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Interaction of memantine and ketamine in morphine- and pentazocine-induced antinociception in mice

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Abstract:
The interaction between uncompetitive NMDA receptor antagonists (memantine and ketamine), and morphine (μ-opioid receptor agonist) and pentazocine (κ-opioid receptor agonist) was studied in the writhing test in mice. Memantine and ketamine, administered at subthreshold doses, potentiated antinociceptive effect of the threshold (1 mg/kg) dose of morphine. The effects of the threshold (6 mg/kg) dose of pentazocine were not significantly changed by ketamine, and were significantly enhanced by the higher dose of memantine (15 mg/kg). Simultaneously performed experiments in the chimney test have shown that combination of morphine or pentazocine with an NMDA receptor antagonist did not induce significant alterations in the motor coordination of mice.
The obtained results have shown that NMDA receptor antagonists (ketamine, memantine) are able to enhance the antinociceptive activity of opioids (morphine, pentazocine). It is necessary to underline that this effect was more apparent for morphine (μ-opioid receptor agonist) + NMDA antagonists than for pentazocine (κ-opioid receptor agonist). These results may have some importance for clinical practice.

Key words:
memantine, ketamine, morphine, pentazocine, antinociception, mice

Introduction

Numerous studies have reported that NMDA receptor antagonists induce antinociceptive action in various animal models of pain [10, 27, 31, 36], and may modulate the effects of opioids. It has been shown that they can attenuate the development of tolerance to analgesic effects of morphine [6, 12, 24, 25, 34, 35]. Antinociceptive actions of opioids are also influenced by NMDA receptor antagonists, although the results of such experiments are not uniform: Kozela et al. [21] presented many literature data indicating that an NMDA receptor antagonist + opioid combinations produced potentiation, inhibition or no effect in the tail-flick or hot plate test in rats. The differences in strength of potentiating effect on opioid analgesia between NMDA receptor antagonists may be also connected with the existence of different opioid receptor types (μ-, δ-, κ-) [2]. Nevertheless, there is evidence from human volunteer studies and small clinical trials that NMDA receptor antagonists relieve some types of neuropathic pain [18]. Moreover, clinical reports indicate that ketamine at low doses as an adjuvant to opioid treatment may improve analgesia with tolerable adverse effects [3, 4, 17, 38].

An uncompetitive NMDA receptor antagonist, memantine (1-amino-3,5-dimethyladamantane), besides its therapeutic potential in numerous CNS disorders [30], has also some analgesic activity in animal models of chronic and neuropathic pain [13–15, 29]. The latest clinical studies have reported that memantine can reduce intensity of phantom limb pain and might prevent its development [32]. Analgesic properties of ketamine, another uncompetitive NMDA receptor antagonist, are well known [3, 16, 39], however, its use is restricted by unpleasant adverse effects, such as hallucinations. Nonetheless, ketamine is recommended as an ideal drug for the use in many prehospital situations, because it is more effective and safer than drugs currently used [33].

The above data prompted us to investigate the interaction between uncompetitive NMDA antagonists (memantine and ketamine), and morphine (μ-opioid receptor agonist) and pentazocine (κ-opioid receptor agonist) in the writhing test in mice. The acetic acid writhing test is employed as visceral or tonic pain model. Administration of an algogenic chemical agent produces a slow, progressive and long-lasting form of stimulation, which makes it the closest in nature to clinical pain. In this test, both central and peripheral analgesics could be estimated and many investigators used it and recommended as a simple screening method. Although the writhing test has low specificity, it possesses high sensitivity and shows good correlation between analgesic effects in humans and animals [22, 37]. Its sensitivity allows for detection of even slight antinociceptive action of drugs, e.g. nootropic [1] or non-steroidal anti-inflammatory drugs [37]. This test in mice is a good test for demonstration of an interaction between morphine and other drug groups (e.g. anti-inflammatory drugs or calcium-channel blockers) [11, 26].

Additionally, we examined possible adverse effects of all used drugs in the chimney test in mice, because the alteration of animal motor performance by drugs may disturb the evaluation of behavioral responses of animals to nociceptive stimuli.

