

Thioperamide-induced Antinociception is Mediated through Endogenous Opioid System in the Dentate Gyrus of Adult Rats

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Abstract

The present study investigated the effects of intra-dentate gyrus microinjection of naloxone (an opioid antagonist) and thioperamide (an antagonist of histamine H₃ receptors) in the formalin test in rats. Subcutaneous injection of formalin (50 µl, 2.5 %) in the ventral surface of right hind paw produced a biphasic pattern (first phase: 0-5 min and second phase: 15 - 60 min) of licking/biting and shaking of the injected paw. Intra-dentate gyrus microinjections of thioperamide (2 and 4 µg) significantly ($P < 0.05$) suppressed the pain responses. Microinjections of naloxone (1, 2 and 4 µg) alone into the dentate gyrus non-significantly increased the intensity of pain. Pretreatment with naloxone (4 µg) significantly ($P < 0.05$) reversed the antinociceptive effect of thioperamide (4 µg). The results indicated that at the level of the dentate gyrus, blockade of histamine H₃ receptors with thioperamide produced an analgesic effect. This thioperamide-induced antinociception may be mediated through the endogenous opioid system.

Key words: Dentate gyrus, Formalin-induced pain, Naloxone, Thioperamide, Rats

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Introduction

Some studies suggest that the dentate gyrus may involve in modulation of pain. Microinjections of acetylcholine and pilocarpine into the dentate gyrus have decreased the discharge frequency of pain-excited neurons, and increased the discharge frequency of pain-inhibited neurons in the sciatic nerve electrical stimulation model of nociception in rats.¹ Besides, lidocaine, a local anesthetic, produced analgesia in the formalin test of rats when microinjected into the dentate gyrus.²

The histamine H₃ receptors are widely distributed in the limbic system areas such as the hippocampus, the dentate gyrus and the amygdale.³ In the dentate gyrus, histamine H₃ receptors play important roles in modulation of excitatory synaptic transmission, information flow and memory consolidation.⁴⁻⁶ On the other hand, opioid receptors are expressed in the hippocampal formation (i.e., the hippocampus and the dentate gyrus), and are involved in mediation of hippocampal functions including adult neurogenesis, the action of gonadal hormones, development of neonatal transmitter system and pain.⁷⁻⁹

By intracerebroventricular route of administration of thioperamide, some researchers have suggested an important role of histamine H₃ receptors in modulation of pain.^{10,11} At the level of the brain, the opioidergic system, through mu (μ), delta (δ), and kappa (κ) receptors, exerts a major role in modulation of pain.¹²⁻¹⁴

The present study was aimed to investigate the implication of histamine H₃ receptor in pain perception by microinjection of a histamine H₃ receptor antagonist, thioperamide, into the dentate gyrus using formalin test in rats. In addition, to identify the mechanism that possibly mediating the effect of thioperamide on pain, we assessed the contribution of the endogenous analgesic opioid system using microinjection of

naloxone prior to thioperamide. Formalin test has been used frequently to study pain mechanisms in laboratory animals and according to these studies a biphasic pattern of pain-related behaviors was produced by subcutaneous injection of small amounts (20–100 μ l) of dilute solutions (0.1–10 %) of formalin into the various parts of the body.^{15,16} The first phase in turn may be attributed to a direct algogenic effect of formalin on the nociceptors and the second phase to release of local inflammatory mediators responsible for sensitization of primary and spinal sensory neurons and subsequent signal transduction into the brain.¹⁵⁻¹⁷

Materials and Methods

Animals. Healthy adult male Wistar rats, weighing 300–350 g were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum* in a laboratory with controlled ambient temperature (22 ± 0.5 °C) and under a 12 h light-dark cycle (lights on from 07:00 a.m.). Six rats were used in each experiment. Experiments were performed between 12:00 and 15:00 o'clock. All research and animal care procedures were approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Drugs. Drugs used in the present study included thioperamide maleate (Sigma-Aldrich) and naloxone dihydrochloride (Sigma-Aldrich). All drugs were dissolved in sterile normal saline 30 min before intra-dentate gyrus microinjection.

Surgical procedure. To deliver the drugs, two 23-gauge guide cannulas were bilaterally implanted in the dentate gyrus of the brain using a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The tip of cannulas was aimed at the following coordinates: 3.8 mm posterior to the

bregma, 2 mm left and right sides of the midline and 3.6 mm below the top of the skull.¹⁸ The cannulas were then fixed to the skull using three screws and dental acrylic (Acropars, Tehran, Iran). At least 14 days were allowed for recovery from the surgery.

Intra-dentate gyrus microinjection. Intra-dentate gyrus microinjections of normal saline (control), thioperamide (1, 2 and 4 µg) and naloxone (1, 2 and 4 µg) were performed using a 5 µl Hamilton syringe. The volume of 0.5 µl of each solution was injected slowly into each dentate gyrus over a period of 1 min. Intra-dentate gyrus microinjections of thioperamide and naloxone were performed 10 and 5 min before intraplantar injection of formalin, respectively.

