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Blockade of the antinociceptive activity of centrally administered ketorolac by nor-binaltorphimine

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Abstract

Antinociceptive activity of intracerebroventricularly administered ketorolac tromethamine was evaluated in mice by measuring inhibition of abdominal stretching induced by *p*-phenylquinone. Ketorolac tromethamine produced dose-dependent antinociception with an ED_{50} of 7.34 μg/mouse (4.97–10.82) and a maximal effect at 30 μg. Selective antagonists of opioid receptors were used to determine ketorolac's mechanism of action. The ketorolac tromethamine-induced antinociception was not blocked by the μ- and δ-opioid receptor antagonists, naloxone and ICI-174,864 (*N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu), respectively; however, the κ-opioid receptor antagonist nor-binaltorphimine dihydrochloride significantly blocked this effect. These findings suggest that activation of κ-opioid receptors appears to play a role in the mechanism of the antinociceptive effect of ketorolac tromethamine. Ketorolac tromethamine may induce the release of endogenous κ-opioids to produce central nervous system antinociception.

Keywords: Ketorolac tromethamine; κ-Opioid receptor; Antinociception; (Intracerebroventricular administration)

1. Introduction

The development of several peripherally acting, nonsteroidal anti-inflammatory drugs (NSAIDS) has been an important advancement in the pharmacotherapeutic management of pain. NSAIDS are established as being as effective as the more potent injectable opioids for certain disease states. Several NSAIDS have been proven effective in nonrheumatic pain, and the development of ketorolac resulted from the search for new cyclooxygenase inhibitors for their analgesic activity. Ketorolac, an NSAID, exhibits moderate anti-inflammatory, potent non-narcotic analgesic, and antipyretic activity (Buckley and Brogden, 1990; Litvak and McEvoy, 1990; Rooks, 1990). Ketorolac tromethamine was approved by the U.S. Food and Drug Administration in late 1989 for the short-term treatment of pain (Litvak and McEvoy, 1990). It has been used for the symptomatic relief of postoperative, abdominal, gynecologic, oral, orthopedic, and urologic surgical pain, and also for the relief of acute renal colic, pain associated with trauma, and visceral pain associated with cancer (Litvak and McEvoy, 1990).

Ketorolac acts peripherally to suppress pain stimuli principally through inhibition of prostaglandin synthesis in body tissues by inhibiting cylooxygenase, an enzyme that catalyzes the formation of prostaglandin precursors (endoperoxides) from arachidonic acid. In addition to this peripheral action, ketorolac may have a potential central effect. Although the exact mechanism of action through which ketorolac exerts its central effects has not been determined, its effects appear to be associated principally with the inhibition of prostaglandin synthesis in the central nervous system (Ferreira et al., 1978; Brooks and Day, 1991). However, several studies suggest that inhibition of cyclooxygenase may not be solely responsible for the analgesic effects of NSAIDs (Malmberg and Yaksh, 1992; Litvak and McEvoy, 1990). The central action of the antinociceptive effects of ketorolac may include an indirect interaction with opioid receptors causing the release of endorphins or enkephalins, which, in turn, could produce pain suppression (Domer, 1990).

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The present study was undertaken to characterize the antinociceptive effects of intracerebroventricularly (i.c.v.) administered ketorolac on the regulation of pain pathways in the central nervous system. To assess the analgesic activity of ketorolac at the cerebral level, ketorolac-induced inhibition of the abdominal stretching induced by *p*-phenylquinone was measured. To determine possible mediation through opioid receptors in the mechanism of the antinociception of ketorolac, the effects of selective antagonists of the μ-, δ-, and κ-opioid receptors upon the antinociception of i.c.v. administered ketorolac were examined.

2. Materials and methods

2.1. Drugs

Ketorolac tromethamine was obtained from Syntex Laboratories (Palo Alto, CA, USA). Naloxone, a selective antagonist of the μ-opioid receptor, and *p*-phenylquinone were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ICI-174,864 (*N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu, molecular weight = 691.88), a specific antagonist of the δ-opioid receptor (Cotton et al., 1984), was obtained from Cambridge Research Biomedicals (Cambridge, UK). Nor-binaltorphimine dihydrochloride (molecular weight = 734.78), a specific antagonist of the κ-opioid receptor (Takemori et al., 1988), was obtained from Research Biomedicals (Natick, MA, USA).

2.2. Animals

Male ICR mice weighing 15–30 g were used. They were obtained from Harlan Laboraties, MD, USA and were housed in a colony room with a 12 h light-dark cycle at a constant temperature of 24 ± 1° C and 55 ± 5% humidity. Animals were allowed food and water ad libitum until the experiment commenced and were acclimated to the laboratory at least 3 h prior to experimentation.

