Department of Pharmacodynamics, Chair of Pharmacology, Medical University of Lodz, Poland

Effect of diazepam and midazolam on the antinociceptive effect of morphine, metamizol and indomethacin in mice

W. PAKULSKA and E. CZARNECKA

The influence of midazolam and diazepam on antinociceptive effect of morphine (10 mg/kg), metamizol (500 mg/kg) and indomethacin (10 mg/kg) was investigated in a mouse model using the tail-flick and hot-plate tests. All drugs were injected intraperitoneally. Benzodiazepines were administered to mice 30 min before applying the analgesic drugs. Measurement of nociception was performed within 2 h after benzodiazepine administration. Diazepam at doses of 0.25 mg/kg and 2.5 mg/kg injected with morphine was found to decrease the antinociceptive effect of morphine. Similarly, diazepam decreased the antinociceptive effect of metamizol (only in the tail-flick test) and indomethacin. Midazolam used at doses of 1.25 mg/kg and 2.5 mg/kg decreased the antinociceptive effect of morphine, metamizol (only in the tail-flick test) and indomethacin.

1. Introduction

The effects of the combined administration of anxiolytic and analgesic drugs are the subject of research and controversy among investigators. Results of this research are incoherent and often contradictory.

Agents that act on GABA subtype $GABA_A$ receptors can produce antinociception. Rat intrathecal (it) administration of $GABA_A$ receptor agonists such as muscimol or isoquavacine produces a modest increase in response latencies in the tail-flick or hot plate test [1–3]. Intrathecal administration of midazolam increases the threshold for transcutaneous electrical stimulation in the rat [4]. Intrathecal administration of diazepam or midazolam also produces a very modest increase of tail-flick response latency [5, 6], although this effect has not been observed by all investigators [7]. The ability of benzodiazepine agonists to produce antinociception is likely to be highly dependent on endogenous GABA concentration. Intrathecal administration of $GABA_B$ receptor agonists also produces antinociception [1, 8]. On the other hand, Moreau and Pieri [9], and Yanez et al. [10] demonstrated that midazolam administered to rats acted antinociceptively only temporarily or not at all. Yet other research studies indicated a hyperanalgetic effect induced by midazolam [5].

The evaluation of interactions between benzodiazepines and opioids after supraspinal or systemic administration is also controversial. The combined administration of benzodiazepine receptor agonists such as midazolam or diazepam with morphine may both decrease [11], and increase [12] the antinociceptive action of the narcotic drug.

Benzodiazepines injected into the cerebral ventricle (ivc) decrease the antinociceptive effect of morphine in the hot-plate test. Rahman et al. [13] think that diazepam does not affect the antinociceptive activity of morphine but only accelerates morphine tolerance. This view contradicts data obtained by other authors, who claim deceleration of morphine tolerance due to combined morphine and diazepam administration [14].

The object of this study was to evaluate the effect of diazepam and midazolam on the antinociceptive action of analgesics of different classes: morphine, metamizol and indomethacin.

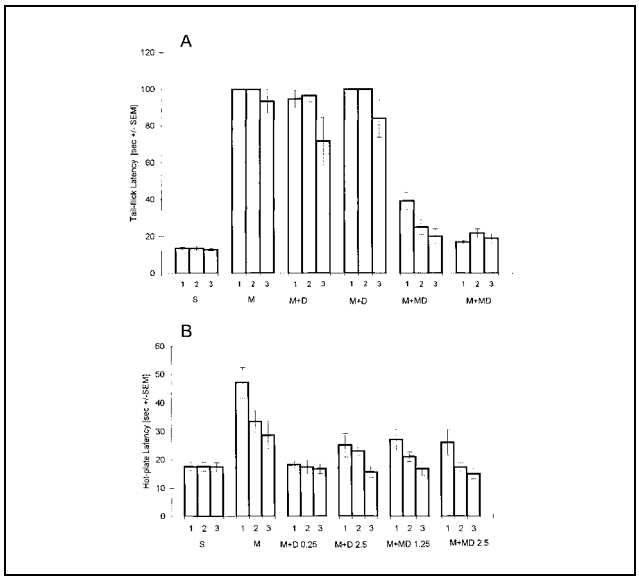

Fig. 1: The antinociceptive effect on (A) tail-flick and (B) hot-plate tests after ip administration of saline (S), morphine 10 mg/kg (M), morphine + diazepam 0.25 mg/kg (M + D 0.25), morphine + diazepam 2.5 mg/kg (M + D 2.5), morphine + midazolam 1.25 mg/kg (M + MD 1.25), morphine + midazolam 2.5 mg/kg (M + MD 2.5) The tail-flick test or the hot-plate test were performed 30 min (1), 60 min (2), and 90 min (3) after administration of analgesic drugs. Significantly different from the morphine group.

