

Synergistic Antinociceptive Effects of Ketamine and Morphine in the Orofacial Capsaicin Test in the Rat

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Background: The clinical efficacy of the noncompetitive *N*-methyl-D-aspartate receptor antagonist ketamine for treating orofacial pain has already been reported. Side effects related to psychotomimetic disturbances, however, limit ketamine use as an analgesic. Theoretically, this limitation could be minimized by using low doses of ketamine in combination with other analgesics. In the present study, the potential synergistic antinociceptive interaction between ketamine and morphine in the orofacial capsaicin test in rats was investigated.

Methods: Male Sprague-Dawley rats were subcutaneously injected with solvent, ketamine, morphine, or combination of both drugs. Thirty minutes later, the orofacial capsaicin test was performed by injecting of 1.5 µg/25 µl of a capsaicin solution into the vibrissa pad. Animal behavior was recorded on videotape and analyzed off-line. The total time spent on rubbing-scratching nociceptive behavior during a period of 42 min was measured.

Results: Subcutaneously administered ketamine (0.4, 1.25, 4, 12.5 mg/kg), morphine (0.5, 1, 2, 4 mg/kg) and ketamine + morphine (0.20 + 0.12, 0.40 + 0.24, 0.80 + 0.49, 1.61 + 0.97, 3.21 + 1.94 mg/kg) reduced the rat facial rubbing-scratching behavior in a dose-dependent manner. Isobolographic analysis showed that the ketamine + morphine association inhibited the studied behavior in a superadditive manner.

Conclusions: These results indicate that ketamine and morphine have antinociceptive effects on the orofacial capsaicin test. Furthermore, their combination produces synergistic antinociception. It is therefore suggested that, used together, ketamine and morphine might be clinically efficient at lower doses than those currently used when administered separately. This could provide a useful strategy for the clinical management of orofacial pain.

THE dissociative anesthetic agent ketamine is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist that can act as an effective analgesic at subanesthetic doses.¹ This property is particularly relevant in pain syndromes characterized by allodynia, hyperalgesia, and prolonged pain responses.¹ A serious limitation for its analgesic use, however, is the frequent presentation of psychotomimetic side effects.^{1,2} To minimize such undesirable effects, the clinical concept of balanced or multimodal analgesia proposes to use a combination of

analgesics to provide better pain relief and to minimize the side effects of each drug.² For instance, ketamine combined with morphine might be clinically efficient at lower doses than those currently used when administered separately.³

In the particular case of orofacial pain, ketamine has shown analgesic efficacy in acute and chronic conditions and also in similar side effect profiles.^{4,5} Whether ketamine combined with morphine is a clinically relevant combination in orofacial pain is still unknown. In the present study, we sought to provide experimental support to this hypothesis by assaying this analgesic combination in a suitable preclinical model of tonic orofacial pain.

Current evidence suggests that persistent nociceptor activity may lead to central sensitization, which is known to underlie many clinical features of tonic orofacial pain.⁶ Because application of capsaicin in the orofacial sensory territory can produce such nociceptor activation,⁷ the rat capsaicin orofacial test can be used as a mechanistic model of tonic orofacial pain. In this test, the intradermal administration of capsaicin into the vibrissa pad produces a stereotyped rubbing-scratching behavior directed to the injected area, which can be submitted to algesimetric quantification.⁸ Accordingly, experimental topical or intradermal application of capsaicin in the orofacial region in humans produces a tonic burning pain sensation and allodynic/hyperalgesic responses.⁹

Thus, to test the proposed hypothesis, rats were treated with ketamine or morphine alone or with the combination of both drugs, and then submitted to the capsaicin orofacial test.

Materials and Methods

Animals

Experiments were performed in male Sprague-Dawley rats weighing 200 to 220 g. They were maintained in a 22°C room with a 12-h daylight regimen (lights on 06:00 to 18:00) and fed standard rat chow and water *ad libitum*. Experimentation was conformed to the *Guide for Care and Use of Laboratory Animals* published by the National Institutes of Health. The experimental protocol was further approved by the Comité de Bioética, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile (Santiago de Chile). All rats were administered a single capsaicin injection and were used only once in this study.