The aim of the present study was to determine whether uncompetitive NMDA receptor antagonists (ketamine, memantine), administered at threshold antinociceptive doses, are able to intensify the antinociceptive activity of the threshold doses of morphine or pentazocine, and which type of opioid receptors, μ or κ, is more involved in such interactions.

Materials and Methods

Animals

The experiments were carried out on male albino Swiss mice (18–35 g). The animals were kept 8–10 to a cage at room temperature of 22 ± 1°C and a 12 h light/dark cycle. Standard food (Murigran pellets, Bacutil, Motycz, Poland) and tap water were available *ad libitum.* All experiments were performed between 9:00 a.m. and 2:00 p.m. Animals were acclimatized to the experimental room for about 2 h before testing, were used only once and sacrificed immediately after the test with a lethal dose of gaseous carbon dioxide (CO_2).

The experiments were performed in accordance with the opinion of Local Ethics Committee.

Drugs

The following drugs were used: uncompetitve NMDA receptor antagonists: memantine (Sigma, USA), ketamine (Ketanest, Parke-Davis, Germany) and opioid receptor agonists: morphine (μ receptor agonist, Polfa, Kutno, Poland), pentazocine (κ receptor agonist, Polfa, Warszawa, Poland)

All drugs were dissolved in saline and administered subcutaneously (*sc*) in a volume of 10 ml/kg. The control animals were injected with an appropriate volume of solvent at the specified time before the test.

Threshold doses and time of injections of both opioids were chosen on the basis of our pilot experiments.

The writhing test

The nociceptive reactions in mice were investigated in the writhing test according to Koster et al. [20]. Each animal received one intraperitoneal (*ip*) injection of 10 ml/kg of 0.6% acetic acid solution to evoke writhing. A writhe is indicated by stretching of the abdomen followed by the extension of the hind limbs. The animals were placed singly in a glass cylinder (35 cm high, 25 cm in diameter) and the number of writhing episodes in a 20 min period was counted, starting 5 min after the acetic acid administration. Other compounds were administered *sc* 20 min (morphine and pentazocine) or 10 min (ketamine and memantine) before injection of acetic acid solution. Abdominal constrictions were not observed in saline-

treated mice. The experimental groups consisted of 10 animals each.

The chimney test

The chimney test of Boissier et al. [7] was used to assess the range of doses of the studied drugs producing motor impairment. The animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm long). Motor impairment was evidenced by the inability of mice to climb backwards up the tube within 60 s.

Statistical analysis

Results are shown as the means ± SEM. Statistical significance of the obtained data was evaluated using χ^2 test with Yates correction (chimney test) and Dunnett's test, following a one-way ANOVA (writhing test); $p > 0.05$ are reported as non-significant.

Results

The writhing test

Morphine administered at the dose of 1 mg/kg decreased the number of writhing episodes by about 40% in comparison to control (saline-treated) animals (Fig. 1 and 2) and this effect was considered to be the threshold effect because a higher dose of morphine (1.5 mg/kg) produced significant antinociceptive action in this test (data not shown).

Ketamine administered at doses of 5, 10 and 15 mg/kg did not show antinociceptive-like action. Analysis (one-way ANOVA) of antinociceptive effects of ketamine and morphine indicated a significant [$F(7, 64) = 9.608$; $p < 0.001$] difference between groups. Dunnett *post-hoc* test showed that only higher doses of ketamine when administered together with morphine decreased significantly ($p < 0.01$) the number of writhing episodes in comparison to morphine-treated group (Fig. 1).

Memantine (10 and 15 mg/kg), when injected alone, had no antinociceptive effect. One-way ANOVA showed significant [$F(5, 49) = 11.23$; $p < 0.001$] differences between groups regarding the antinociceptive effects of memantine and morphine. *Post-hoc* test indicated that both used doses of memantine given together with morphine significantly ($p < 0.01$) decreased the number of writhing episodes in comparison to morphine alone group (Fig. 2).

