

ORIGINAL ARTICLE

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

Esmaeel Tamaddonfard<sup>1\*</sup>, Amir-Abbas Farshid<sup>2</sup>, Sona Seiednejhad<sup>1</sup> and Asghar Morvaridi<sup>2</sup>

<sup>1</sup>Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, <sup>2</sup>Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

### Key words:

Acetylcholine  
Atropine  
Corneal nociception  
Hexamethonium  
Physostigmine  
Rats

### \*Correspondance:

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

Received: 26 December 2010

Accepted: 21 May 2011

### 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.<sup>1-3</sup> 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.<sup>4</sup> Microinjection of a 5-hydroxytryptamine (5-HT)<sub>1A/7</sub> receptor agonist, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), into parafascicular nucleus produced antinociception in rats.<sup>5</sup> 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.<sup>6</sup>

The roles of acetylcholine, cholinergic agonists and cholinesterase inhibitors, collectively termed cholinomimetics, have been established in the modulation of pain and analgesia.<sup>7</sup> 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.<sup>8-10</sup> Naguib and Yaksh<sup>11</sup> 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.<sup>12</sup>

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.<sup>13-16</sup>

## 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 ( $22 \pm 0.5$  °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.<sup>17,18</sup>

**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-para-fascicular nucleus microinjection.

**Surgical procedure.** To deliver the compounds to be tested, two 24-gauge guide cannulas were bilaterally implanted in the para-fascicular 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.<sup>19</sup> 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-para-fascicular nucleus microinjection.** Intra-para-fascicular nucleus microinjections of normal saline (control), acetylcholine and physostigmine at the same doses of 0.25, 0.5, 1 and 2  $\mu\text{g}$  and atropine and hexamethonium at the same doses of 1 and 4  $\mu\text{g}$  were performed using a 1  $\mu\text{L}$  Hamilton syringe. The volume of the drug solution to be injected into each para-fascicular nucleus was 0.25  $\mu\text{L}$ , and the injection was slowly made over a period of 1 min. Intra-para-fascicular 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.

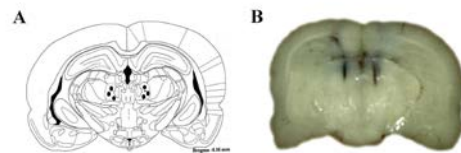
**Corneal nociception.** Corneal nociception was induced according to Farazifard *et al.*<sup>13</sup> and Tamaddonfard *et al.*<sup>14,15</sup> Briefly, rats were placed on wooden tables. After a 15 min adaptation period, one drop (40  $\mu\text{L}$ ) of 5 M NaCl solution was applied locally onto the 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  $\mu\text{L}$  methylene blue was injected into the each para-fascicular 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  $\mu\text{m}$ ), and viewed under a loupe to localize the injection site (Fig. 1).<sup>19</sup>

**Statistical analysis.** To evaluate significance differences among intra-para-fascicular nucleus treated groups, one-way analysis of variance (ANOVA) and Duncan's test were applied. In figures, all values are expressed as the mean  $\pm$  SEM. A value of  $P < 0.05$  was considered statistically significant.

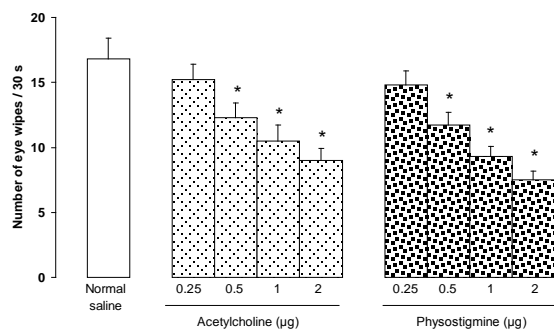
## Results

Placement of the tip of the cannulas in the para-fascicular nucleus of the brain of rats is shown in Fig. 1. The rat brain section was modified from the atlas of Paxinos and Watson<sup>19</sup> (Fig. 1A). The location of the cannula tip placements in the para-fascicular nucleus was confirmed with intra-para-fascicular nucleus injection of methylene blue (Fig. 1B).



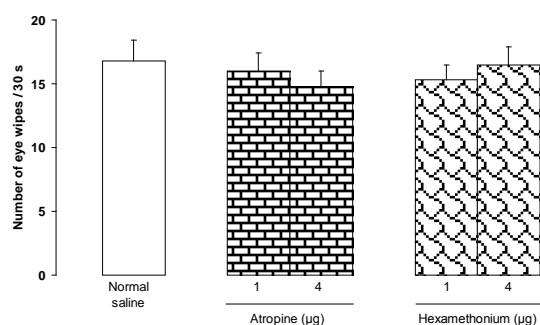
**Fig 1.** Verified section was taken from the atlas of Paxinos and Watson<sup>17</sup> (A). The black circles represent the cannula tip placement. Location of the injection cannula tips in the para-fascicular nucleus of all rats were included in the data analysis (B).