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Experimental Procedures

The general procedure was essentially similar to that described previously by Pelissier *et al.*⁸ Briefly, each rat was placed in an individual plexiglas observation cage endowed with four mirrors so that the animal behavior could be visualized from the front of the cage no matter where the rat was situated. To minimize stress, the animal was kept in the cage for 10 min before the beginning of the experiment.

Capsaicin (Sigma Chemical, St. Louis, MO) was dissolved in a lipidic emulsion for venous perfusion (Endolipide; Braun Medical SA, Boulogne, France), whereas ketamine (Imalgene 1000; Merial, Lyon, France) and morphine (Morfina clorhidrato; Laboratorio Biosano, Santiago, Chile) were dissolved in isotonic saline solution (solvent). The drug to be studied or the solvent alone was then administered subcutaneously in a volume of 1 ml/kg in the dorsal region of the neck. Thirty minutes later, a 1.5 $\mu\text{g}/25 \mu\text{l}$ capsaicin solution was injected intradermally in the dorsal right lip of the rat, using a 27-g \times 4-mm massotherapy needle (PIC indolor; Artsana, Grandate, Italy) attached to a 50- μl microsyringe (Hamilton, Reno, NV). The rat was placed back in the box and the behavior was filmed using a video camera for 42 min. Immediately after the end of the recording session, the animal was killed with an overdose of intraperitoneal sodium pentobarbital (100 mg/kg). Nociceptive behavior was analyzed off-line by an experimenter blind to the drug treatment, and the total time spent on rubbing-scratching behavior was measured. Only nociceptive behavior executed with the ipsilateral fore or hind paw was considered as valid.

The following experimental groups were designed: solvent; ketamine: 0.4, 1.25, 4, and 12.5 mg/kg; morphine: 0.5, 1, 2, and 4 mg/kg; ketamine + morphine: 0.20 + 0.12, 0.40 + 0.24, 0.80 + 0.49, 1.61 + 0.97, and 3.21 + 1.94 mg/kg. Ketamine + morphine ratios were calculated as indicated later in this article.

Assessment of psychomotor effects was made in separate groups of rats using the same protocol of drugs and doses ($n = 5/\text{group}$). In these animals, the time spent in exploratory activity and the rearing counts were measured during 42 min.

Statistical Analysis

Results were expressed as mean percentages of antinociceptive effect ($\text{AE} \pm \text{SD}$ ($n \geq 5/\text{group}$)). The percentage AE was calculated as: $\% \text{AE} = 100 - (\text{total time of face rubbing-scratching in drug assay} \div \text{total time of face rubbing-scratching in solvent assay}) \times 100$. Data were analyzed by using one-way ANOVA, followed by the Student-Newman-Keuls *post hoc* test. Differences were considered significant at $P < 0.05$. The doses that produced 50% of AE (ED_{50}) and their 95% confidence limits (CL) were calculated by linear regression analysis of the log dose-response curve.

