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Original research article

Effects of microinjection of histamine into the anterior cingulate cortex on pain-related behaviors induced by formalin in rats

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ABSTRACT

Background: The present study was aimed to investigate the effects of microinjection of histamine and its H_1, H_2 and H_3 receptor antagonists, mepyramine, ranitidine and thioperamide, respectively, into the anterior cingulate cortex (ACC) on pain-related behaviors induced by formalin in rats.
Methods: Two stainless steel guide canulas were bilaterally implanted into the ACC of anaesthetized rats. For induction of pain, intraplantar (*ipl*) injection of a 2.5% formalin solution was performed. The duration of paw licking/biting and the number of paw flinching were recorded in 5 min blocks for 60 min. Locomotor activity was assessed using an open-field test.
Results: Formalin produced a marked biphasic pattern of pain. Histamine reduced the second phases of paw licking/biting and flinching. Mepyramine (2 μg/side) prevented the suppressive effect of histamine (1 μg/side) on second phase of pain, but at a dose of 8 μg/side it did not inhibit the suppressive effects of 4 μg/side of histamine. Ranitidine at doses of 2 and 8 μg/side prevented histamine (1 and 4 μg/side)-induced antinociception. Thioperamide not only suppressed the second phases of pain, but also increased the suppressive effect of histamine. Naloxone prevented suppressive effects of histamine and thioperamide on pain. Mepyramine (8 μg/side) suppressed locomotor activity.
Conclusion: The results of the present study showed pain suppressing effects for histamine. Histamine H_2 and H_3, and to a lesser extent, H_1 receptors might be involved in histamine-induced antinociception. Opioid receptors might be involved in suppressive effects of histamine and thioperamide.

Introduction

Histamine, as a biogenic amine, found throughout the body in neuronal and non-neuronal sources, can modify pain transmission in the peripheral organs, spinal cord and brain by action at its H_1, H_2, H_3 and H_4 receptors [1–3]. At the supraspinal level, the involvement of histamine H_1, H_2 and H_3 receptors have been reported in the antinociceptive effects induced by microinjection of histamine into the brain regions such as periaqueductal gray (PAG), raphe nucleus (RN), locus coeruleus (LC), dorsal hippocampus (DH), dentate gyrus (DG) and primary somatosensory cortex (PSC) [4–8]. By using of naloxone (an opioid receptors antagonist), the involvement of opioid receptors in centrally administered histamine-induced antinociception have been reported [8,9].

The anterior cingulate cortex (ACC) plays a key role in performance monitoring, value encoding, decision making, emotion, learning, motivation and pain perception [10–13]. Electrophysiological recordings from both human and animals demonstrate that neurons within the ACC respond to noxious stimuli and are activated during pain anticipation or pain avoidance behavior [14,15]. A variety of neurotransmitters and neuropeptides including glutamate, dopamine, gamma-amino butyric acid (GABA), opioids and cholecystokinin (CCK) are involved in the ACC processing of pain [12,13,16].

The present study was aimed to investigation of the effects of microinjection of histamine and it is H_1, H_2 and H_3 receptor antagonists into the ACC on pain related-behaviors induced by formalin. In addition, we assessed the contribution of the endogenous analgesic opioid system by microinjection of naloxone. We also

Abbreviations: ACC, anterior cingulate cortex; TMN, tuberomammillary nucleus; DH, dorsal hippocampus; DG, dentate gyrus; PSC, primary somatosensory cortex; PAG, periaqueductal gray; RN, raphe nucleus; LC, locus coeruleus; CABA, gama-amino butyric acid; CCK, cholecystokinin; *icv*, intracerebroventricular; *sc*, subcutaneous; *ip*, intraperitoneal; *ipl*, intraplantar.

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examined the effects of histaminergic agents on locomotor activity using an open-field test.

Materials and methods

Animals

Male Wistar rats (280–320 g) were obtained from Rat House of Laboratory of Physiology of Faculty of Veterinary Medicine of Urmia University and maintained in groups of six per cage in a light-dark cycle (light on at 07:00 h) at a controlled ambient temperature (22 ± 0.5 °C) with *ad libitum* food and water. 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 histamine dihydrochloride, mepyramine maleate, ranitidine hydrochloride, thioperamide maleate and naloxone hydrochloride. The drugs were purchased from Sigma–Aldrich Co., St. Louis, MO, USA. All drugs were dissolved in sterile normal saline 30 min prior to intra-anterior cingulate cortex (intra–ACC) microinjection.