2. Investigations and results

Midazolam in both doses used (1.25 and 2.5 mg/kg ip) administered 30 min before morphine shortened the time of pain response when compared to morphine alone (Figs. 1A, 1B). This influence was observed in both the tail-flick and hot- plate tests. Diazepam (0.25 and 2.5 mg/kg ip) demonstrated such an effect only in the hot-plate test (Fig. 1B). Both diazepam and midazolam, administered before metamizol at either of the doses used, shortened the time of pain response when compared to metamizol alone (Figs. 2A, 2B). This activity was noted in the tail-flick test (Fig. 2A) but was not observed in the hot-plate test (Fig. 2B). Diazepam and midazolam administered prior to indomethacin at either dose shortened the time of pain response when compared to indomethacin alone (Fig. 3A, 3B). This effect was observed in both tests. Mid-

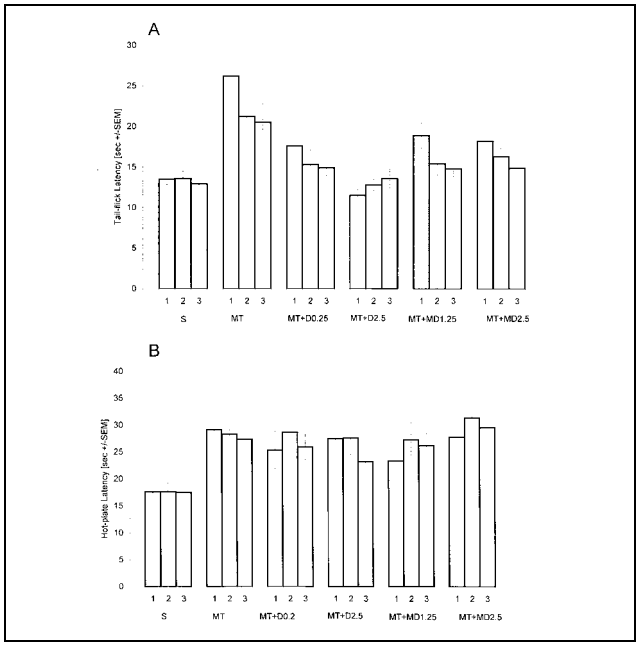

Fig. 2: The antinociceptive effect on (A) tail-flick and (B) hot-plate tests after ip administration of saline (S), metamizol 500 mg/kg (MT), metamizol + diazepam 0.25 mg/kg (MT + D 0.25), metamizol + diazepam 2.5 mg/kg (MT + D 2.5), metamizol + midazolam 1.25 mg/kg (MT + MD 1.25), metamizol + midazolam 2.5 mg/kg (MT + MD 2.5). The tail-flick test or the hot-plate test were performed 30 min (1), 60 min (2), and 90 min (3) after administration of analgesic drugs. Significantly different from the metamizol group.