2.3. Intracerebroventricular injections

Ketorolac and all opioid receptor antagonist drugs were dissolved in sterile distilled water and administered in a volume of 5 μl per mouse. Six mice were used per group. All injections were administered using a 50-μl glass Hamilton syringe with a 26-gauge, 3/8 inch stainless steel needle modified with a shaft so that the depth of injection was consistently 2 mm. Mice were lightly anesthetized with anhydrous ether and transversely incised across the scalp to expose the reference point where the two skull plates meet, the bregma. The injection was made into the lateral ventricle 2 mm caudal and 2 mm lateral from the bregma. Doses of 1, 5, 10, 15, and 30 μg of i.c.v. ketorolac were used for the dose-response curve, and these doses did not produce any behavioral or motor deficits in mice. To test for reversal of the antinociceptive response induced by ketorolac (30 μg), naloxone (10 μg), nor-binaltorphimine (70 μg), and ICI-174,864 (10 μg) were also injected i.c.v. Naloxone and ICI-174,864 were administered i.c.v. 10 min prior to the ketorolac injection. Nor-binaltorphimine was administered i.c.v. 60 min prior to ketorolac, a pretreatment time shown previously to block the κ-opioid receptor agonist, U-50,488H (Takemori et al., 1988).

2.4. Abdominal stretching test

The nociceptive test used in this study was the *p*-phenylquinone abdominal stretching test developed by Hendershot and Forsaith (1958). The agent used to induce abdominal stretching was 0.02% solution (w/v) of *p*-phenylquinone in ethanol/distilled water (6% ethanol). Ten minutes after the i.c.v. injection of ketorolac or vehicle, *p*-phenylquinone (2 mg/kg) was administered intraperitoneally. The number of abdominal constrictor responses displayed by each mouse was counted for 2 min beginning 10 min after the administration of *p*-phenylquinone. An abdominal stretch is characterized by an elongation of the mouse's body, the development of tension in the lower region of the abdomen, and an extension of the forelegs. The percentage of inhibition of the stretch response was calculated as follows: (1 − {[Total number of stretching motions in test group]/[Total number of stretching motions in control group]} × 100). The average percent inhibition (±S.E.M.) was determined using at least 18 mice per dosage group.

2.5. Statistical analysis

The ED_{50} and correlation coefficient for the ketorolac dose-response curve were calculated using a modification of the method of Litchfield and Wilcoxon (1949). Significant difference between treatment groups was determined by analyses of variance followed by Dunnett's *t*-test (Dunnett, 1955).

3. Results

The inhibition of the frequency of abdominal stretching induced by *p*-phenylquinone was measured after the i.c.v. injection of ketorolac. The mean number of abdominal stretches observed in vehicle-injected control mice was 45 ± 10. As shown in Fig. 1, ketorolac was administered i.c.v. at doses of 1, 5, 10, 15, and 30 μg. At these doses inhibition of *p*-phenylquinone-in-

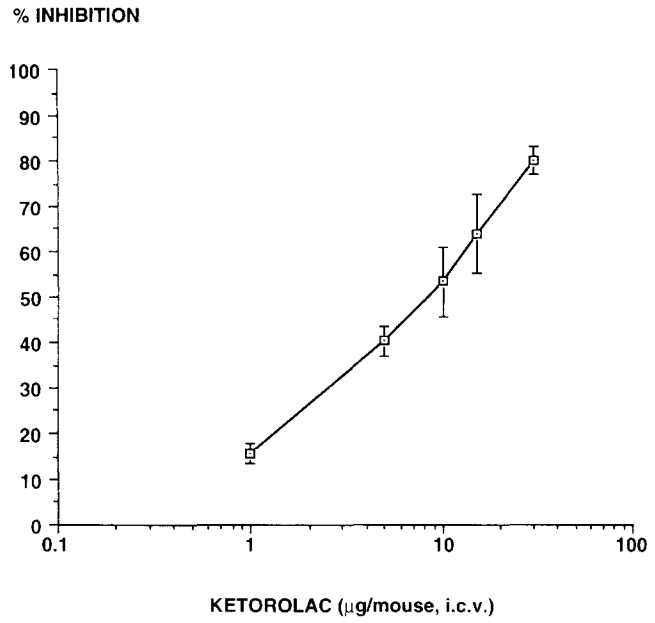

Fig. 1. The effect of intracerebroventricular ketorolac on the frequency of abdominal stretching induced by *p*-phenylquinone. Mice were injected intracerebroventricularly with ketorolac in the doses listed. Ten minutes after the administration of ketorolac, the mice were injected with *p*-phenylquinone as described in Materials and methods. The percent inhibition of abdominal stretching (±S.E.M.) and ED_{50} were calculated as previously described using at least 24 mice per dose.

