Interaction of histamine and calcitonin gene-related peptide in the formalin-induced pain perception in rats

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ABSTRACT

Histamine and calcitonin gene-related peptide (CGRP) contribute to the pain perception. The aim of the present study is to clarify the interaction of histamine and CGRP in the perception of inflammatory pain. The effects of a histamine H1 receptor antagonist (pyrilamine, i.p.), an H2 receptor antagonist (ranitidine, i.p.) and a CGRP antagonist (CGRP 8-37, i.t.) on the formalin-induced pain was studied in rats. Pyrilamine and ranitidine produced a dose-dependent antinociceptive response in the first and the second phases of the formalin test. A single administration of pyrilamine (1 mg/kg, i.p.), ranitidine (10 mg/kg, i.p.) or CGRP 8-37 (10 μg/μL, i.t.) had no significant effects on the pain perception in the second phase. A combination of CGRP 8-37 and pyrilamine or ranitidine at these sub-effective doses, however, showed nociceptive response in the second phase. Moreover, a histamine (i.t.)-induced hyperalgesia was completely prevented by treatment with GGRP 8-37 at this dose. Our findings have raised the possibility that the CGRP system has interaction with histamine in the perception of inflammatory pain.

Pain perception is modulated in the spinal cord by many bioactive substances, such as histamine (38, 39), substance P and calcitonin gene-related peptide (CGRP) (12, 28). Histamine is mostly derived from two cell types, neurons and mast cells. The cell bodies of histaminergic neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus (15). The descending histaminergic neurons terminate at the periaqueductal gray and the dorsal horn of the spinal cord (30, 31, 43), which is an important site for nociceptive transmission (2). All three histamine H1, H2 and H3 receptors (16) are found in the dorsal horn (26, 33, 42) and play important roles in the pain perception (22–25).

CGRP is a 37-amino-acid neuropeptide widely distributed in the peripheral and central nervous systems including the dorsal root ganglion (DRG) and its primary afferent terminals in the spinal cord (1, 37). The actions mediated by CGRP type 1 receptor, which is considered to consist of calcitonin receptor-like receptor and receptor activity modifying protein-1 (21), is antagonized by CGRP 8-37 (8). CGRP and its receptors are involved in many stages of pain neurotransmission, especially in the spinal cord (12, 28). As a neurotransmitter, CGRP itself had no obvious effect in pain modulation, but potentiated the excitatory effect induced by substance P or other nociceptive stimuli in the dorsal horn of the spinal cord (3). CGRP stimulated histamine release from mast cells and potentiated histamine ac-
Before the test, the animals were

First, dose response effects of H1 antagonist (CGRP 8-37), and their combinations on the pain perception induced by formalin in rats.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats (200–220 g) (Pasteur Institute of Iran, Tehran, Iran) were used in the experiments. Animals (four per cage) were housed in a controlled colony room (temperature 21 ± 3°C), which was maintained under a 12:12 h light/dark cycle with water and food provided *ad libitum*. Six to eight animals were used in each experiment. Experiments were approved by the ethics committee of Pasteur Institute of Iran (Document No. 60657; Mar. 2008).

**Drugs.** The following drugs were used: Ranitidine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), pyrilamine maleate salt (Sigma Chemical Co.), human calcitonin gene-related peptide 8-37 (CGRP 8-37) (Sigma Chemical Co.), and histamine dihydrochloride (Sigma Chemical Co.). Ranitidine hydrochloride and pyrilamine maleate salt were resolved in sterile 0.9% NaCl solution. CGRP 8-37 and histamine were dissolved in sterile artificial cerebrospinal fluid (CSF) containing 126.6 mM NaCl, 2.5 mM KCl, 2.0 mM MgCl₂, and 1.3 mM CaCl₂.

**Formalin test.** Before the test, the animals were

placed individually in Plexiglas testing chambers (30 × 20 × 20) and allowed to acclimate for at least 35–40 min. A mirror was placed at a 45° angle below the observation box to allow the experimenter to have an unobstructed view of the injected paw. After the drug injection, 100 µL of 2.5% formalin was injected subcutaneously (s.c.) into the plantar surface of the left hind paw using a microsyringe with a 29-gauge needle. Each rat was immediately returned to the observation box, and the recording of early response (phase 1) started immediately and lasted for 10 min (0–10 min). The recording of the late response (phase 2) started 10 min after the formalin injection and lasted for 20 min (10–30 min). In both phases, only licking or biting of the injected hindpaw was defined as a nociceptive response and the total time of the response was measured with a hand-held stopwatch during the test period (22).