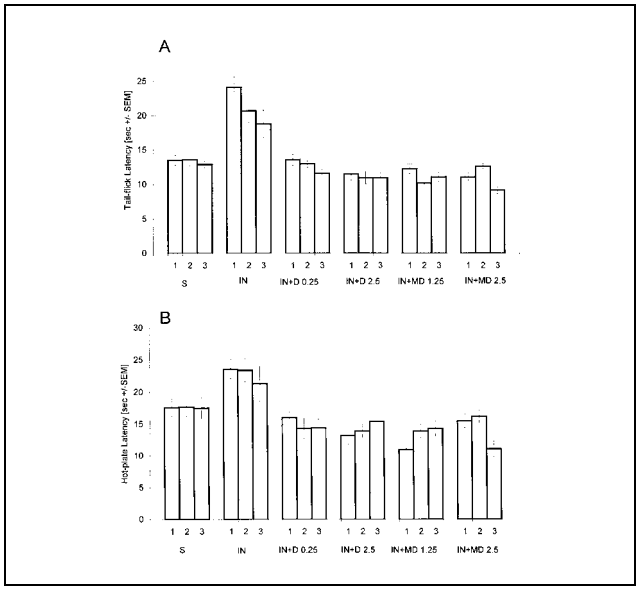

Fig. 3: The antinociceptive effect on (A) tail-flick and (B) hot-plate tests after ip administration of saline (S), indomethacin 10 mg/kg (IN), indomethacin + diazepam 0.25 mg/kg (IN + D 0.25), indomethacin + diazepam 2.5 mg/kg (IN + D 2.5), indomethacin + midazolam 1.25 mg/kg (IN + MD 1.25), indomethacin + midazolam 2.5 mg/kg (IN + MD 2.5). The tail-flick test or the hot-plate test were performed 30 min (1), 60 min (2), and 90 min (3) after administration of analgesic drugs. Significantly different from the indomethacin group.

azolam alone, at doses of 1.25 and 2.5 mg/kg, shortened the time of latencies to paw licking during the hot-plate test (Fig. 4B). Similar effects were observed at both doses of diazepam. These effects were not seen during the tail-flick test for either drug (Fig. 4A).

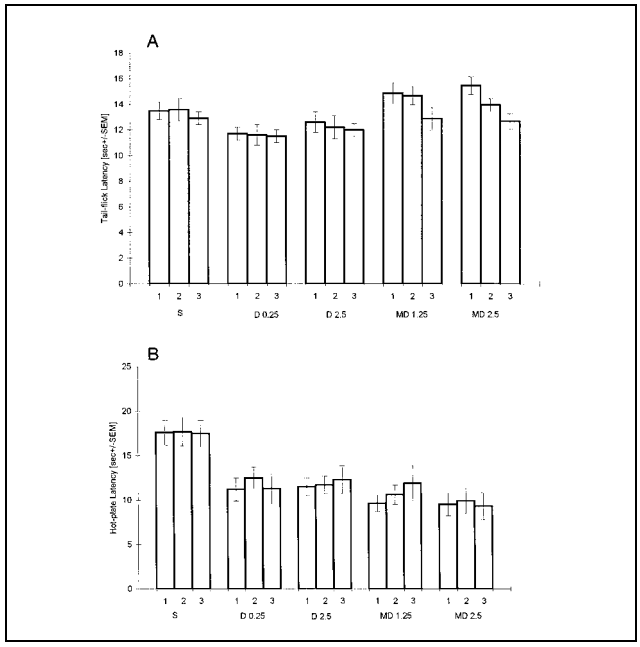

Fig. 4: The antinociceptive effect on (A) tail-flick and (B) hot-plate tests after ip administration of saline (S), diazepam 0.25 mg/kg (D 0.25), diazepam 2.5 mg/kg (D 2.5), midazolam 1.25 mg/kg (MD 1.25), midazolam 2.5 mg/kg (MD 2.5). The tail-flick test or the hot-plate test were performed 30 min (1), 60 min (2), and 90 min (3) after anxiolytic drug administration. Significantly different from the saline control group.

3. Discussion

The experiments performed have shown that diazepam and midazolam administered intraperitoneally prior to administration of morphine, metamizol and indomethacin by the same route decrease the antinociceptive effect of these drugs. The effect does not appear consistently in both of the tests used. Midazolam and diazepam decreased the antinociceptive effect of morphine in the hot-plate test, while in the tail-flick test only midazolam produced such an action. In the experiments with metamizol, both midazolam and diazepam decreased the analgesic of effect metamizol in the tail-flick test only. Both anxiolytics reduced the antinociceptive effect of indomethacin in both tests.

The analgesic drugs evaluated in our study belong to classes with different mechanisms of action. Morphine's antinociceptive action is mediated through type μ opioid receptors.