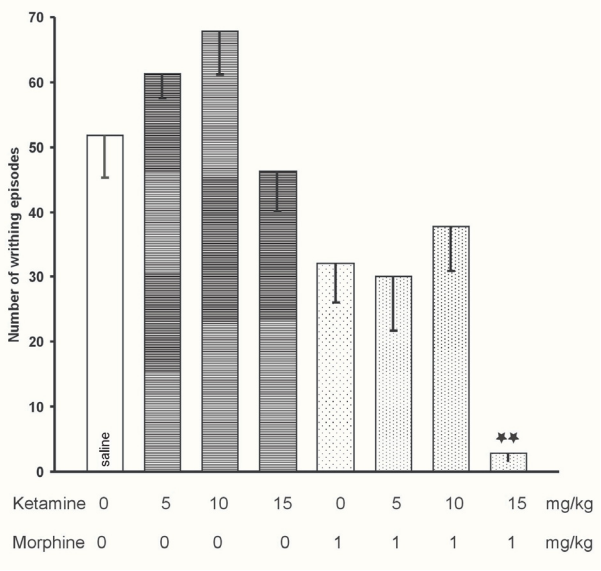

Fig. 1. The influence of ketamine on antinociceptive effects of the subthreshold dose of morphine (1 mg/kg) in the writhing test in mice. Each bar represents the mean ± SEM of a group of 10 mice. ** $p < 0.01$ *vs.* morphine (Dunnett's test)

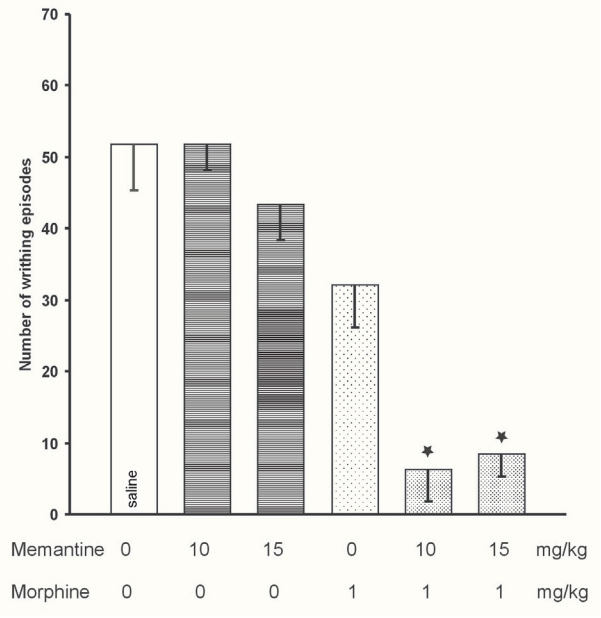

Fig. 2. The influence of memantine on antinociceptive effects the subthreshold dose of morphine (1 mg/kg) in the writhing test in mice. Each bar represents the mean ± SEM of a group of 10 mice. * $p < 0.02$ *vs.* morphine (Dunnett's test)

Pentazocine administered at the dose of 6 mg/kg (subthreshold dose) slightly decreased the number of writhing episodes in mice (Fig. 3 and 4). The analysis (one-way ANOVA) of antinociceptive effects of ketamine and pentazocine indicated a significant [$F(5, 42) = 4.494$; $p < 0.01$] difference between groups. Dunnett *post-hoc* test showed that ketamine (10 and 15 mg/kg) did not change significantly antinociceptive action of pentazocine (Fig. 3).

One-way ANOVA showed significant [$F(5, 45) = 5.750$; $p < 0.001$] differences between groups regarding the antinociceptive effects of memantine and pentazocine. *Post-hoc* test indicated that only a higher (15 mg/kg) dose of memantine given together with pentazocine significantly ($p < 0.05$) decreased the number of writhing episodes in comparison to pentazocine alone group (Fig. 4).

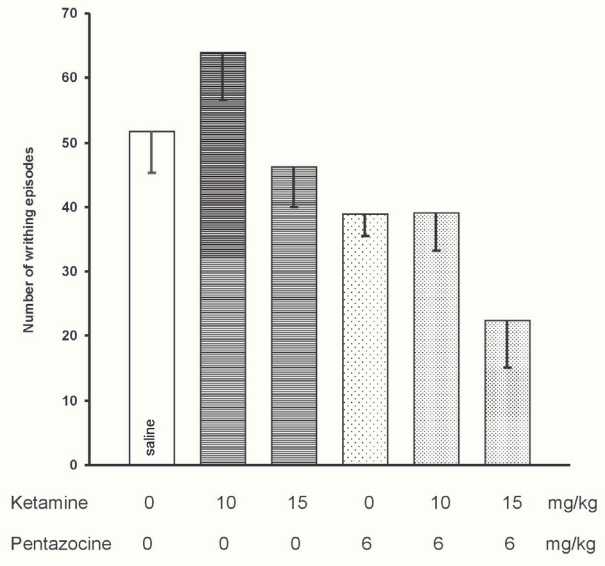

Fig. 3. The influence of ketamine on antinociceptive effects of the subthreshold dose of pentazocine (6 mg/kg) in the writhing test in mice. Each bar represents the mean ± SEM of a group of 10 mice