To identify drug synergism in the drug combination, the isobolographic analysis proposed by Tallarida¹⁰ was performed. Using ketamine + morphine fixed ratios, the isobologram was constructed by connecting with a straight line the ketamine ED_{50} point in the abscissa with the morphine ED_{50} point in the ordinates to obtain an additivity line. For each drug mixture, the ED_{50} and their associated 95% CL were determined by linear regression analysis of the log dose-response curve and compared by means of a Student *t* test to a theoretical additive ED_{50} ($\text{ED}_{50} \text{ add}$). The $\text{ED}_{50} \text{ add}$ was calculated as: $\text{ED}_{50} \text{ add} = \text{ED}_{50} \text{ ketamine} \div (\text{P1} + \text{R} \times \text{P2})$, where R is the potency ratio of ketamine alone to other drug alone (morphine), whereas P1 is the proportion of ketamine and P2 is the proportion of morphine in total combination. In this study, the proportions of fixed ratios were selected by combining first the ED_{50} of ketamine + the ED_{50} of morphine, and then one half, one quarter, one eighth, one sixteenth, and one thirty-second of the respective ED_{50} . In that equation, $\text{ED}_{50} \text{ add}$ is the total dose, and the variance of $\text{ED}_{50} \text{ add}$ was calculated from the fraction of the ED_{50} s (*i.e.*, 0.5) in the combination as: $\text{Var ED}_{50} \text{ add} = (0.5)^2 \times \text{Var ED}_{50} \text{ morphine} + (0.5)^2 \times \text{Var ED}_{50} \text{ ketamine}$. From these variances, CLs were calculated and resolved according to the ratio of the individual drugs in the combination. Superadditivity or synergistic effect was considered as the effect of a drug combination that is higher and statistically different (significantly lower experimental ED_{50}) than the theoretical calculated equi-effect of a drug combination with the same proportions.

Results

Orofacial Capsaicin Test

Injection of capsaicin into the right vibrissa pad produced an immediate rubbing-scratching of the injected area. This behavior was performed with the ipsilateral forepaw, often accompanied by contralateral forepaw movements. The amplitude of the rubbing-scratching response reached a maximum during the 12- to 18-min interval after capsaicin injection. The cumulated total time of response was $232 \pm 56.1 \text{ s}$ ($n = 14$). Subcutaneous administration of saline (1 ml/kg) 30 min before application of capsaicin did not modify the rubbing-scratching response ($231 \pm 30.9 \text{ s}$, $n = 6$, $P > 0.05\%$). Time spent in exploratory activity in this group was $470.8 \pm 56.1 \text{ s}$, whereas rearing number was 80.3 ± 15.2 .

Effect of Ketamine

Ketamine produced a dose-dependent decrease of the face rubbing-scratching response. The time-course of this behavior after the administration of different doses of ketamine is presented in figure 1A. The decrease of

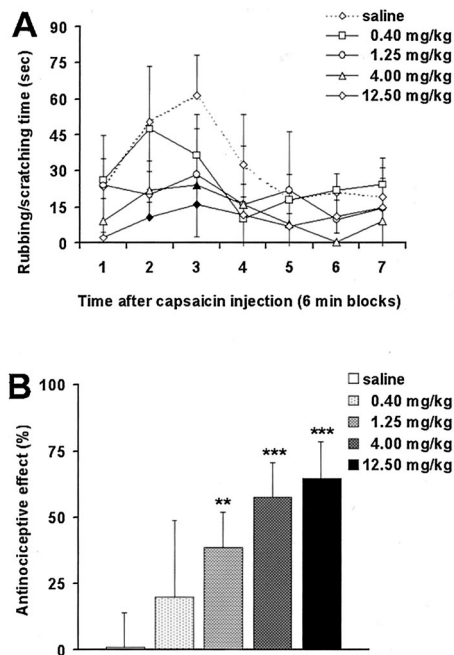


Fig. 1. Temporal evolution (A) and percentage of antinociceptive effect (B) of subcutaneously administered ketamine on the orofacial capsaicin test. Data are presented as mean \pm SD, $n = 6$. Significant differences are represented by filled symbols (see details in Results) or as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the behavioral response reached statistical significance with the 4-mg/kg dose at the 12- to 18-min interval ($P < 0.05$) and with the 12.5-mg dose at the 6- to 12-min ($P < 0.05$) and 12- to 18-min ($P < 0.01$) intervals. The percentage AE obtained with the doses of 0.4, 1.25, 4, and 12.5 mg/kg were 19.8 ± 28.9 ($n = 6$; $P > 0.05$), 38.6 ± 13.2 ($n = 6$; $P < 0.01$), 57.7 ± 12.8 ($n = 6$; $P < 0.001$), and 64.6 ± 13.8 ($n = 6$; $P < 0.001$), respectively (fig. 1B). Rearing number was significantly inhibited only by 12.5 mg/kg of ketamine (25 ± 6.3 ; $P < 0.001$) with respect to saline-treated rats; besides, ketamine did not produce significant effects on exploratory activity at any dose. Ketamine ED_{50} was calculated at 3.21 mg/kg (lower 95% CL = 1.485 mg/kg and upper 95% CL = 6.932 mg/kg).