Surgical procedure

Each rat was anaesthetized with an intraperitoneal (*ip*) injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), and then placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The scalp was incised, and the skull was leveled off around the bregma. Two 24 gauge, 10 mm stainless steel guide cannulas were bilaterally implanted 1 mm over the right and left ACC according to the following coordinates: 2–2.4 mm anterior to the bregma, 0.6 mm left and right sides of the midline and 1.2–1.4 mm below the top of the skull [17]. The cannulas were then fixed to the skull using three screws and dental acrylic (Acropars, Tehran, Iran). A 10 mm stylet was inserted to each cannula to keep them patent prior to microinjection. All animals were allowed 10 days to recover from surgery.

Intra-ACC microinjection

A 30-gauge, 11 mm injection needle attached to a 30 cm polyethylene tube fitted to a 1 μl Hamilton syringe was used for intra-ACC microinjections. The volume of the drug solution to be injected into each ACC was 0.25 μl and the injection was slowly made over a period of 60 s. The injection needle was left in place for a further 60 s after the completion of the injection to facilitate the diffusion of the drug. Intra-ACC microinjections of normal saline (0.25 μl/side, control), histamine (0.063, 0.25, 1 and 4 μg/side), mepyramine, ranitidine and thioperamide at the same doses of 2 and 8 μg/side alone and before 1 and 4 μg/side of histamine and naloxone (4 μg/side) alone and before 4 μg/side of histamine and 8 μg/side thioperamide were performed. Microinjections of mepyramine, ranitidine and thioperamide were performed six min and histamine was done three min before locomotor and or pain recording. In the case of naloxone, it was microinjected 3 min before histamine and thioperamide. The drug doses and treatment schedule used here were according to our previous studies [5–9].

Formalin-induced pain test

The formalin test was applied as follows. Fifty μl of 2.5% formalin was injected *sc* into the ventral surface of right hind paw using a 30-gauge injection needle. Formalin was injected one and two times in each rat over a 1 week interval to assure disappearance of local inflammation [18]. The nociceptive behaviors including licking/biting durations and the number of paw flinching of the injected paw were recorded in 5-min blocks for 60 min. We recorded licking/biting and flinching to clarify supraspinally- and spinally-mediated mechanisms of histaminergic system on pain. It is well known that licking/biting is a supra-spinal mediating behavior and paw flinching is a spinal controlling response [7,8,19,20]. Flinching, as a limb flexion response, is characterized as a rapid and brief withdrawal or flexion of the injected paw [20,21]. The frequency, duration and level of formalin-induced pain behaviors depend on the specific concentration used and the site of injection [22]. In the present study, data collected between 0 and 5 min and between 15 and 60 min after formalin injection represented the first (early) and the second (late) phases, respectively [8,19–22].

Locomotor activity

Ten days after the end of pain study, locomotor activity was assessed in an open-field test as described previously [23]. The apparatus consisted of a wooden box measuring 120 cm × 120 cm × 50 cm. The floor of the arena was divided into 16 equal squares. To monitor the activity, animals were removed from the home cage and placed directly into one corner of the open field apparatus. The number of squares crossed with all paws (crossings) and the number of rearing were counted in a 5-min session.

Cannula verification

At the end of each experiment, anesthetized rats were intracardially perfused with physiological saline followed by 10% formalin solution. The brains were removed and placed in a formalin solution (10%). One week later, thin coronal sections (4–5 μm) were provided and stained with Hematoxylin and Eosin. The sections were viewed under a light microscope to localize the injection site according to the atlas of [17]. The results obtained from rats with guide cannulas outside the ACC were eliminated from the data analysis.

Statistical analysis

The results were analyzed using Graph Pad Prism software V. 5. Data were analyzed using one-way and factorial ANOVA followed by Tukey's test. In figures, all values are expressed as the mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

Cannulas tip verification

The placements of the tip of the cannulas in the ACC of rats are shown in Fig. 1. The locations of the cannulas tip placements in the ACC were confirmed in the ACC sections (Fig. 1A). The rat brain section (Fig. 1B) was modified with permission from the atlas of Paxinos and Watson [17].

Nociceptive behaviors

The *ipl* injection of normal saline produced negligible licking/biting (Fig. 2A) and flinching (Fig. 2B), whereas formalin produced significant ($p < 0.05$) licking/biting (Fig. 2A) and flinching (Fig. 2B) at the first and 4th–12th 5 min blocks.