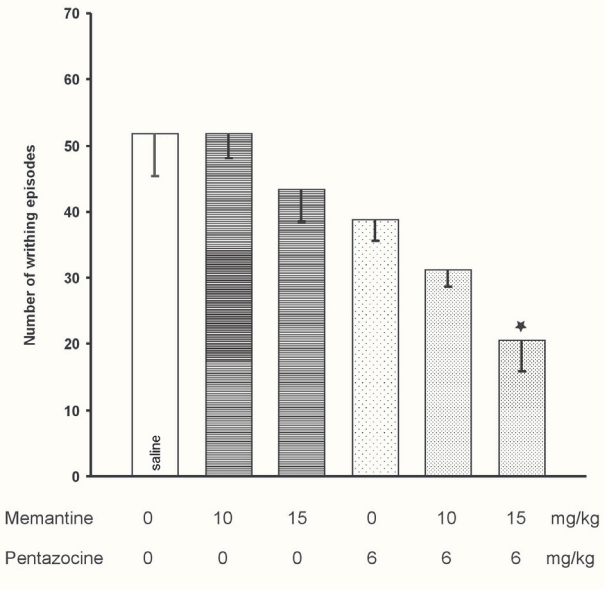

Fig. 4. The influence of memantine on antinociceptive effects of the subthreshold dose of pentazocine (6 mg/kg) in the writhing test in mice. Each bar represents the mean ± SEM of a group of 10 mice. * $p < 0.05$ *vs.* pentazocine (Dunnett's test)

The chimney test

Reaction time of mice in this test was not changed by any drug injected alone (Tab. 1).

Ketamine administered at a higher dose of (15 mg/kg) together with morphine (1 mg/kg) increased the reaction time of mice 20 min after the injection of ketamine. This effect was not higher than 60 s and disappeared at 60 min of observation

Tab. 1. The influence of morphine, pentazocine, ketamine and memantine alone and in combination on the reactivity of mice in the chimney test in mice

Substance	Number of mice leaving the chimney within 60 s (mean reaction time (s) ± SEM)	
	20'	60'
0.9% NaCl	$^{10}/_{10}$* (10.27 ± 1.04)	$^{10}/_{10}$ (11.4 ± 1.32)
Ketamine 10 mg/kg	$^{10}/_{10}$ (9.98 ± 2.29)	$^{10}/_{10}$ (10.07 ± 1.56)
Ketamine 15 mg/kg	$^{10}/_{10}$ (21.42 ± 6.87)	$^{10}/_{10}$ (8.63 ± 0.84)
Memantine 10 mg/kg	$^{10}/_{10}$ (8.47 ± 1.87)	$^{10}/_{10}$ (8.77 ± 1.53)
Memantine 15 mg/kg	$^{10}/_{10}$ (8.27 ± 0.74)	$^{10}/_{10}$ (9.48 ± 1.02)
Morphine 1 mg/kg	$^{10}/_{10}$ (7.76 ± 1.27)	$^{10}/_{10}$ (12.42 ± 6.88)
+ Ketamine 15 mg/kg	$^{10}/_{10}$ (39.93 ± 8.28)	$^{10}/_{10}$ (5.86 ± 0.68)
+ Memantine 15 mg/kg	$^{10}/_{10}$ (7.08 ± 0.67)	$^{10}/_{10}$ (6.04 ± 0.57)
Pentazocine 6 mg/kg	$^{10}/_{10}$ (9.68 ± 0.81)	$^{10}/_{10}$ (7.89 ± 1.09)
+ Ketamine 15 mg/kg	$^{10}/_{10}$ (44.45 ± 6.94)	$^{10}/_{10}$ (8.33 ± 0.73)
+ Memantine 15 mg/kg	$^{10}/_{10}$ (36.28 ± 9.24)	$^{10}/_{10}$ (15.64 ± 3.87)

N = 10 mice per group. * The data were evaluated using χ^2 test with Yates correction

(Tab. 1). Memantine administered together with morphine did not influence the reactivity of mice.