Effect of Morphine

Morphine produced a dose-dependent inhibition of the face rubbing-scratching behavior. The evolution of this behavior after the administration of different doses of morphine is presented in figure 2A. This decrease reached statistical significance with the 0.5-mg/kg ($P < 0.05$), 1-mg/kg ($P < 0.01$), and 2-mg/kg ($P < 0.01$) doses at the 12- to 18-min interval and with the 4-mg/kg dose at the 6- to 12-min ($P < 0.05$) and 12- to 18-min ($P < 0.001$) intervals. The percent AE obtained with the 0.5, 1, 2, and 4 mg/kg doses were 13.2 ± 16.3 ($n = 6$; $P > 0.05$), 26.5 ± 21.2 ($n = 6$; $P > 0.05$), 43.2 ± 28.3 ($n = 6$; $P < 0.01$), and 78.4 ± 16.7 ($n = 6$; $P < 0.001$), respectively (fig. 2B). Even assayed in a dose of 4 mg/kg,

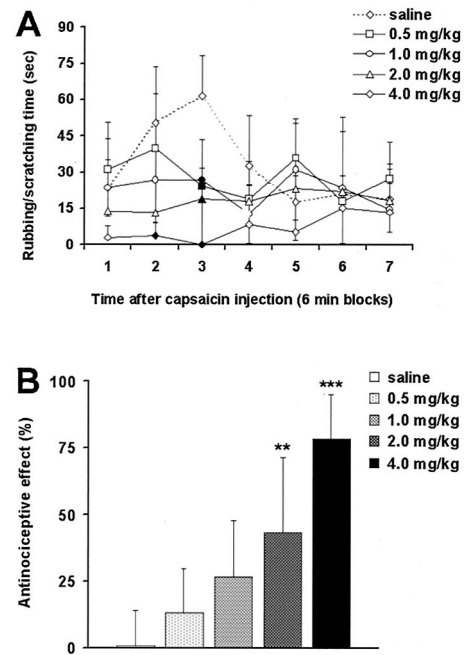


Fig. 2. Temporal evolution (A) and percentage of antinociceptive effect (B) of subcutaneously administered morphine on the orofacial capsaicin test. Data are presented as mean \pm SD, $n = 6$. Significant differences are represented by filled symbols (see details in Results) or as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

morphine had no significant effects on exploratory activity or on rearing behavior respective to saline-treated rats. Morphine ED_{50} was calculated as 1.94 mg/kg (lower 95% CL = 1.050 mg/kg and upper 95% CL = 3.586 mg/kg).