**Drug treatment.** First, dose response effects of H1 receptor antagonist (pyrilamine) and H2 receptor antagonist (ranitidine) on the pain perception were studied in the formalin test. Rats were injected intraperitoneally (i.p.) either with saline (2 mL/kg), pyrilamine (1, 2.5, 5 and 10 mg/kg) or ranitidine (10, 20 and 40 mg/kg) 20 min before the formalin injection. Antinociceptive effects during 0–10 min (phase 1) and 10–30 min (phase 2) after the formalin injection were recorded.

CGRP 8-37 was injected intrathecally (i.t.) in a volume of 10 µL. The intrathecal injection procedure was adapted from the method of Hylden and Wilcox (17). A 28-gauge stainless-steel needle attached to a 50-µL Hamilton microsyringe was inserted between lumbar 5 and lumbar 6 in unanesthetized rats. A combination of sub-effective doses of CGRP 8-37 (10 µg/10 µL) and pyrilamine (1 mg/kg) or ranitidine (10 mg/kg) was studied in the formalin test. Rats were administered with either CGRP 8-37 (5 and 10 µg/10 µL, i.t.), CGRP 8-37 plus pyrilamine (1 mg/kg, i.p.), or CGRP 8-37 plus ranitidine (10 mg/kg). CGRP 8-37 was injected i.t. 10 min before the formalin injection. Pyrilamine or ranitidine was administered i.p. 20 min prior to the formalin injection. Antinociceptive effects during the phases 1 and 2 after the formalin injection were recorded.

Finally, effects of a subeffective dose of CGRP 8-37 (10 µg/10 µL, i.t.) on histamine-induced hyperalgesia were studied in the formalin test. Rats were administered i.t. with artificial CSF (control) (10 µL), histamine (1 nmol/10 µL), CGRP 8-37 (10 µg/10 µL), or CGRP 8-37 plus histamine (a total volume of 10 µL containing 10 µg CGRP 8-37 and
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Statistical analysis. Data are shown as means ± S.E.M. The statistical significance of differences between groups was obtained by means of one-way analysis of variance (ANOVA) followed by Newman-Keuls test. Differences with \( P < 0.05 \) between experimental groups at each point were considered statistically significant.

RESULTS

Effects of an H1 receptor antagonist (pyrilamine) and an H2 receptor antagonist (ranitidine) on the pain perception in the formalin test

Intraperitoneal injection of pyrilamine (Fig. 1A and 1B) or ranitidine (Fig. 1C and 1D) induced an antinociception in the phases 1 and 2 of the formalin test in a dose-dependent manner (Fig. 1). Pyrilamine decreased the licking time significantly at a dose of 2.5–10 mg/kg in the phases 1 and 2 of the formalin test (Fig. 1A and 1B). Ranitidine decreased the licking time significantly at a dose of 10 and 40 mg/kg in the phase 1, and at a dose of 20 and 40 mg/kg in the phase 2 (Fig. 1C and 1D).

Effects of a CGRP antagonist on the pain perception in the presence or absence of pyrilamine or ranitidine in the formalin test

In a preliminary study, we confirmed that intrathecal injection of CGRP 8-37 induced an antinociception in a dose-dependent manner. CGRP 8-37 decreased the licking time significantly at a dose of 20 μg/10 μL, but not at 10 μg/10 μL, in the phases 1 and 2 of the test (data not shown). A single administration of pyrilamine (1 mg/kg, i.p.) or ranitidine (10 mg/kg, i.p.) had small or no significant effects on the pain perception in both phases of the formalin test, as shown in Fig. 1. Effects of a combination of CGRP 8-37 and pyrilamine or ranitidine at these sub-effective doses on the pain perception were then studied in the formalin test.

