

Figure 1(a) Antinociceptive effect of morphine (●) in rats (6–7 per group) treated with diazepam (□). Morphine (1.25 mg/kg) was administered s.c. simultaneously with diazepam (0.5 μg/ventricle, ○). Controls (▲). Mean values are shown; vertical lines indicate s.e.mean. The morphine group compared to the diazepam/morphine group: $^{**}P<0.01$. **(b)** Antinociceptive effect of morphine (●) in rats (6–8 per group) treated with midazolam (□). Morphine (1.25 mg/kg) was administered s.c. simultaneously with midazolam (0.5 μg/ventricle, ○). Controls (▲). Mean values are shown; vertical lines indicate s.e.mean. The morphine group compared to the midazolam/morphine group: $^{*}P<0.05$; $^{**}P<0.01$.

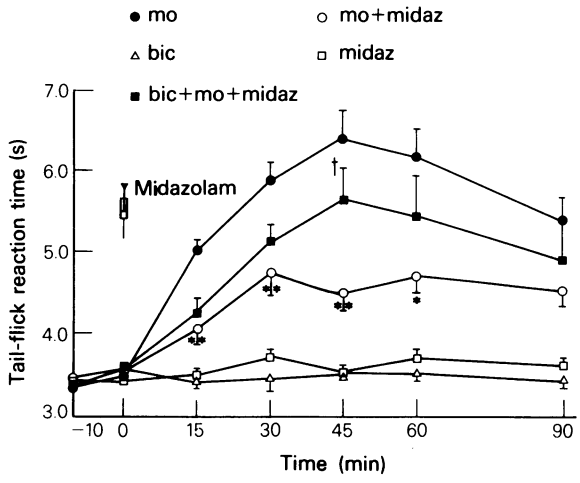

Figure 2 Antagonism by bicuculline (△) of the inhibitory effect of midazolam (□) on the antinociceptive effect of morphine (●) in rats (7–8 per group). Morphine (2 mg/kg) was injected s.c. (0 time) 10 min after the microinjection of bicuculline (6 μg/PAG) and immediately after midazolam (■). Mean values are shown; vertical lines indicate s.e.mean. Morphine group (●) compared to morphine/midazolam (○) group and to bicuculline/morphine/midazolam (■) group; $*P<0.05$; $**P<0.01$. Morphine/midazolam group (○) compared to bicuculline/morphine/midazolam group (■): $\dagger P<0.05$.

midazolam. Since we found that muscimol injected into the lateral ventricles and locally into the PAG reduces morphine analgesia through a mechanism that involves bicuculline-sensitive receptors (Mantegazza *et al.*, 1979; Zonta *et al.*, 1981; Zambotti, Zonta, Parenti, Vicentini, Tammiso, Conci & Mantegazza, 1981), it is possible that muscimol and midazolam activate the same neuronal subtrates. This suggestion is supported by studies which have demonstrated that glutamate decarboxylase activity and GABAergic neurones are located in the PAG (Belin, Aguera, Tappaz, MacRae-Deguerce, Bobillier & Pujol, 1979).

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