Ketamine-Induced Potentiation of Morphine Analgesia in Rat Tail-Flick Test: Role of Opioid-, α₂-Adrenoceptors and ATP-Sensitive Potassium Channels

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Ketamine is known to improve opioid efficacy, reduce postoperative opioid requirement and oppose opioid associated pain hypersensitivity and tolerance. However, the mechanisms underlying these beneficial effects are not clear. This study investigated the effects of ketamine at a non-analgesic dose (30 mg/kg, i.p.) on analgesia induced by morphine (2.5, 5.0, 7.5 mg/kg, s.c.), using rat tail-flick test as an animal model of acute pain. Further, the role of opioid-, α₂-adrenoceptors and ATP-sensitive potassium channels was examined on the potentiating effect of ketamine. Male rats received morphine alone at 5.0 and 7.5 but not at 2.5 mg/kg showed a dose-related increase in tail-flick latencies. The combination of morphine and ketamine resulted in dose-related increase in morphine analgesia, both on the intensity as well as on duration. The ketamine-induced potentiation of morphine (7.5 mg/kg) analgesia was unaffected by glibenclamide (3 mg/kg, s.c.) and only partially blocked by yohimbine (2 mg/kg, i.p.), but more completely abolished by naloxone (2 mg/kg, i.p.). Both morphine (5.0 mg/kg) and ketamine (30 mg/kg) alone did not evoke catalepsy in rats but on combination produced a synergistic effect, which was however, abolished by naloxone pretreatment. In the open-field test, while morphine (5.0 mg/kg) caused a depressant effect, ketamine (30 mg/kg) enhanced the locomotor activity. Nevertheless, in combination potentiated the morphine’s depressant effect on locomotion, which was also antagonized by naloxone. These results indicate that ketamine at a non-analgesic dose can potentiate morphine analgesia, induce catalepsy and cause locomotor depression, possibly involving an opioid mechanism. This potentiation, although favorable in acute pain management, may have some adverse clinical implications.

Key words morphine; ketamine; tail-flick test; analgesia; naloxone antagonism

MATERIALS AND METHODS

Animals The experiments were performed using male Wistar rats (160—180 g). The animals were housed at a room temperature of 22±2°C subjected to a 12 h light–dark cycle, and 55±5% relative humidity with food and water available ad libitum. Each animal was used only once for experimentation. All experiments were carried out between 8:00 and 15:30 h in a room with controlled temperature (23±1°C) and light intensity of 20 lux. The Institutional Animal Care Committee approved the experimental protocols in accordance with the guidelines of NIH, U.S.A.

Chemicals and Drugs Morphine hydrochloride was obtained from Secretaria de Saúde (Fortaleza, CE, Brazil). Ketamine (Ketalar®) was purchased from Parke-Davis, and naloxone hydrochloride, yohimbine hydrochloride, and glibenclamide were obtained from Sigma, Brazil. All drugs were dissolved in saline (0.9%).

Tail-Flick Test To evaluate the antinociceptive activity of the drugs and their combinations, rat tail-flick apparatus (Ugo-Basile) was used (D’Amour and Smith, 1941). The distal part of the tail (except 2 mm) was placed over a heated nichrome wire and the time taken for tail-flick response was recorded both before and after drug administration (at 30, 60, 90, 120 or 150 min). The heat intensity was adjusted such that the average tail-flick latency was 3.0—4.0 s and a maximum cut-off time of 15 s was followed to avoid undue tissue damage of the tail to radiant heat.

Morphine was administered subcutaneously and all other drugs by intraperitoneal route. All the test doses of drugs were chosen based on our pilot experiments. Three series of experiments were done using 14 groups of animals, each
containing 8 rats. In the first series, tail-flick latencies were recorded with saline (10 ml/kg, s.c.) or after morphine at different doses (2.5, 5.0 or 7.5 mg/kg, s.c.). In the second series, the effects of co-administration of morphine (2.5, 5.0, 7.5 mg/kg, i.p.) with ketamine (30 mg/kg, i.p.) on tail-flick latencies were compared with responses obtained with ketamine (30 mg/kg, i.p.) alone or with saline. This dose of ketamine (30 mg/kg, i.p.) did not manifest per se antinociception in tail-flick test. In the third series of experiments, groups of animals were pretreated respectively, with glibenclamide (3 mg/kg, i.p.), yohimbine (2 mg/kg, i.p.) or naloxone (2 mg/kg, i.p.) to test their influence on the tail-flick latencies of morphine (7.5 mg/kg, i.p.) + ketamine (30 mg/kg, i.p.) combination. All pretreatments were given 15 min prior to morphine + ketamine injections.

