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

**Esmaeal Tamaddonfard[1*], Amir-Abbas Farshid[2], Sona Seiednejhad[1] and Asghar Morvaridi[2]**

*[1]Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia. Iran, [2]Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*

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***Correspondance:**
Esmaeal Tamaddonfard, DVM, DVSc
Department of Basic Sciences,
Faculty of Veterinary Medicine,
Urmia University, Urmia, Iran
E-mail: etamaddonfard@yahoo.com

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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-hydroxytriptamine (5-HT) $_{1A/7}$ 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-opiate 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 Yaksh[11] 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.[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.[17,18]

**Drugs.** Drugs used in the present study included acetylcholine hydrobromide (Sigma-Aldrich), phsostigmine (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.[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.*[13] and Tamaddonfard *et al.*[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 30s. 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).[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[19] (Fig. 1A). The location of the cannula tip placements in the parafascicular nucleus was confirmed with intra-parafascicular nucleus injection of methylene blue (Fig. 1B).

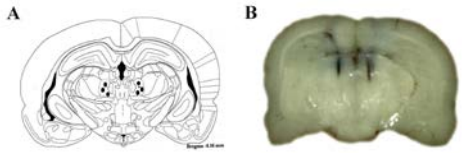


**Fig 1.** Verified section was taken from the atlas of Paxinos and Watson[17] **(A).** The black circles represent the cannula tip placement. Location of the injection cannula tips in the parafascicular nucleus of all rats were included in the data analysis **(B).**

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).

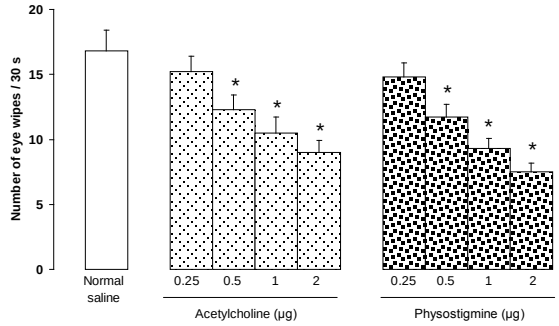


**Fig 2.** Effects of intra-parafascicular microinjection of acetylcholine and physostigmine on the hypertonic saline-induced acute corneal pain response in rats. * $P < 0.05$ as compared with normal saline (control) group, n = 6 rats in each group.

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).

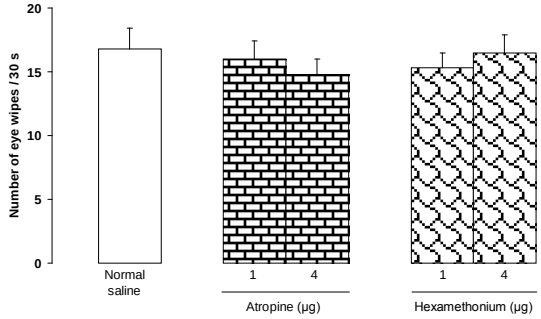


**Fig 3.** Effects of intra-parafascicular microinjection of atropine and hexamethonium on the hypertonic saline-induced acute corneal pain response in rats. n = 6 rats in each group.

Figure 4 shows the effects of intra-parafascicular microinjections of atropine and hexamethonium on acetylcholine- and physostigmine-induced antinociception in the corneal pain. Microinjection of atropine (4 µg) prior to acetylcholine (2 µg) and physostigmine (2 µg) into the parafascicular nucleus significantly ($P < 0.05$) inhibited the suppressive effect of acetylcholine and physostigmine on the corneal nociception. Hexamethonium (4 µg) did not prevent acetylcholine (2 µg) - and physostigmine (2 µg)-induced corneal antinociception before acetylcholine and physostigmine had been microinjected into the parafascicular nucleus (Fig. 4).