Effect of the Ketamine-Morphine Association

Association of ketamine + morphine in doses of 0.2 + 0.12, 0.4 + 0.24, 0.8 + 0.49, 1.61 + 0.97, and 3.21 + 1.94 mg/kg also produced a dose-dependent inhibition of the studied behavior. This inhibition reached statistical significance at the 12- to 18-min interval for all doses in combination (0.2 + 0.12 mg/kg, $P < 0.05$; 0.4 + 0.24 mg/kg, $P < 0.01$; 0.8 + 0.49 mg/kg, $P < 0.01$; 1.61 + 0.97 mg/kg, $P < 0.01$; 3.21 + 1.94 mg/kg, $P < 0.01$) (fig. 3A). The percent AE exhibited by ketamine + morphine was of 37.4 ± 19.6 for 0.2 + 0.12 mg/kg ($n = 5$, $P < 0.01$), 46.4 ± 14.3 for 0.4 + 0.24 mg/kg ($n = 5$, $P < 0.001$), 55.1 ± 4.4 for 0.8 + 0.49 mg/kg ($n = 5$, $P < 0.001$), 59.6 ± 18.8 for 1.61 + 0.97 mg/kg ($n = 5$, $P < 0.001$), and 78.6 ± 15.2 for 3.21 + 1.94 mg/kg ($n = 5$, $P < 0.001$) (fig. 3B). Ketamine + morphine combinations did not produce significant inhibitory effects on exploratory activity or on rearing counts. Ketamine + morphine ED_{50} was 0.87 mg/kg (lower 95% CL = 0.556 mg/kg and upper 95% CL = 1.351 mg/kg), which was significantly different from ED_{50} *add*, calculated as 2.57 mg/kg (lower 95% CL = 1.446 mg/kg and upper 95% CL = 4.581 mg/kg), indicating a superadditive effect ($P < 0.05$) (fig. 4).

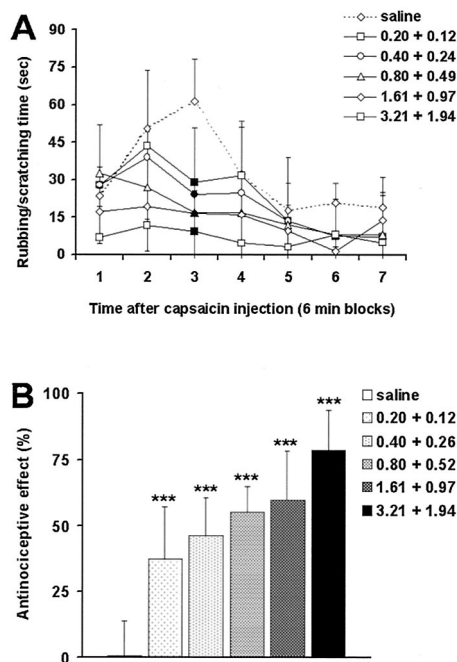


Fig. 3. Temporal evolution (*A*) and percentage of antinociceptive effect (*B*) of the association ketamine + morphine (mg/kg) on the orofacial capsaicin test. Data are presented as mean \pm SD, $n = 5$. Significant differences are represented by filled symbols (see details in Results) or as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Discussion

In the present work, we sought to provide experimental support to the hypothesis that ketamine combined with morphine may be a clinically relevant combination in orofacial pain. Antinociceptive effects of ketamine, morphine, and the interaction of both drugs were thus studied in the orofacial capsaicin test in rats. Results show that whereas each drug exhibited antinociceptive effects by itself, the ketamine + morphine association was superadditive in this model of acute tonic orofacial pain.

Orofacial Capsaicin Test

Strong evidence suggests that the behavioral pattern observed in the rat orofacial capsaicin test is related to

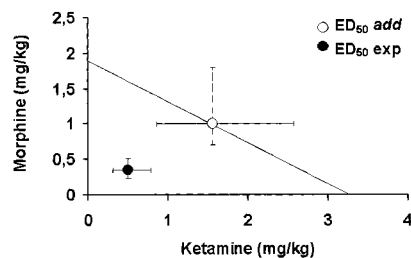


Fig. 4. Isobologram showing the interaction between subcutaneous ketamine + morphine association on capsaicin orofacial test. Values of ED_{50}^{add} (○) and experimental ED_{50} (●) \pm 95% CL, were calculated by linear regression analysis. Significant difference is represented as * $P < 0.05$.

pain. Indeed, application of control innocuous procedures to the orofacial region is not followed by persistent face rubbing-scratching, as observed here.¹¹ Conversely, chemical, thermal, mechanical, or electrical noxious stimulus applied to the orofacial region induce a behavioral response similar to the one observed in the orofacial capsaicin test.^{8,11-13} In humans, psychophysical studies have revealed that the application of capsaicin into the orofacial territory evokes a tonic "burning" pain perception of monophasic time course.⁹ The closely similar time course of capsaicin-evoked behavioral response in rats and oral pain in humans further validates the test used here as a preclinical model of human acute tonic orofacial pain.^{8,9}