Fig. 2 shows antinociceptive effects of intrathecal administration of CGRP 8-37 in the presence or absence of pyrilamine (1 mg/kg, i.p.) in the formalin test. In the phase 1 of the formalin test (Fig. 2A, upper column), a combination of CGRP 8-37 (5 and 10 μg/10 μL) and pyrilamine did not show any interaction. In the phase 2 (Fig. 2B, lower column), however, the licking time measured after pretreatment with CGRP 8-37 (10 μg/10 μL) and pyrilamine was significantly shorter than that after pretreatment with CGRP 8-37 alone, suggesting that CGRP 8-37 and pyrilamine exert synergistic antinociceptive effects on the behavior induced by formalin in the phase 2.

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Fig. 3 shows antinociceptive effects of intrathecal administration of CGRP 8-37 in the presence or absence of ranitidine (10 mg/kg, i.p.) in the formalin test. In the phase 1 of the formalin test (Fig. 3A, upper column), a combination of CGRP 8-37 (5 and 10 μg/10 μL) and ranitidine did not show any interaction. In the phase 2 again (Fig. 3B, lower column), the licking time measured after pretreatment with CGRP 8-37 and ranitidine was significantly shorter than that after pretreatment with CGRP 8-37 alone, suggesting that CGRP 8-37 and ranitidine also exert synergistic antinociceptive effects on the behavior induced by formalin in the phase 2.
**DISCUSSION**

In the present study, we at first confirmed anti-nociceptive actions of the intraperitoneally-administered H1 receptor antagonist (pyrilamine) and H2 receptor antagonist (ranitidine), and the intrathecally-administered CGRP antagonist (CGRP 8-37). It was reported that intrathecal administration of CGRP 8-37 induced very strong inhibition on the pain-related responses to thermal and mechanical noxious stimulations in rats, indicating that CGRP 8-37 induced strong analgesic effects in the spinal cord (45–47). The present study has shown that the CGRP antagonist is also effective against inflammatory pain in-
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Produced by formalin. We have shown for the first time that a combination of sub-effective doses of CGRP 8-37 (10 μg/10 μL) and pyrilamine (1 mg/kg), or ranitidine (10 mg/kg) showed nociceptive response in the phase 2 of the formalin test. Moreover, histamine-induced hyperalgesia was completely prevented by treatment with a sub-effective dose of GGRP 8-37 (10 μg/10 μL) in both the phases 1 and 2. These findings suggested that histamine and CGRP acted synergistically in the pain perception of the formalin test, particularly in the phase 2.

The formalin test is a model of injury-produced pain (11, 41), which produces a distinct biphasic response; the early response (phase 1) recorded during the 5 min after formalin injection, and the late response (phase 2) recorded 20–50 min after formalin injection. The actions of analgesics are different between the first phase and the second phase. The first phase seems to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the second phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (41). In response to experimentally induced inflammation, the biosynthesis of CGRP increased in the DRG (13), and release of CGRP-like immunoreactive substance increased in the spinal cord (4, 5, 35). Thus, there is accumulating evidence which suggests that CGRP is related to the pathophysiology of inflammatory pain.

Furthermore, both histamine and CGRP stimulate the release of each other and act synergistically in the process of inflammation. CGRP stimulated the release of histamine from mast cells in the dura mater (29, 36). Mast cells in the rat dura are located close to sensory nerve fibers (34), and histamine and CGRP have frequently been implicated in the pathogenesis of migraines and other primary headaches via the vasodilator action. Bileviciute et al. (6) reported that an injection of histamine induced the release of CGRP together with substance P and neurokinin A in the rat knee joint or in the cerebrospinal fluid. The synergistic action of histamine and CGRP was reported in the edema formation via the increase of blood flow in the skin. Intradermal injection of CGRP increased blood flow in rabbit skin, but had no direct effect on edema formation in the skin (7). However, CGRP produced significant potentiation of edema formation when co-injected with histamine, a potent mediator of increased vascular permeability (7). The present study has suggested that histamine and CGRP act synergistically also in the nociception of inflammatory pain. These findings raised a possibility that a combination of a histamine antagonist and a CGRP antagonist may be a novel therapeutic strategy against inflammatory pain.

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REFERENCES