## Discussion

In the present study, local corneal surface application of a 5 M NaCl solution produced corneal nociception. Hypertonic saline was applied to the tongue, and cornea transiently activated wide dynamic range nociceptive neurons in the trigeminal subnucleus caudalis.[20] Farazifard

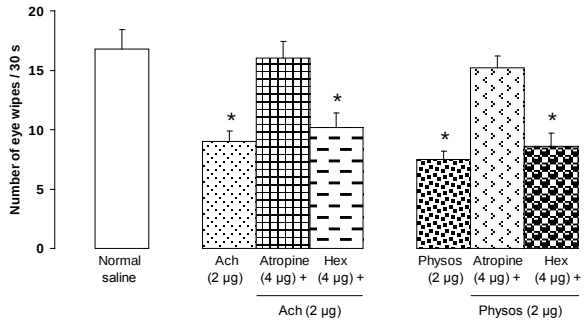


**Fig 4.** Effects of intra-parafascicular microinjection pretreatments with atropine and hexamethonium on the acetylcholine- and physostigmine-induced antinociceptive effects in the acute corneal pain in rats. * $P < 0.05$ as compared with other groups, n = 6 rats in each group. Ach: acetylcholine, Hex: hexamethonium, Physos: physostigmine.

*et al.*[13] introduced the eye wiping induced by local application of 5 M NaCl on the corneal surface, as a sensitive animal model for behavioral study of acute trigeminal pain originating from cornea. In hypertonic saline model of acute corneal pain, the antinociceptive effect of intracerebroventricular (ICV) injection of histamine was reported.[14,15] However, the results presented here is in agreement with the other findings.[13-15] In this study, intra-parafascicular nucleus microinjections of acetylcholine and physostigmine produced antinociceptive effects in the acute corneal pain. This finding indicated that in the thalamic nuclei such as parafascicular nucleus, the cholinergic system might modulate the corneal nociception. Intraventricular administration of acetylcholine attenuated the increased firing of parafascicular nucleus neurons in response to noxious stimulation.[21] Moreover, distribution of acetylcholine in the thalamic nuclei including dorsolateral geniculate, parafascicular nucleus and reticular nuclei has been reported in rats.[22] In addition, microinjection of carbachol, a cholinergic agonist, into the parafascicular nucleus increased vocalization threshold induced by tail-shock in rats.[23] Physostigmine is a major alkaloid found in seeds of the fabaceous plant *Physostigma venenosum*, and is a powerful and reversible acetylcholine esterase inhibitor that effectively increases concentration of acetylcholine in the sites of cholinergic transmission.[24,25] Most studies have focused on effects of acetylcholine esterase inhibitors such as physostigmine and neostigmine at the spinal cord levels.[11,26,27] By ICV route of administration of physostigmine, it was reported that physostigmine produced an antinociceptive effect in the formalin test in rats.[12] Moreover, physostigmine reduced synaptic transmission in periaqueductal grey, the area involved in organizing the behavioral responses to threat, stress and pain.[28] In the present study, prior microinjected atropine, but not hexamethonium, prevented the antinociceptive effects induced by acetylcholine and physostigmine. This

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.[27] In the formalin test in rats, the aninociceptive effects induced by ICV injection of physostigmine was blocked by prior ICV injection of atropine.[12] Moreover, atropine blocked the antinociceptive effect of carbachol when microinjected prior to carbachol into the parafascicular nucleus of thalamus.[23] In the present study, hexamethonium did not prevent physostigmine-induced antinociception. Hexamethonium acts at the peripheral tissue levels.[30] 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.[31] Moreover, the intrathecal injection of mechamylamine did not prevent physostigmine- and neostigmine-induced antinociception in the formalin test in rats.[26] It has been reported that the nociceptive information from the cornea conveys to the spinal trigeminal nucleus part caudalis.[32] 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.[33,34] The parafascicular nucleus is the main intralaminar nucleus of the thalamus, and various neurotransmitters and neuropeptides including serotonin, acetylcholine, neuropeptide FF and opiate system are involved in parafascicular nucleus mediating pain and analgesia mechanisms.[5,6,21,23,35]

In conclusion, the results of the present study indicate that in the parafasciular 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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