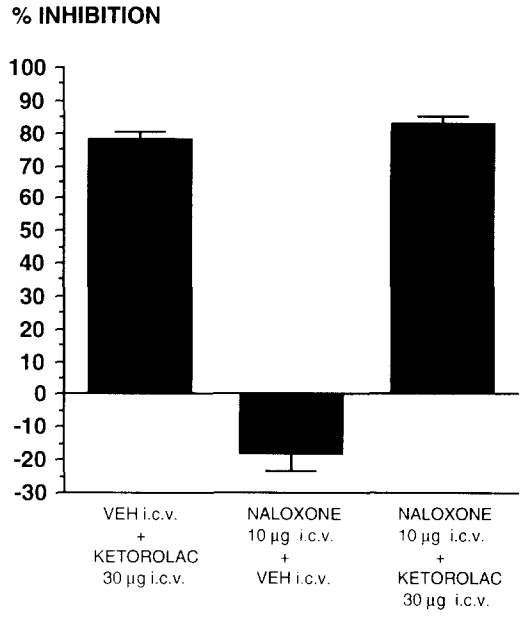

Fig. 2. The effect of the specific μ-opioid receptor antagonist naloxone on the antinociceptive effect of intracerebroventricular ketorolac. Mice were injected intracerebroventricularly with naloxone. Ten minutes later, ketorolac was given intracerebroventricularly. Ten minutes after the administration of ketorolac, the mice were injected with *p*-phenylquinone and tested for abdominal stretching as described in Materials and methods. The percent inhibition (±S.E.M.) was calculated as described in Materials and methods for each treatment group using at least 24 mice per group.

duced stretching was 16%, 40%, 53%, 64%, and 80%, respectively. The ED_{50} (CL) for i.c.v. administered ketorolac to inhibit abdominal stretching was 7.34 μg/mouse (4.97–10.82) and the correlation coefficient for the dose-effect curve was 0.99. Ketorolac inhibited abdominal stretching in a dose-related manner. However, doses as high as 50 μg produced no greater inhibition than that produced by the 30 μg dose (data not shown). Since the 30 μg dose of ketorolac produced approximately 80% antinociception, this dose was chosen to study the effects of the opioid receptor antagonists: naloxone, nor-binaltorphimine, and ICI-174,864.

Intracerebroventricular administration of the μ-opioid receptor antagonist, naloxone, at a dose of 10 μg, followed by ketorolac (30 μg), failed to reverse the antinociceptive effects of ketorolac, as shown in Fig. 2. Naloxone (10 μg) injected with ketorolac produced 82% inhibition, no greater inhibition than that produced by ketorolac (30 μg, inhibition = 78%) alone. Naloxone administered with the vehicle produced no effective percent inhibition.

The effect of the δ-opioid receptor antagonist, ICI-174,864 (10 μg), on the antinociceptive effect of ketorolac (30 μg) is shown in Fig. 3. ICI-174,864 administered with the vehicle produced an increase in *p*-phenylquinone-induced abdominal stretching. The inhibition produced by the i.c.v. injection of ICI-174,864

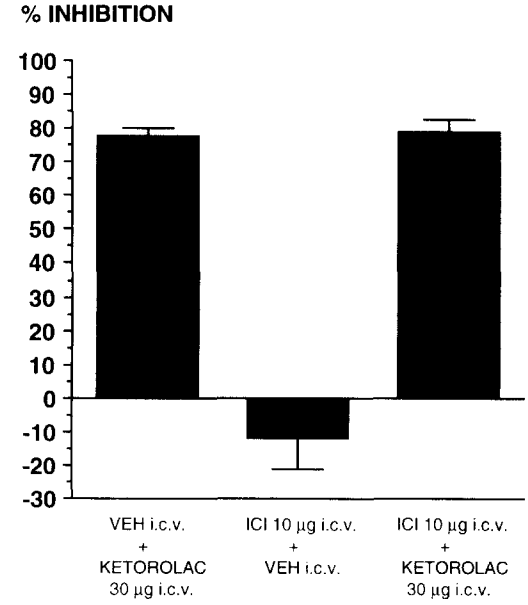

Fig. 3. The effect of the specific δ-opioid receptor antagonist ICI-174,864 on the antinociceptive effect of intracerebroventricular ketorolac. Mice were injected intracerebroventricularly with ICI-174,864. Ten minutes later, ketorolac was given intracerebroventricularly. Ten minutes after the administration of ketorolac, the mice were injected with *p*-phenylquinone and tested for abdominal stretching as described in Materials and methods. The percent inhibition (±S.E.M.) was calculated as described in Materials and methods for each treatment group using at least 36 mice per treatment group.