Formalin test. The formalin test was applied as follows: Fifty microlitres of 2.5% formalin was injected subcutaneously into the ventral surface of right hind paw using a 29-gauge injection needle.¹⁹⁻²² Nociceptive behaviors including licking/biting and shaking of the injected paw were observed every 5 min for 1 h. In the present study, data collected between 0-5 min after formalin injection represented the first (early) phase and data collected between 15-60 min after injection of formalin represented the second (late) phase of pain.^{15, 20, 23}

Cannula verification. At the end of each experiment, 0.25 µl methylene blue was injected into the each dentate gyrus. The animals were killed with the high dose ether, and perfused intracardially with physiological saline followed by 10 % formalin solution. Brains were removed and placed in the formalin (10%) solution. At least 3 days later, the brains were sectioned coronally (50-100 µm), and viewed under a loupe to localize the injection site (Fig. 1).¹⁸

Statistical analysis. To evaluate significance differences among intra-dentate gyrus treated groups, one-way analysis of variance (ANOVA) and Duncan's test were applied. In figures, all

values are expressed as the mean ± SEM. A value of $P < 0.05$ was considered statistically significant.

Results

The placements of the tip of the cannulas in the dentate gyrus of rats are shown in Fig. 1. The rat brain section was modified from the atlas of Paxinos and Watson¹⁸ (Fig. 1A).

The location of the cannula tip placements in the dentate gyrus was confirmed with intra-dentate gyrus injection of methylene blue (Fig. 1B).

Fig. 2 shows the effects of intra-dentate gyrus microinjection of thioperamide on the formalin-induced nociceptive behaviors. Intra-dentate gyrus microinjection of thioperamide at dose of 2 and 4 µg, but not at a dose of 1 µg, significantly decreased the duration of licking/biting of the formalin-injected paw in the first ($F_{(3,20)} = 3.319, P < 0.05$) and second ($F_{(3,20)} = 9.223, P < 0.05$) phases (Fig. 2A).

The number of shakes of the formalin-injected paw was significantly decreased in the first ($F_{(3,20)} = 2.942, P < 0.05$) and second ($F_{(3,20)} = 6.483, P < 0.05$) phases when 2 and 4 µg, but not 1 µg, of thioperamide were microinjected into the dentate gyrus (Fig. 2B).

Fig. 3 shows the effects of intra-dentate gyrus microinjection of naloxone on the formalin-induced nociceptive behaviors. Microinjections of naloxone (1, 2 and 4 µg) non-significantly increased the first ($F_{(3,20)} = 0.882, P > 0.05$) and second ($F_{(3,20)} = 0.642, P > 0.05$) phases of formalin-induced licking/biting of the injected paw (Fig. 3A).

Naloxone at doses of 1, 2 and 4 µg also non-significantly increased the number of shakes in the first ($F_{(3,20)} = 0.882, P > 0.05$) and second ($F_{(3,20)} = 0.642, P > 0.05$) phases (Fig. 3B).

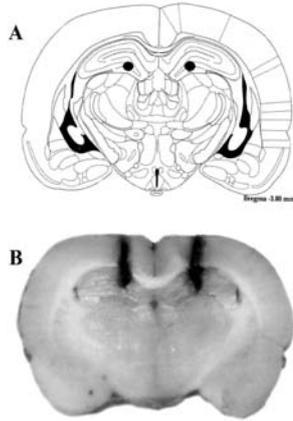


Fig 1. Verified section was taken from the atlas of Paxinos and Watson¹⁸ (A). The black circles represent the cannulas tip placements. Location of the injection cannula tips in the dentate gyrus of all rats included in the data analysis (B). DG: dentate gyrus.¹⁸

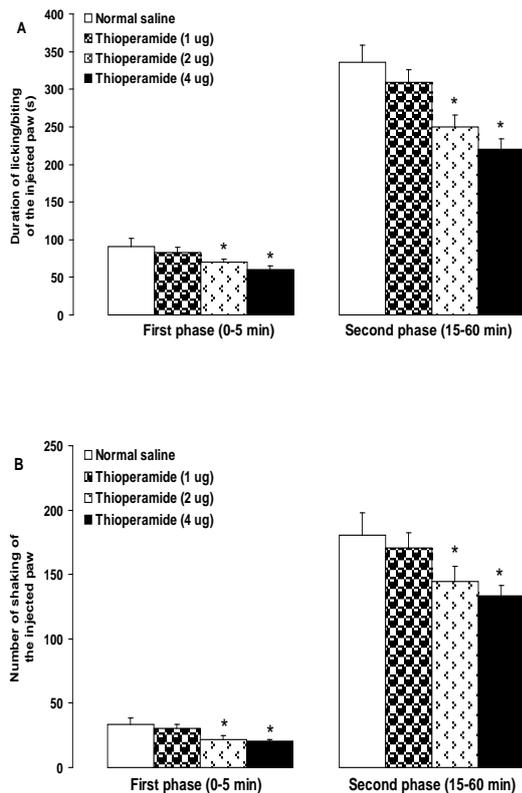


Fig 2. Effects of intra-dentate gyrus microinjection of thioperamide on the formalin-induced licking/biting (A) and shaking (B) of the hind paw in rats. * $P < 0.05$ as compared with normal saline (control) group, $n = 6$ rats in each group.

