Role of the thalamic parafascicular nucleus cholinergic system in the modulation of acute corneal nociception in rats

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Abstract

The present study investigated the effects of microinjections of acetylcholine (a cholinergic agonist), physostigmine (a cholinesterase inhibitor), atropine (an antagonist of muscarinic cholinergic receptors) and hexamethonium (an antagonist of nicotinic cholinergic receptors) into the parafascicular nucleus of thalamus on the acute corneal nociception in rats. Acute corneal nociception was induced by putting a drop of 5 M NaCl solution onto the corneal surface of the eye and the number of eye wipes was counted during the first 30s. Both acetylcholine and physostigmine at the same doses of 0.5, 1 and 2 μg significantly (\(P < 0.05\)) reduced the number of eye wipes. The intensity of corneal nociception was not changed when atropine and hexamethonium were used alone. Atropine (4 μg), but not hexamethonium (4 μg) significantly (\(P < 0.05\)) prevented acetylcholine (2 μg)- and physostigmine (2 μg)-induced antinociceptive effects. The results indicated that at the level of the parafascicular nucleus of thalamus, the muscarinic cholinergic receptors might be involved in the antinociceptive effects of acetylcholine and physostigmine.

Introduction

Parafascicular nucleus, the main intralaminar nucleus of the thalamus, is involved in modulating many functions of brain such as learning and memory, processing of motor information and control of epileptic seizures.1-3 Several studies suggest important roles for parafascicular nucleus in mediating pain and analgesia. Noxious peripheral stimulation increases c-fos-like protein expression in parafascicular nucleus.4 Microinjection of a 5-hydroxytryptamine (5-HT) \(1_A/7\) receptor agonist, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), into parafascicular nucleus produced antinociception in rats.5 Moreover, intra-parafascicular nucleus administration of morphine increased the vocalization threshold induced by noxious tail-shock, and methylnaloxonium, a mu-opioid receptor antagonist, reversed the antinociceptive effect of morphine.6 The roles of acetylcholine, cholinergic agonists and cholinesterase inhibitors, collectively termed cholinomimetics, have been established in the modulation of pain and analgesia.7 The antinociceptive effects induced by microinjection of acetylcholine, pilocarpine and charbacol in the brain nuclei and regions including central amygdale, hippocampus and dentate gyrus have been reported.8-10 Naguib and Yaksh11 reported the involvement of muscarinic, but not nicotinic cholinergic receptors in the antinociception induced by intrathecal injections of neostigmine and edrophonium in the radiant heat-evoked hind paw withdrawal in rats. Thus far, only one study has investigated the involvement of brain muscarinic receptors in the physostigmine-induced antinociception in the formalin test in rats.12
The present study was aimed to investigate the effects of microinjections of acetylcholine (a cholinergic agonist), physostigmine (a cholinesterase inhibitor), atropine (an antagonist of muscarinic cholinergic receptors) and hexamethonium (an antagonist of nicotinic cholinergic receptors) on the acute corneal pain in rats. The eye wiping induced by local application of 5 M NaCl solution and capsaicin onto the corneal surface has been introduced as a sensitive animal model for the study of acute corneal pain.\textsuperscript{13-16}

Materials and Methods

Animals. Healthy adult male Wistar rats, weighing 280-320 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 h). Six rats were used in each experiment. Experiments were performed between 13:00 h and 16:00 h. 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.\textsuperscript{17,18}

Drugs. Drugs used in the present study included acetylcholine hydrobromide (Sigma-Aldrich), physostigmine (Sigma-Aldrich), atropine sulphate (Sigma–Aldrich) and hexamethonium chloride (Sigma–Aldrich). All drugs were dissolved in sterile normal saline 30 min before intra-parafascicular nucleus microinjection.

Surgical procedure. To deliver the compounds to be tested, two 24-gauge guide cannulas were bilaterally implanted in the parafascicular nucleus of thalamus of the brain using a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The tip of cannulas was aimed at the following coordinates: 4.3 mm posterior to the bregma, 1.4 mm left and right sides of the midline and 5.4-6.2 mm below the top of the skull.\textsuperscript{19} 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-parafascicular nucleus microinjection. Intra-parafascicular nucleus microinjections of normal saline (control), acetylcholine and physostigmine at the same doses of 0.25, 0.5, 1 and 2 µg and atropine and hexamethonium at the same doses of 1 and 4 µg were performed using a 1 µL Hamilton syringe. The volume of the drug solution to be injected into each parafascicular nucleus was 0.25 µL, and the injection was slowly made over a period of 1 min. Intra-parafascicular nucleus microinjections of atropine and hexamethonium were performed 10 min before topical corneal surface application of hypertonic saline. Acetylcholine and physostigmine were microinjected 5 min before induction of corneal pain.