**Effect of Morphine and Ketamine in Catalepsy Test**
Catalepsy was evaluated by placing the animal with both forelegs over a horizontal bar (diameter, 0.5 cm; length, 10.5 cm) fixed at 9.0 cm above the floor (Sanberg et al., 1996). The time (s) during which the rat maintained this position was recorded for up to 300 s. Recovery from catalepsy was considered when the forepaw touched the floor or when the rat climbed upon the bar. Five groups of rats (eight in each) were randomly assigned to the following treatments. (1) vehicle (normal control, saline, 5 ml/kg) alone (2) morphine control, vehicle + morphine (5 mg/kg, s.c.), (3) ketamine control, vehicle + ketamine (30 mg/kg, i.p.), (4) co-administration of morphine + ketamine, and (5) pretreatment with naloxone (2 mg/kg, i.p., 15 min before) followed by co-administration of morphine + ketamine.

**Effect of Morphine and Ketamine on Ambulation in Open-Field Test**
The apparatus consists of a square field of 96 cm and 47 cm high with an open top, which was divided into 12 equal size squares. Groups of animals were treated as above and 30 min later, each animal was placed in the center of open-field arena, and persons who were unaware of treatments counted the number of floor units entered with the four paws during 4 min of observation.

**Statistical Analysis** Results are presented as mean ± standard error of the mean (S.E.M.). Analysis of variance, followed by Tukey’s test, was used to detect differences between treatments. Differences were considered significant when \( p < 0.05 \).

**RESULTS**
Subcutaneous administration of morphine significantly enhanced the tail-flick latency at the doses of 5.0 and 7.5 mg/kg when compared to saline-treated control (Fig. 1). The duration of analgesia at 5.0 mg/kg dose was up to 60 min whereas at 7.5 mg/kg, it was 90 min. However, at 2.5 mg/kg dose, no antinociceptive effect of morphine was evidenced. Figure 2 shows the potentiating effect of ketamine on morphine antinociception at three dose levels. Ketamine at 30 mg/kg dose produced no per se antinociception but its combination with morphine (2.5, 5.0, 7.5 mg/kg, s.c.) showed significant potentiation/synergistic effect, both in maximal effect as well as on duration (Fig. 2). Although morphine (2.5 mg/kg) produced no per se antinociception, its combination with ketamine manifested significant analgesic activity at time points of 60 and 90 min (Fig. 2A). Higher dose combination of morphine

![Fig. 1. Time Course of Antinociception Induced by Morphine (Morph; 2.5, 5.0, 7.5 mg/kg, s.c.) in Rat Tail-Flick Test](image1)

All treatments were given at time 0. Control group was treated with saline. Data are expressed as means±S.E.M. for eight animals. ∗ \( p < 0.05 \) and ∗∗∗ \( p < 0.001 \) vs. saline-treated controls (ANOVA and Tukey’s test).

![Fig. 2. The Antinociceptive Effect of Morphine (Morph; 2.5, 5.0, 7.5 mg/kg, s.c.) in Association with Ketamine (Ket, 30 mg/kg, i.p.) in Rat Tail-Flick Test](image2)

All treatments were given at time 0. Control group and ketamine alone groups were treated with saline. Data are expressed as means±S.E.M. for eight animals. Upper panel (A): ∗∗∗ \( p < 0.001 \) vs. control, ketamine or morphine alone; middle panel (B): ∗ \( p < 0.05 \), ∗∗ \( p < 0.01 \) vs. control, ∗∗∗ \( p < 0.001 \) vs. ketamine alone and ∗∗∗∗ \( p < 0.001 \) vs. morphine alone; lower panel (C): + \( p < 0.05 \), +++ + \( p < 0.001 \) vs. control and + + + + \( p < 0.001 \) vs. control, ketamine or morphine alone (ANOVA and Tukey’s test).
(5.0, 7.5 mg/kg) with ketamine also showed significant enhancements in the duration of analgesia (Figs. 2B, C). Combination of 7.5 mg/kg dose morphine with 30 mg/kg ketamine showed sustained analgesia beyond 120 min (Fig. 2C). To verify the possible mechanism of this potentiation, animals were pre-treated with naloxone (2 mg/kg, i.p.), a non-selective opioid receptor antagonist, or glibenclamide (3 mg/kg, i.p.), an ATP-sensitive potassium channel blocker or yohimbine (2 mg/kg, i.p.), an alpha-2 adrenergic blocking agent, 15 min before morphine (7.5 mg/kg, s.c.)+ketamine (30 mg/kg, i.p.) injections. Naloxone completely and yohimbine only partially blocked the antinociceptive effect produced by morphine+ketamine, whereas glibenclamide was without any significant influence (Fig. 3).