Figure 2 shows the effects of intra- para-fascicular nucleus microinjections of acetylcholine and physostigmine on the hypertonic saline-induced corneal pain. Intra-para-fascicular nucleus microinjections of acetylcholine and physostigmine at the same dose of 0.25  $\mu\text{g}$  produced no significant effects on the corneal pain intensity, whereas at the same doses of 0.5, 1 and 2  $\mu\text{g}$ , 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).

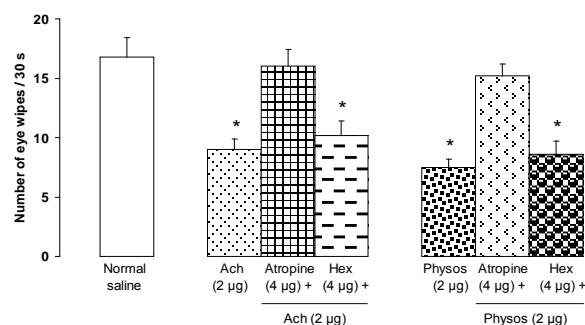


**Fig 3.** Effects of intra-parafascicular microinjection of atropine and hexamethonium on the hypertonic saline-induced acute corneal pain response in rats.  $n = 6$  rats in each group.

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.<sup>20</sup> 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.*<sup>13</sup> 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.<sup>14,15</sup> However, the results presented here is in agreement with the other findings.<sup>13-15</sup> 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.<sup>21</sup> Moreover, distribution of acetylcholine in the thalamic nuclei including dorsolateral geniculate, parafascicular nucleus and reticular nuclei has been reported in rats.<sup>22</sup> In addition, microinjection of carbachol, a cholinergic agonist, into the parafascicular nucleus increased vocalization threshold induced by tail-shock in rats.<sup>23</sup> 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.<sup>24,25</sup> Most studies have focused on effects of acetylcholine esterase inhibitors such as physostigmine and neostigmine at the spinal cord levels.<sup>11,26,27</sup> By ICV route of administration of physostigmine, it was reported that physostigmine produced an antinociceptive effect in the formalin test in rats.<sup>12</sup> Moreover, physostigmine reduced synaptic transmission in periaqueductal grey, the area involved in organizing the behavioral responses to threat, stress and pain.<sup>28</sup> 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.<sup>27</sup> In the formalin test in rats, the antinociceptive effects induced by ICV injection of physostigmine was blocked by prior ICV injection of atropine.<sup>12</sup> Moreover, atropine blocked the antinociceptive effect of carbachol when microinjected prior to carbachol into the parafascicular nucleus of thalamus.<sup>23</sup> In the present study, hexamethonium did not prevent physostigmine-induced antinociception. Hexamethonium acts at the peripheral tissue levels.<sup>30</sup> 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.<sup>31</sup> Moreover, the intrathecal injection of mechamylamine did not prevent physostigmine- and neostigmine-induced antinociception in the formalin test in rats.<sup>26</sup> It has been reported that the nociceptive information from the cornea conveys to the spinal trigeminal nucleus part caudalis.<sup>32</sup> 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.<sup>33,34</sup> 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.<sup>5,6,21,23,35</sup>

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.

### Acknowledgment

This study was supported by the Office of Vice Chancellor for Research of the Urmia University Research Project No. 001/D/88.