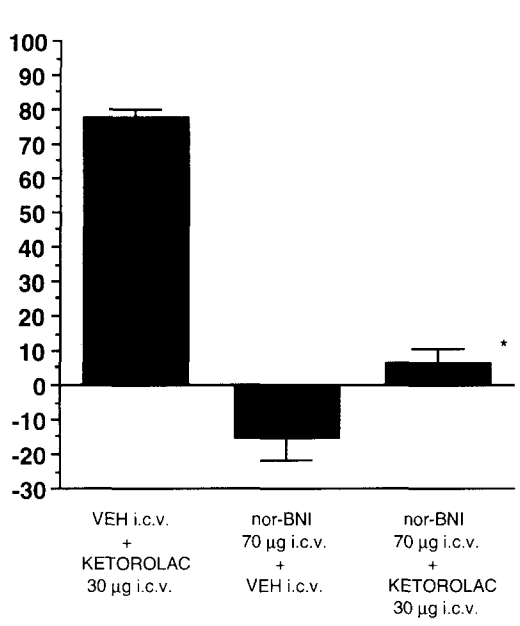

Fig. 4. Blockade of the antinociceptive effect of intracerebroventricular ketorolac by the specific κ-opioid receptor antagonist nor-binaltorphimine. Mice were injected intracerebroventricularly with nor-binaltorphimine. One hour later, ketorolac was given intracerebroventricularly. Ten minutes after the administration of ketorolac, the mice were injected with *p*-phenylquinone and tested for abdominal stretching as described in Materials and methods. The percent inhibition (±S.E.M.) and statistical significance were calculated as described in Materials and methods using at least 30 mice per treatment group. The significant blockage of the antinociceptive effects of ketorolac by nor-binaltorphimine was not an additive effect since the administration of nor-binaltorphimine with vehicle produced no effective percent inhibition when compared to the vehicle (i.c.v.)/vehicle (i.c.v.) group (data not shown). $^{*}P < 0.01$ from vehicle + ketorolac.

followed by the i.c.v. injection of ketorolac was 79%. When compared to the ketorolac control (inhibition = 78%), no attenuation of the antinociceptive effects of ketorolac was produced by ICI-174,864. In addition, ICI-174,864 (i.c.v.) administered with vehicle produced no effective percent inhibition.

As illustrated in Fig. 4, nor-binaltorphimine (70 μg), a specific antagonist of the κ-opioid receptor, significantly blocked the antinociceptive effects of ketorolac (30 μg). Following pretreatment with vehicle (i.c.v.), ketorolac (i.c.v.) produced 78% inhibition. Similarly, pretreatment with nor-binaltorphimine (i.c.v.) followed by injection of ketorolac (i.c.v.) produced 6.4% inhibition. This inhibition is significant at the $P < 0.01$ level when compared to the vehicle + ketorolac group. The significant blockage of the antinociceptive effects of ketorolac by nor-binaltorphimine was not an additive effect since the administration of nor-binaltorphimine with vehicle produced no effective percent inhibition when compared to the vehicle (i.c.v.)/vehicle (i.c.v.) group (data not shown).

4. Discussion

The abdominal stretching test is useful for evaluating nonsteroidal anti-inflammatory agents (Chau and Weichman, 1989). Ketorolac has been reported to be effective in humans as an opioid analgesic (Chau and Weichman, 1989; Lopez et al., 1987; Yee et al., 1986). Opioid analgesics have been the principal agents in controlling postoperative pain for years; however, these potent drugs are associated with potentially serious side effects that limit their use in providing analgesia. Unlike opiate analgesics, ketorolac does not appear to cause development of tolerance, physical dependence in long-term therapy, respiratory depressant effects, sedation, cardiovascular effects, or smooth muscle effects on the gastrointestinal tract and ureter (Litvak and McEvoy, 1990; Rubin et al., 1987). Several lines of evidence indicate that ketorolac is not a centrally acting opiate-like drug. Studies with oral doses of ketorolac in rodents suggest that ketorolac lacks central nervous system effects (Rooks et al., 1985). Ketorolac does not bind to μ-, δ-, or κ-opioid receptors in vitro (Lopez et al., 1987; Yee and Waterbury, 1987). It is inactive in pain models in which opioids are efficacious (Rooks et al., 1982). In studies involving the addictive potential of ketorolac, subjective withdrawal symptoms were not observed following the termination of the drug (Lopez et al., 1987; Yee and Waterbury, 1987). Domer (1990) reported that naloxone reversed the effects of ketorolac in mice using the abdominal stretching test. These lines of evidence imply that ketorolac indirectly activates opioid receptors by actuation of an endogenous pain suppression system.