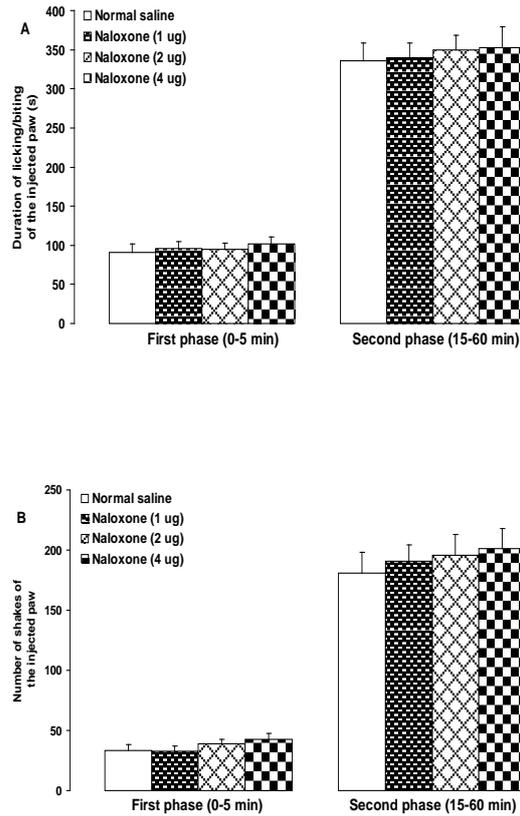


Fig 3. Effects of intra-dentate gyrus microinjection of naloxone on the formalin-induced licking/biting (A) and shaking (B) of the hind paw in rats. $n = 6$ rats in each group.

Fig. 4 shows the effects of intra-dentate gyrus microinjection of naloxone on the thioperamide-induced antinociception in the formalin test. Microinjection of naloxone ($4 \mu\text{g}$) prior to thioperamide ($4 \mu\text{g}$) significantly reversed the suppressive effect of thioperamide on the licking/biting of the formalin-injected paw in the first ($F_{(3,20)} = 4.522, P < 0.05$) and second ($F_{(3,20)} = 7.461, P < 0.05$) phases (Fig. 4A).

The suppressive effects of intra-dentate gyrus microinjected thioperamide ($4 \mu\text{g}$) on shaking behavior induced by formalin were significantly inhibited by prior microinjection of naloxone ($4 \mu\text{g}$) in the first ($F_{(3,20)} = 3.396, P < 0.05$) and second ($F_{(3,20)} = 5.531, P < 0.05$) phases (Fig. 4B).

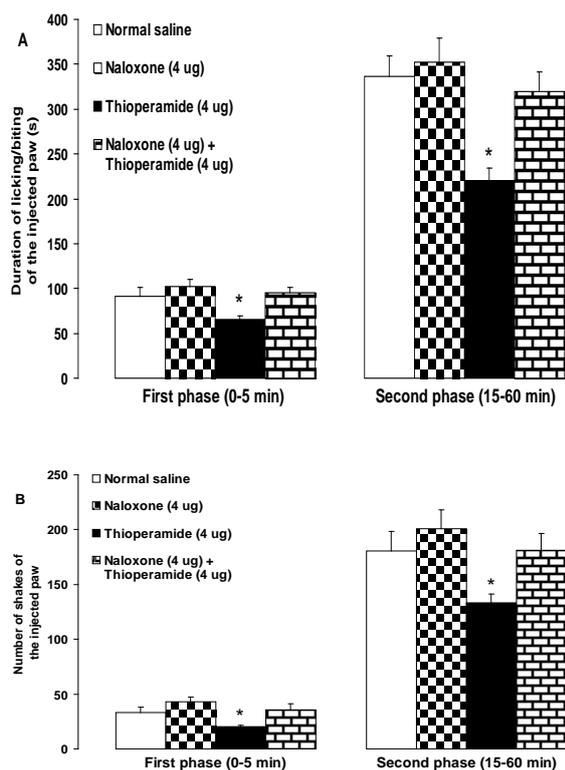


Fig 4. Effects of intra-dentate gyrus microinjection pretreatment with naloxone on the thioperamide-induced antinociception in the formalin-induced licking/biting (A) and shaking (B) of the hind paw in rats. * $P < 0.05$ as compared with other groups, $n = 6$ rats in each group.