Antinociceptive Effects of Ketamine

Ketamine significantly reduced nociceptive behavior studied here, and at the lower doses needed to produce significant psychomotor effects. Accordingly, results of other behavioral studies have shown that NMDA-receptor antagonists produced antinociception in animal models of acute tonic pain. For instance, it has been shown that systemically administered memantine produced a significant inhibition of the tonic phase of the orofacial formalin test,¹⁴ whereas intrathecally administered dizocilpine (MK-801) had antinociceptive effects on the second phase of the rat formalin test.¹⁵ In addition, systemically delivered ketamine and MK-801 were found to reduce the thermal hyperalgesia behavior displayed after chemical injury of the rat hind paw by formalin¹⁶ or carragenine.¹⁷ At higher doses (12.5 mg/kg), however, ketamine was found to produce significant psychomotor disturbances in parallel with stronger antinociception. This experimental feature resembles the clinical apparition of psychotomimetic side effects that impede the use of large doses of ketamine for analgesic effect.²

Because ketamine was systemically administered in this study, it was not possible to identify the sites of action related to its antinociceptive effects. Central mechanisms, however, may be involved because quantitative binding studies have identified a dense labeling for NMDA-glutamate receptors in the spinal trigeminal nucleus, which is the main brainstem relay for orofacial pain.¹⁸ Blockade of these receptors by specific antagonists was found to inhibit spinal trigeminal nucleus nociceptive neurons^{19,20} and to prevent central sensitization evoked by chemical injury of the orofacial territory.²¹ Activation of the monoaminergic descending inhibitory system might also be implied in the central antinociceptive effect produced by ketamine.^{22,23} Finally, because the peripheral participation of NMDA receptors in chemical nociception has been demonstrated, a peripheral component in the antinociceptive effect of ketamine cannot be discarded.²⁴ Actual evidence for such effect, however, remains controversial.^{25,26}

Antinociceptive Effects of Morphine

The antinociceptive effects of morphine in the rat orofacial capsaicin test are closely concordant with the results of other behavioral studies on acute tonic orofacial nociception. In fact, either systemically^{8,12,27} or intrathecally administered, at the medullary²⁸ or the cervico-medullary²⁹ level, morphine always produced a significant inhibition of tonic orofacial nociception. As for ketamine, it is not possible to ascertain the antinociceptive effects produced by systemic administration of morphine to a specific site of action. Because several brain areas exhibiting a high density of μ -opioid receptors have been shown to be related to the modulation of orofacial nociceptive information,³⁰ central mechanisms may be involved, including modulation of nociceptive information relay within the spinal trigeminal nucleus.³⁰⁻³² Peripheral effects can also contribute to the antinociception exhibited by morphine, because the existence of peripheral opioid receptors has been demonstrated.³³ Accordingly, peripherally applied morphine at orofacial level has also shown antinociceptive effects.^{8,27}

Drug Synergism

The main finding of this study was that combination of ketamine with morphine produced synergistic antinociception in the rat orofacial capsaicin test. First, the ketamine + morphine association produced a significant diminution of the persistent face rubbing-scratching behavior. Second, isobolographic analysis showed that the experimentally obtained ED₅₀ of the combination was significantly lower than the theoretic ED₅₀ add, indicating that the drug association was superadditive (fig. 3). These results are in agreement with previous dose-response studies reporting a similar synergy using animal models of phasic³⁴ or tonic pain.^{35,36}

Different mechanisms may be involved in the complementary action of both drugs. Persistent noxious stimulus induces NMDA receptor activation leading to central sensitization, a phenomenon whose behavioral correlate is hyperalgesia. Opioids produce their antinociceptive effects acting presynaptically on C-fiber terminals, which inhibit neurotransmitter release and produce a synergistic inhibition with postsynaptically acting NMDA antagonists.³ Postsynaptic influx of calcium ions produced by activated NMDA receptor channels is a critical step in the subsequent translocation and activation of protein kinase C (PKC), induction of nitric oxide synthase, and increase of intracellular nitric oxide, which in total are implied in blunted responsiveness of the μ -opioid receptor to its agonists.^{37,38} Therefore, an inhibition of NMDA-mediated calcium influx by ketamine can also prevent inhibitory effects of PKC and intracellular NO on μ -opioid receptor activity.