Indomethacine and metamizol are inhibitors of prostaglandin synthesis. GABA-ergic receptors play an important role in pain control. GABA receptor agonists may modulate pain conduction. It seems that this effect may depend on their route of administration.

Luger et al. [15] showed that intrathecal injection of midazolam enhanced antinociception induced by intrathecal morphine. The intracerebroventricular injection of midazolam inhibited morphine antinociception. They suggest that different mechanisms are involved in the spinal cord and brain.

Morphine can increase the amount of GABA and the activity of glutamate decarboxylase in dorsal parts of rat spinal cord [16]. Potentiation of morphine induced antinociception by midazolam in the spinal cord may be the result of GABA-ergic receptor stimulation by morphine.

Benzodiazepines given supraspinally reduce morphine antinociception. Rosland and Hole [17] attribute this to

their high CNS level. According to Ding et al. [18] GABA A receptor is involved in modulation of the supraspinal action of opioid receptor occupancy.

The antinociceptive action of morphine is connected with its effect on different levels of pain conduction. Benzodiazepines given intracerebroventricularly probably inhibit descending impulsation on a higher level and thus may reduce morphine action. The dose of benzodiazepine may also be of importance. It has been demonstrated that a low midazolam dose can have an analgetic action while at higher doses it may cause hyperalgesia [5, 19]. Metamizol's antinociceptive action is mainly related to the effect on a higher CUN level.

The differences in the effect of midazolam and diazepam on the antinociceptive action of metamizol in the hot-plate and tail-flick tests which we report may result from this mechanism. Sribanditmongkol et al. [20] have shown that the mechanism and neuronal pathway of these tests are different. Hot-plate test results are associated with central analgesia while the tail-flick test measures both spinal and central analgesia.

Indomethacin's antinociceptive activity is connected with peripheral and central effects.

The benzodiazepines diazepam and midazolam, injected intraperitoneally reduce the antinociceptive effect of analgetics independently of their mechanism of action. GABA-ergic receptors are involved in the processes of pain and their agonists may affect the action of analgetic drugs.

4. Experimental

4.1. Animals and treatment

The experiments were carried out on Swiss male mice (18–24 g). The mice were housed in group cages under normal laboratory conditions at a temperature of 20–21 °C, and natural day/night cycle and they had free access to commercial chow food and water. All experiments were performed between 11.00 a.m. and 2.00 p.m. The drugs were injected intraperitoneally (ip) and solutions were in 0.9% NaCl. Diazepam (Relanium® "Polfa" Warsaw) at doses of 0.25 and 2.5 mg/kg, and midazolam (Dormicum® "La Roche") at doses of 1.25 and 2.5 mg/kg were given 30 min before the analgesic drugs; morphine (Morphinum hydrochloricum "Polfa" Warsaw) was given at a dose of 10 mg/kg, metamizol (Pyralgin® "Polpharma" S.A.) at 500 mg/kg, indomethacin (Metindol® "Polfa" Krakow) at 10 mg/kg.

4.2. Nociception tests

The hot-plate test was derived from that of Eddy and Leimbach [21]. A plastic cylinder (height: 20 cm, diameter: 14 cm) was used to confine a mouse to a heated surface of the plate. The temperature of the plate was maintained at 52 ± 0.4 °C. Latencies to low paw licking were determined 30, 60 and 90 min after treatment with analgesic. The groups consisted of 7–10 mice each and the control group of 14 animals.

The tail-flick test of D'Amour and Smith [22] modified for mice was used. Mice were placed in retention boxes. The latency of tail withdrawal was determined by focusing a radiant heat source on the tail at about 3 cm from the tip of the tail. The latency was measured 30, 60 and 90 min after administration of analgesic drugs. Each group consisted of 7–10 mice.

4.3. Statistical analysis

The normality of the distribution was checked with the Kolmogorow-Smirnow test with the Lillieforse correction and then variance equality was tested by Fisher's test. Student's t test was used for statistical evaluation.

This study was supported by a research grant from the Medical University, Lodz, Poland nr 502-13-523 (196).

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Received December 28, 1999
Accepted May 16, 1000

Dr. Wanda Pakulska
Department of Pharmacodynamics
Chair of Pharmacology
Medical University
ul. Muszyñskiego 1,
90-151 Lodz
Poland