Table 1 shows the results obtained in behavioral studies. In open-field test, compared to saline-treated controls, morphine (5 mg/kg, s.c.) produced a significant decrease whereas ketamine (30 mg/kg, i.p.) caused an increase in locomotor activity. But the combination treatment with morphine (5 mg/kg) and ketamine (30 mg/kg) resulted in significant potentiation of morphine’s effect, which was however reversed by prior administration of naloxone (2 mg/kg, i.p.). In catelepsy test, morphine (5 mg/kg), ketamine 30 mg/kg and saline administrations produced apparently no per se cataleptic effect. However, combination treatment with morphine and ketamine induced very profound and statistically significant cataleptic behavior, which was abolished by naloxone pre-treatment.

DISCUSSION

The main finding of this study was that a non-analgesic dose of ketamine (30 mg/kg, i.p.) potentiates morphine (2.5, 5.0, 7.5 mg/kg, s.c) antinociception in a naloxone reversible manner in rat tail-flick test, the most commonly used animal model of acute pain. These data confirm previous observations on the ability of ketamine to potentiate the effect of morphine in other types of experimental models as well as in clinical situations. The mechanism of this potentiating interaction between morphine and ketamine is unclear. In pain transmission, numerous neurotransmitters and ion channels are involved. In order to verify the possible mechanism in the potentiating effect of morphine antinociception by ketamine, animals were pretreated with naloxone, a non-selective opioid receptor antagonist; glibenclamide, an ATP-sensitive K+ channel blocker; or yohimbine, an α2-adrenoceptor antagonist. It was observed that pretreatment of rats with naloxone abolished the ketamine-induced potentiation of morphine antinociception, suggesting a possible mediation by opioid receptor. The primary mechanism of ketamine is to block N-methyl-D-aspartate (NMDA) receptors and there may be interactions between ketamine and the endogenous opiate systems, which could be antagonized by naloxone. On the other hand, pretreatment with yohimbine only partially antagonized the potentiating effect of ketamine. Ketamine seems to elevate the levels of noradrenaline in the spinal cord suggesting that ketamine-induced analgesia involves an activation of noradrenergic inhibitory system. In fact, spinal level antinociception involves the activation of opioid, noradrenergic and serotonergic systems. At the dose employed in this study, yohimbine (2 mg/kg, i.p.) although do not produce per se effect on nociception, it has been shown to limit the opioid antinociception. The mitigating effect of yohimbine on ketamine-induced potentiation of morphine antinociception is consistent with the hypothesis that adrenergic agonists either prolong the opiate analgesia or reduce the need for postoperative opioid analgesics. Many studies have suggested that the opening of ATP-sensitive K-channels plays an important role in the antinociception induced by morphine at supraspinal, spinal and peripheral levels. In our studies, glibenclamide, an antagonist failed to modify the ketamine response on morphine antinociception, indicating that ATP-sensitive K-channels play no role.

It has been shown in rats that both ketamine and morphine induce catalepsy at larger doses, which could be reversed by naloxone. At the doses employed, ketamine (30 mg/kg) and morphine (5.0 mg/kg) alone failed to produce catalepsy but when administered together in combination, these subeffective doses provoked naloxone-reversible catalepsy, indicating the participation of opioid receptors. In the open-field test, morphine at 5 mg/kg significantly diminished the ambulation frequency whereas ketamine (30 mg/kg) enhanced it. In contrast, combination treatment of morphine+ketamine showed only a depressant effect on locomotor activity, and no ambulation-enhancing effect of ketamine, which could be reversed in a significant manner by naloxone pretreatment.

Thus the data obtained in this study suggest the possible
involvement of ketamine stimulated endogenous opioids and the increased noradrenaline release at the spinal sites contributes to its enhancement effect on morphine’s antinociception, catalepsy and ambulation. In clinical situations, the purpose of drug combination is to optimize dose regimens so that greater analgesic effects are obtained with decreased unwanted side effects. Ketamine combination with morphine therefore may not only reduce the requirements of morphine, but also may provide rapid and sustained improvement in morphine analgesia and subjective feeling of well being without unacceptable side effects.

In summary, the results of this study indicate that ketamine potentiates morphine antinociception and support the use of opioid agonists in combination with NMDA antagonists for the management of acute pain. However, caution must be exercised in choosing the right dosage for combination therapy strategy for pain relief so that excessive sedation, and ketamine-induced hallucinations and respiratory depression can be avoided. Further, behavioral data of mice in open-field and catalepsy tests indicate that low dose ketamine combination with morphine may be helpful in clinical management of opiate withdrawal in humans using antagonist-assisted abstinence strategy.

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