However, μ -opioids can produce acute antinociception by binding to opioid receptors, and they can also induce hyperalgesia by acting on NMDA receptors.^{37,38}

This hyperalgesic effect of μ -opioids can be blocked by NMDA receptor antagonists.³⁹⁻⁴² In fact, behavioral studies have shown that naloxone administered after morphine or fentanyl antagonizes the antinociceptive effects of these substances, thereby precipitating an state of hyperresponsiveness to noxious stimulation.³⁹ This hyperalgesic state can be prevented by administration of noncompetitive NMDA antagonists such as MK801,³⁹ which suggests that opioid-activated NMDA receptors might account for acute opioid-induced hyperalgesia. Electrophysiologic evidence shows that the selective μ -opioid agonist D-Ala²-MePhe⁴-Gly-ol⁵-enkephalin enhances the glutamate synaptic effectiveness on NMDA receptors in neurons of the spinal trigeminal nucleus, an effect that can be interrupted by inhibition of PKC and mimicked by intracellular administration of functional PKC.⁴³ Activated PKC can trigger NMDA receptor activation by reducing Mg²⁺ block and by increasing the probability of channel opening.⁴⁴ Therefore, in addition to the inhibitory effect of ketamine on the establishment of central sensitization when administered alone, this drug could also enhance the antinociceptive effect of morphine by a reduction of its hyperalgesic effect.

The present results are not contradictory with an earlier experimental study performed in humans in which only simple additivity between ketamine and alfentanil was found.⁴⁵ In this study, subjects were treated with ketamine and/or alfentanil when allodynia and hyperalgesia induced by intradermal capsaicin were already present.⁴⁵ These symptoms reveal an installed central sensitization process, a state in which NMDA antagonists and opioid agonists are significantly less efficacious.^{15,46,47} Thus, it is not surprising to find in the literature a dose-response antinociceptive synergy between NMDA antagonists and opioids on tonic pain when drugs are administered preemptively,³⁵ as in the present study.

Clinical Implications

Psychotomimetic side effects produced by ketamine are dose-related events^{1,2,4} that have been reported to be the primary cause for discontinuation of ketamine in pain clinics.¹ Because this drug is efficacious to treat persistent orofacial pain,^{1,4} the identification of strategies to diminish the amount necessary to produce analgesia is highly relevant. In this sense, our findings suggest that the addition of ketamine to opioid analgesic therapy is promising. Despite the controversy with the use of NMDA receptor antagonists as adjuncts to opioids in pain management,^{48,49} several studies have reported that these drugs can enhance the analgesic efficacy of opioids.^{1,4,50} In addition, reports indicate that ketamine allows treatment of severe pain in opioid-tolerant/opioid-addicted patients.⁵¹ Actually, experimental evidence shows that NMDA antagonists can prevent, delay, or

revert the apparition of analgesic tolerance and physical dependence to opioids.⁵² Besides, in a reciprocal manner, NMDA receptor antagonists could also inhibit the ongoing analgesic tolerance and physical dependence produced by μ -opioid receptor agonists, thus limiting their prolonged clinical use.³⁸

In conclusion, this study shows that ketamine and morphine individually exhibit antinociceptive effects in the rat capsaicin orofacial test, whereas their combination produces superadditive antinociceptive effects. On the whole, however, these results support the idea that the use of opioid and ketamine combinations may be clinically relevant to manage orofacial pain in humans at doses lower than those currently used when administered separately.

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