Inhibition of cyclooxygenase by ketorolac in the periphery was previously accepted as the primary mechanism by which it produces attenuation of pain. Recently, however, studies have shown significant central actions of ketorolac. Malmberg and Yaksh (1992) reported that spinal injection of ketorolac blocks the hyperalgesia induced by the activation of spinal glutamate and substance P receptors. In another study, they found that intrathecal ketorolac potentiates the antinociceptive effects of intrathecal morphine in the formalin test in rats (Malmberg and Yaksh, 1993). Maves et al. (1994) reported a synergistic interaction between ketorolac and morphine in which intravenous ketorolac enhances the analgesic effects of intravenous morphine, thereby suggesting that ketorolac's mechanism of action may involve a central effect on an opioid receptor. These findings clearly support a central mechanism of action of ketorolac. This study has shown that the i.c.v. injection of ketorolac produced dose-related antinociception in mice in the *p*-phenylquinone abdominal stretching test, although no greater antinociceptive effect could be elicited with a dose as high as 50 μg.

The present results indicate that the μ-opioid receptor antagonist, naloxone, and the δ-opioid receptor antagonist, ICI-174,864, did not block the antinociception induced by i.c.v. ketorolac. However, the κ-opioid receptor antagonist, nor-binaltorphimine, significantly blocked i.c.v. ketorolac. These results are in agreement with the results of Uphouse et al. (1993) who found that subcutaneously injected naloxone and intrathecally administered ICI-174,864 failed to block the effects of subcutaneous ketorolac; however, the κ-opioid receptor antagonist, nor-binaltorphimine, administered intrathecally, blocked the antinociceptive effects of both systemic and intrathecally administered ketorolac. The lack of blockade by naloxone and ICI-174,864 suggests that antinociceptive effects of i.c.v. ketorolac are not mediated through μ- and δ-opioid receptors. Ketorolac failed to produce withdrawal jumping in morphine-tolerant mice suggesting that ketorolac does not possess properties of an opioid antagonist and does not act as a mixed agonist-antagonist at the opioid receptor (Uphouse et al., 1993).

The ability of nor-binaltorphimine to significantly block the antinociceptive effects of i.c.v. ketorolac indicates that the interaction of nor-binaltorphimine may be either non-opioid in nature or via ketorolac-induced release of another substance that is blocked by nor-binaltorphimine, but not by naloxone or ICI-174,864. Welch (1993) recently reported that nor-binaltorphimine blocked cannabinoid-induced antinociception, which was not blocked by naloxone. Shukla and Lemaire (1993) reported that nor-binaltorphimine is a potent blocker of NMDA (*N*-methyl-D-aspartic acid) receptor-mediated activity. Nor-binaltorphimine also blocked dynorphin-A-induced non-opioid effects such as spinal cord injury, loss of tailflick reflex, and hind limb paralysis (Faden and Jacobs, 1984; Bakshi and Faden, 1990; Long et al., 1989; Bakshi et al., 1990). If ketorolac interacts with the κ-opioid receptor system in the production of antinociception, the primary site appears to be at the central nervous system level. The ketorolac-induced antinociceptive activity appears to be mediated through the release of endogenous κ-opioid receptor agonists to produce central antinociception. The exact nature of such interaction remains to be determined.

In conclusion, the results of this study have established a phenomenon of antinociception induced by i.c.v. administered ketorolac and identified the central nature of this response for the first time. Inhibition of *p*-phenylquinone-induced abdominal stretching by i.c.v. ketorolac was dose-dependent with a maximal effect at 30 μg. The antinociception produced by i.c.v. ketorolac was not blocked by the μ-opioid receptor antagonist, naloxone, or the δ-opioid receptor antagonist, ICI-174,864; however, it was significantly blocked by the κ-opioid receptor antagonist, nor-binaltorphimine. The present study demonstrates that the interaction of ketorolac with nor-binaltorphimine is an integral element in the mechanism of action of ketorolac at the central nervous system level.

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