Discussion

In this study, intra-dentate gyrus microinjection of thioperamide produced an antinociceptive effect in the formalin-induced pain. Histamine H_3 receptors act as pre-synaptic auto-receptors as well as post-synaptic hetero-receptors.^{24,25} Activation of histamine H_3 auto-receptors by R- α -methylhistamine, immpip and imetit (histamine H_3 receptor agonists) results in the inhibition of histamine synthesis and release from histaminergic neurons.^{26,27} On the other hand, blockade of histamine H_3 auto-receptors with histamine H_3 receptor antagonists including clobenpropit, ciproxifan and thioperamide can increase the release of histamine from histaminergic endings.^{26,27} Although the majority of histamine H_3 receptors are located in brain,³ histamine H_3 receptor mRNA is also found in various non-brain tissues including skin, stomach,

intestines, brown adipose tissue, dorsal root ganglion and spinal cord.^{28,29} The evidences taken from acute and chronic pain tests have suggested peripheral, spinal and supraspinal roles for histamine H_3 receptor in modulation of pain. Local activation of histamine H_3 receptor with sub-plantar injection of R- α -methylhistamine potentiated the suppressive effect of fentanyl in thermal hyperalgesia induced by sub-plantar injection of Complete Freund's Adjuvant in mice.³⁰ In addition, administration of immpip and thioperamide to the cholestatic rats increased and decreased tail-flick latencies, respectively.³¹ It has been reported that activation of histamine H_3 receptors by immpip on peripheral and spinal sites of pain pathways attenuates formalin-induced swelling and flinching.³² Using histamine H_3 receptor gene knockout mice, Mobarakeh *et al.* (2009)³³ reported an inhibitory effect of histamine through its H_3 receptors on the morphine-induced antinociception in hot-plate, tail-flick, paw-withdrawal and formalin tests of nociception at the spinal level. At the supraspinal level, intracerebroventricular injection of thioperamide increased the nociceptive threshold in a rat model of neuropathic pain.¹¹ In contrast, intracerebroventricular injection of thioperamide did not exert any analgesic activities in the tail flick and hot plate tests of nociception in rats.³⁴ However, Malmberg-Aiello *et al.* (1994).¹⁰ reported analgesic and hyperalgesic effects after intracerebroventricular injection of thioperamide and R- α -methylhistamine, respectively, in rats and mice.

In this study, naloxone alone non-significantly increased the intensity of pain, and reversed thioperamide-induced antinociception. This means that the endogenous opioid analgesic system may be involved in thioperamide-induced antinociception. Naloxone, as a competitive antagonist of mu-, kappa- and sigma-opioid receptors with higher affinity for the mu-opioid receptors,³⁵ has been

frequently used to explore the role of endogenous opioid analgesic system in pain modulation.^{9, 36-38} Several interactions exist between histamine H₃ and opioid receptors in modulation of pain. Local activation of histamine H₃ receptor with sub-plantar injection of R- α -methylhistamine potentiated the suppressive effect of fentanyl in thermal hyperalgesia induced by sub-plantar injection of Complete Freund's Adjuvant in mice.³⁰ Subcutaneous injection of naloxone attenuated immepip-induced antinociception in cholestatic rats.³¹ Using the histamine H₃ receptors gene knockout mice, Mobarakeh *et al.* (2009)³³ reported that histamine, through its H₃ receptors, exerted inhibitory effects on the morphine-induced antinociception at the spinal level. In addition, microinjection of naloxone into periaqueductal gray reversed the antinociceptive effect induced by microinjection of histamine into the same site.³⁹

In the present study, intra-dentate gyrus microinjection of thioperamide, without any significant effect on interphase (data not shown) suppressed the first and the second phases of formalin-induced licking/biting and shaking responses. The first phase of formalin-induced pain may be attributed to a direct algogenic effect of formalin on the nociceptors and the second phase to release of local inflammatory mediators responsible for sensitization of primary and spinal sensory neurons and subsequent signal transduction into the brain.¹⁵⁻¹⁷ The interphase of formalin test is under active inhibition of spinal cord mechanisms.⁴⁰ The pain-related behaviors can be associated with distinct brain structures including spinal, brainstem and cerebrally mediated responses to nociceptive stimulation.⁴¹ Regarding the formalin-induced nociceptive behaviors including licking/biting and shaking of the injected paw, it was found that these behaviors are mediated by supraspinal structures.⁴²

In conclusion, the results of the present study indicated that blockade of brain histamine H₃ receptor by thioperamide in the dentate gyrus of the brain could produce an antinociceptive effect. The endogenous opioid analgesic system may be involved in thioperamide-induced antinociception.

Acknowledgments

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