Ketamine and memantine injected together with pentazocine prolonged the time of climbing up of the chimney (but not over 60 s) at 20 min of observation and this effect was also short lasting (Tab. 1).

Discussion

Many previous studies investigating interactions of NMDA receptor antagonists with opioids have indicated that rats appear to respond to this treatment with a potentiation of morphine analgesia (see Kozela et al. [21]), while miscellaneous results may be observed in mice, for example, Lutfy et al. [23] demonstrated even the blockade of morphine analgesia by MK-801 in the tail-flick test in mice. Moreover, NMDA-induced modulation of morphine analgesia is influenced by sex, site of analgesia, morphine dose and time after injection [28].

In our study, we used a clinically available drug with NMDA antagonist properties, i.e. the anesthetic ketamine and the anti-Parkinsonian agent memantine, and common opioid analgesics – morphine (μ-) and pentazocine (κ-opioid receptor agonist). The obtained results demonstrate that both memantine and ketamine, administered at subthreshold doses, potentiate antinociceptive action of the threshold (1 mg/kg) dose of morphine in the writhing test in mice. Memantine increased morphine effects when injected at both doses used (10 and 15 mg/kg), but ketamine enhanced it only at the highest dose of 15 mg/kg. Thus, the memantine + morphine combination seems to produce a stronger synergism than that elicited by ketamine + morphine. These results are consistent with the findings of Baker et al. [2] who observed potentiation of morphine antinociception by ketamine in the hot plate test, which is the mouse model of acute pain. Statistically significant potentiation of morphine antinociception was also observed in mice pretreated with morphine (20 mg/kg) and memantine (10 mg/kg) in the tail-flick test [5]. Dambisya and Lee [9] studied the antinociceptive effects of a ketamine + morphine combination in the tail-flick test in mice but concluded that the net effect of this combination was rather a simple additive one. Our observations in mice seem to be partially consistent with the findings of Kozela et al. [21] in rats suggesting that uncompetitive NMDA receptor antagonists (memantine, dextromethorphan and compound MRZ 2/579) potentiated morphine-induced antinociception recorded from the tail although it did not change that from hind paws.

In the present study, the antinociceptive effects of the threshold dose (6 mg/kg) of pentazocine were slightly, and to a similar degree increased by higher doses of both NMDA antagonists used. The enhancing effect of memantine was significant while that of ketamine was not. Pentazocine acts as a weak μ-opioid receptor antagonist or partial agonist, but its analgesic effects are due to agonistic action at κ-opioid receptors [19]. Our results are consistent with the observations of Baker et al. [2] that NMDA receptor antagonists, ketamine and dextromethorphan potentiated the antinociceptive effects of μ- (morphine) but not δ- or κ-opioid agonists in the mouse hot plate test. Similarly, Chen et al. [8] have shown that dextromethorphan potentiated the antinociceptive effects of some μ-opioid agonists (morphine and meperidine) but not codeine or κ-opioid agonists (nalbuphine and U-50,488H) in the tail-flick test in rats.

Nonetheless, in our experiments pentazocine antinociception was significantly enhanced by a higher dose of memantine (15 mg/kg), and it means that memantine is able to increase the effects of both used opioid drugs although its intensifying activity on morphine antinociception is markedly higher than on that of pentazocine.

Our simultaneously performed experiments in the chimney test have shown that combinations of morphine + an NMDA receptor antagonist did not induce significant alterations in the motor coordination of mice although we observed some short-lasting prolongation of the time of climbing out of the chimney in the groups treated with ketamine + opioids. This prolongation was below 60 s (established time of normal coordination) and was only seen at 20 min of observation.

Summing up, our results have shown that NMDA receptor antagonists (ketamine, memantine) are able to intensify antinociceptive activity of opioids (morphine, pentazocine) in the writhing test in mice. This antinociceptive interaction was more apparent for morphine (μ receptor agonist) + NMDA receptor antagonists than for pentazocine. Such results may have some implications in clinical practice.

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