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The Antinociceptive Activity of Intrathecally Administered Amiloride and Its Interactions With Morphine and Clonidine in Rats

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Abstract: **In this study, we aimed to evaluate the antinociceptive interaction between intrathecally administered amiloride and morphine or clonidine. Using rats chronically implanted with lumbar intrathecal catheters, we examined the ability of intrathecal amiloride, morphine, clonidine, and mixtures of amiloride-morphine and amiloride-clonidine to alter tail-flick latency. To characterize any interactions, isobolographic analysis was performed. The effects of pretreatment with intrathecally administered naloxone or yohimbine were tested. Intrathecal administration of amiloride (25–150 μg), morphine (.25–10 μg), or clonidine (.5–10 μg) alone produced significant dose-dependent antinociception in the tail-flick test. The median effective dose (ED_{50}) values for intrathecally administered amiloride, morphine, and clonidine were 120.5 μg, 5.0 μg, and 4.4 μg, respectively. Isobolographic analysis exhibited a synergistic interaction after coadministration of amiloride-morphine and amiloride-clonidine. Intrathecal pretreatment with naloxone (10 μg) completely blocked the antinociceptive effects of morphine and the amiloride-morphine mixture. Intrathecal pretreatment with yohimbine (20 μg) completely blocked the antinociceptive effect of clonidine and antagonized the effect of the amiloride-clonidine mixture. There was no motor dysfunction or significant change in blood pressure or heart rate after the intrathecal administration of amiloride, amiloride-morphine, and amiloride-clonidine. The synergistic effect observed after the coadministration of amiloride and morphine or clonidine suggests a functional interaction among calcium channels, μ-receptors and α_2-receptors at the spinal cord level of the nociceptive processing system.**

Perspective: ***Although intrathecal morphine and clonidine produces pronounced analgesia, antinociceptive doses of intrathecal morphine and clonidine produce several side effects, including hypotension, bradycardia, sedation, and tolerance. This article presents antinociceptive synergistic interaction between amiloride and morphine, amiloride, and clonidine on thermal nociceptive tests in the rat.***

Key words: *Analgesics, amiloride, opioid, α_2-adrenergic agonist, T-type calcium channel.*

T-type calcium channels enhance neuronal excitability by allowing Ca^{2+} entry when the membrane is near its resting potential,[19] which strengthens synaptic inputs and decreases the activation threshold required for action potential generation.[14] Thus, T-type channel blockade may be expected to lead to a decrease in overall neuronal excitability. Matthews and Dickenson[21] have suggested that blocking T-type channels may

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lead to decreased neurotransmitter release, thereby preventing depolarization. Amiloride is known to inhibit T-type calcium channels,[34] block the epithelial TTX-insensitive Na^+ channel,[25,28] and inhibit the Na^+-H^+ and Na^+-Ca^{2+} exchange system.[20] Due to its ability to interact with a number of ion transport systems, receptors, and enzymes, amiloride has been a useful drug for elucidating the molecular mechanisms involved in Na^+ translocation across cell membranes.[1] The amiloride-sensitive Na^+-channel/degenerin family constitutes a group of proteins that is thought to be involved in a wide variety of functions, such as sodium and pH homeostasis, transduction of mechanical stimuli, and even nociception.[10] A previous study provided unequivocal evidence that amiloride, at doses that do not cause side effects (motor dysfunction), produces systemic, spinal, and supraspinal antinociceptive and antihyperalgesic effects in chemical models of nociception (ie, acetic-acid-induced writhing, capsaicin- and formalin-induced licking, and glutamate-mediated hyperalgesia).[8] Another study that tested amiloride in a behavioral nociceptive assay was performed by Sluka et al[31] who reported dose-dependent amiloride inhibition of acid-induced mechanical hypersensitivity in male C57BL/6 mice. Dube et al[7] also demonstrated dose-dependent amiloride reversal of both the thermal and mechanical hypersensitivities produced by inflammation or skin incision, respectively, in male Sprague Dawley rats. Chanda and Mogil[4] have shown that 30 mg/kg of amiloride robustly blocks formalin-induced licking behavior throughout phase II in female mice but not in males.