Corneal nociception. Corneal nociception was induced according to Farazifard et al.\textsuperscript{13} and Tamaddonfard et al.\textsuperscript{14,15} Briefly, rats were placed on wooden tables. After a 15 min adaptation period, one drop (40 µL) of 5 M NaCl solution was applied locally onto the corneal surface using a fine dropper and then the number of eye wipes performed with the ipsilateral forelimb was counted for a period of 30 s. Thereafter, the eye was washed by local corneal surface application of distilled water. Control groups received one drop of distilled water applied locally on the corneal surface.

Cannula verification. At the end of each experiment, 0.25 µL metylene blue was injected into the each parafascicular nucleus. The animals were euthanized with 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).\textsuperscript{19}

Statistical analysis. To evaluate significance differences among intra-parafascicular nucleus 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

Placement of the tip of the cannulas in the parafascicular nucleus of the brain of rats is shown in Fig. 1. The rat brain section was modified from the atlas of Paxinos and Watson\textsuperscript{19} (Fig. 1A). The location of the cannula tip placements in the parafascicular nucleus was confirmed with intra-parafascicular nucleus injection of metylene blue (Fig. 1B).

Figure 2 shows the effects of intra-parafascicular nucleus microinjections of acetylcholine and physostigmine on the hypertonic saline-induced corneal pain. Intra-parafascicular nucleus microinjections of acetylcholine and physostigmine at the same dose of 0.25 µg produced no significant effects on the corneal pain intensity, whereas at the same doses of 0.5, 1 and 2 µg acetylcholine and physostigmine significantly ($P < 0.05$) decreased the number of eye wipes induced by local corneal surface application of hypertonic saline (Fig. 2).
In the present study, prior microinjected atropine, as a cholinergic receptor antagonist, and hexamethonium, as a powerful and reversible acetylcholine esterase inhibitor, prevented the antinociceptive effects induced by acetylcholine and physostigmine. This pretreatments with atropine and hexamethonium on the hypertonic saline- and physostigmine-induced antinociceptive effects in the acute corneal pain in rats. *P < 0.05 as compared with other groups, n = 6 rats in each group. Acetylcholine (Ach) and physostigmine (Physos) are known to modulate the corneal nociception. Moreover, physostigmine reduced synaptic transmission in periaqueductal gray, the area involved in organizing the behavioral responses to threat, stress, and pain. In the present study, prior microinjected atropine, but not hexamethonium, prevented the antinociceptive effects induced by acetylcholine and physostigmine. This study, microinjection of atropine and physostigmine at the same doses of 1 and 4 μg into the parafascicular nucleus of thalamus did not change intensity of corneal pain (Fig. 3).

Figure 3 shows the effects of intra-parafascicular nucleus microinjection of atropine and hexamethonium alone on the corneal nociception. Microinjections of atropine and hexamethonium at the same doses of 1 and 4 μg into the parafascicular nucleus of thalamus did not change intensity of corneal pain (Fig. 3).

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result indicates that the muscarinic, but not nicotinic, cholinergic receptors in the parafascicular nucleus are involved in acetylcholine- and physostigmine-induced antinociceptive effects. An ultrastructural examination of the muscarinic receptor population in parafascicular nucleus does not exist, nonetheless, the observation that muscarinic induced antinociception persists following the depletion of acetylcholine suggests that post-synaptic muscarinic receptors are responsible for pain modulation. In the formalin test in rats, the antinociceptive effects induced by ICV injection of physostigmine was blocked by prior ICV injection of atropine. Moreover, atropine blocked the antinociceptive effect of carbachol when microinjected prior to carbachol into the parafascicular nucleus of thalamus. In the present study, hexamethonium did not prevent physostigmine-induced antinociception. Hexamethonium acts at the peripheral tissue levels. On the other hand, subcutaneous injection of mecamylamine, a centrally acting antagonist of nicotinic cholinergic receptors, did not inhibit the antiallodynic effect of SC injected of physostigmine in a rat model of neuropathic pain. Moreover, the intrathecal injection of mecamylamine did not prevent physostigmine- and neostigmine-induced antinociception in the formalin test in rats. It has been reported that the nociceptive information from the cornea conveys to the spinal trigeminal nucleus part caudalis. The spinal trigeminal nucleus part caudalis projects directly to higher brain structures involved in pain processing, such as medial division of the ventroposterior thalamic nucleus, the posterior thalamic nuclear group and intralaminar nuclei. The parafascicular nucleus is the main intralaminar nucleus of the thalamus, and various neurotransmitters and neuropeptides including serotonin, acetylcholine, neuropeptide FF and opiates system are involved in parafascicular nucleus mediating pain and analgesia mechanisms.

In conclusion, the results of the present study indicate that in the parafascicular nucleus of thalamus, endogenously activated acetylcholine by microinjection of physostigmine or exogenous microinjection of acetylcholine produced antinociception in the hypertonic saline induced corneal nociception. The muscarinic receptors, but not nicotinic receptors, may be involved in the antinociceptive effect of activated acetylcholine.

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