Spinal opioid and α_2 receptor agonists have played important roles in the management of acute and chronic pain. However, the side effects associated with intrathecal or epidural opioid and α_2 receptor agonists are dose-dependent respiratory depression and hypotension, which are life threatening and limit the clinical use of these substances for pain management. Dogrul et al[6] showed that mibefradil, which has also been found to produce a potent and selective blockade of T-type voltage-dependent calcium channels (VDCCs) in several neuronal preparations,[22] potentiates the antinociceptive effects of morphine at the spinal level, suggesting that there is likely an interaction between μ receptor agonists and T-type VDCCs in the production of analgesia. If an intrathecal T-type calcium channel blocker amiloride can produce antinociception or potentiate the analgesic effects of opioids or α_2-receptor agonists, then their combinations may be extremely useful for pain management. This study examined the effects of amiloride on the antinociception of morphine or clonidine at the spinal cord level.

Methods

Animal Preparation and Surgical Procedure

With approval from the Gifu University Animal Care and Use Committee, a study was conducted on male Sprague Dawley rats weighing 250 to 350 g (n = 5–7/group). All the surgical procedures were performed with the rats under intraperitoneal midazolam (2 mg/kg) and ketamine (40 mg/kg) anesthesia. Using the method described by Zeng et al,[37] an intrathecal catheter (PE-10, 8.5 cm) was inserted into the lumbar subarachnoid space through an opening in the cisternal magna. After the surgery, the animals were allowed to recover for 1 week before drug administration. Rats kept awake when they were given intrathecal injection. Each animal was studied 1 or 2 times in an experimental series, with a 4-day interval between studies. After the last experimental period, the rats were sacrificed with an overdose of pentobarbital, and an injection of 1% methylene blue was given to confirm the position of the catheter and the likely spread of the injectate.

Nociception and Motor Function Tests

The nociceptive thresholds were assessed using the tail-flick (TF) test. In the tail-flick test, the response to a noxious somatic stimulus was measured by monitoring the latency to withdrawal from the heat source (a 50-W projection lamp bulb, KN-205E; Natsume, Tokyo, Japan) focused on the dorsal surface of the tail. The same portion of the tail was exposed to the stimulus in each test. The mean baseline TF latency was 3.5 secondds (3.3–3.8 sec). A cut-off time of 10.0 seconds was imposed to minimize tail skin damage during the experiment. TF latencies were determined 5, 10, 15, 20, 30, 40, 50, and 60 minutes after the intrathecal drug administration. The motor blockade was graded according to the scale proposed by Langerman et al[18] for rabbits, which we modified for rats as follows: 0 = free hind limb movement without limitations; 1 = limited or asymmetrical hind limb movement to support the body and walk; 2 = inability to move the hind limbs and respond to pain stimuli; and 3 = total hind limb paralysis.

Drugs and Injections

After the baseline TF latencies had been obtained, each animal received an intrathecal injection of amiloride (25, 50, 100, 150 μg), morphine (.25, .5, 1.0, 2.0, 5.0, 10.0 μg), or clonidine (.5, 1.0, 2.0, 5.0, 10.0 μg). Amiloride-morphine combinations and amiloride-clonidine combinations were administered in a fixed ratio for each drug (amiloride/morphine = 25:1 μg, amiloride/clonidine = 25:1 μg). Rats randomly received 1 of the following combinations of amiloride-morphine (50 μg:2 μg, 25 μg:1μg, 12.5 μg:.5 μg) or amiloride-clonidine (50 μg:2 μg, 25 μg:1 μg, 12.5 μg:.5 μg). A naloxone (10 μg) pretreatment was administered 10 minutes before the intrathecal administration of morphine (10 μg) or a mixture of amiloride (50 μg)-morphine (2 μg), and yohimbine (20 μg) was administered 10 minutes before the intrathecal administration of clonidine (10 μg) or a mixture of amiloride (50 μg)-clonidine (2 μg). Naloxone (10 μg) and yohimbine (20 μg) alone were tested. Physiological saline (20 μL or 40 μL) served as the vehicle control. All the drugs were administered in a total volume of 10 μL followed by 10 μL of physiological saline to flush out the contents of the catheter.