### References

1. Quiroz-Padilla MF, Guillazo-Blanch G, Vale-Martinez A, et al. Effects of parafascicular excitotoxic lesions on

- two-way active avoidance and odor-discrimination. *Neurobiol Learn Mem* 2007; 88: 198-207.
2. Sadikot AF, Rymar W. The primate centromedian-parafascicular complex: anatomical organization with a note on neuromodulation. *Brain Res Bull* 2009; 78: 122-130.
3. Nail-Boucherie K, Le-Pham BT, Gobaille S, et al. Evidence for a role of the parafascicular nucleus of thalamus in the control of epileptic seizures by the superior culliculus. *Epilepsia* 2005; 46: 141-145.
4. Bullitt E. Induction of c-fos-like protein within the lumbar spinal cord and thalamus of the rat following peripheral stimulation. *Brain Res* 1989; 493: 391-397.
5. Harte SE, Kender RG, Borszcz GS. Activation of 5HT1a and 5HT7 receptors in the parafascicular nucleus suppresses the affective reaction of rats to noxious stimulation. *Pain* 2005; 113: 405-415.
6. Harte SE, Lagman AL, Borszcz GS. Antinociceptive effects of morphine injected into the nucleus parafascicularis thalami of the rat. *Brain Res* 2000; 874: 78-86.
7. Jones PG, Dunlop J. Targeting the cholinergic system as a therapeutic strategy for the treatment of pain. *Neuropharmacology* 2007; 53: 197-206.
8. Leite-Panissi CRA, Brentegani MK, Menescal-de-Oliveira L. Cholinergic-opioidergic interaction in the central amygdala induces antinociception in the guinea pig. *Braz J Med Biol Res* 2004; 37: 1571-1579.
9. Yang XF, Xiao Y, Xu MY. Both endogenous and exogenous ACh plays antinociceptive role in the hippocampus CA1 of rats. *J Neural Transm* 2008; 115: 1-6.
10. Jiao R, Yang C, Zhang Y, et al. Cholinergic mechanism involved in the nociceptive modulation of dentate gyrus. *Biochem Biophys Res Commun* 2009; 379: 975-979.
11. Naguib M, Yaksh TL. Antinociceptive effects of spinal cholinesterase inhibition and isobolographic analysis of the interaction with mu and alpha 2 receptor system. *Anesthesiology* 1994; 80: 1338-1348.
12. Mojtahedin A, Tamaddonfard E, Zambouri A. Role of central muscarinic cholinergic receptors in the formalin-induced pain in rats. *Indian J Pharmacol* 2008; 41: 143-146.
13. Farazifard R, Safarpour F, Sheibani V, et al. Eye wiping test: a sensitive animal model for acute trigeminal pain studies. *Brain Res Brain Res Protocol* 2005; 16: 44-49.
14. Tamaddonfard E, Khalilzadeh E, Hamzeh-Gooshchi N, et al. Central effect of histamine in a rat model of acute trigeminal pain. *Pharmacol Rep* 2008; 60: 219-224.
15. Tamaddonfard E, Hamzeh-Gooshchi N. Effects of intraperitoneal and intracerebroventricular injection of crocin on acute corneal pain in rats. *Phytother Res* 2010; 24: 1463-1467.
16. Bates BD, Mitchell K, Keller JM, et al. Prolonged analgesic

- response of cornea to topical resiniferatoxin: a potent TRPV1 agonist. *Pain* 2010; 149: 522-528.
17. Bayne K. Revised guide for the care and use of laboratory animals available. American physiological society. *Physiologist* 1996; 39: 208-211.
  18. Clark JD, Gebhart GF, Gonder JC, et al. Special report: the 1996 guide for the care and use of laboratory animals. *ILAR J* 1997; 38: 41-48.
  19. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Compact Third Edition, Academic Press, San Diego, USA, 1997.
  20. Carstens E, Kuenzier N, Handwerker HO. Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to the oral and ocular mucosa. *J Neurophysiol* 1998; 80: 465-492.
  21. Di-Cheng Z, Tun X, Ming-Zhi S. The effects of acetylcholine on the electric activities of pain reaction neurons in nucleus parafascicularis of thalamus and midbrain reticular formation in rats. *Acta Physiol Sin* 1988; 40: 326-334.
  22. Parent M, Descarries L. Acetylcholine innervation of the adult rat thalamus: distribution and ultrastructural features in dorsomedial geniculate, parafascicular and reticular thalamic nuclei. *J Comp Neurol* 2008; 511: 678-691.
  23. Harte SE, Hoot MR, Borszcz GS. Involvement of the intralaminar parafascicular nucleus in muscarinic-induced antinociception in rats. *Brain Res* 2004; 1019: 152-161.
  24. Zhao B, Moochhala SM, Tham SY. Biologically active components of *Physostigma venenosum*. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; 812: 183-192.
  25. Cummings JL. Cholinesterase inhibitors: a new class of psychotropic compounds. *Am J Psychiatry* 2000; 157: 4-15.
  26. Yoon MH, Choi JI, Jeong SW. Antinociception of intrathecal cholinesterase inhibitors and cholinergic receptors in rats. *Acta Anaesthesiol Scand* 2003; 47: 1079-1084.
  27. Buerkle H, Boschin M, Marcus MAE, et al., Central and peripheral analgesia mediated by the acetylcholinesterase-inhibitor neostigmine in the rat inflamed knee joint model. *Anaesth Analg* 1998; 86: 1027-1032.
  28. Lau BK, Vaughan CW. Muscarinic modulation of synaptic transmission via endocannabinoid signaling in the rat mid brain periaqueductal grey. *Mol Pharmacol*. 2008; 74: 1392-1398.
  29. Bartolini A, Ghelardini L, Fantetti M, et al. Role of muscarinic receptor subtypes in central antinociception. *Br J Pharmacol* 1992; 105: 77-82.
  30. Ueno K, Togashi H, Matsumoto M, et al.  $\alpha 4\beta 2$  nicotinic acetylcholinereceptor activation ameliorates impairment of spontaneous hypertensive rats, an animal model of attention deficit hyperactivity disorder. *J Pharmacol Exp Ther* 2002; 302: 95-100.
  31. Poyhia R, Xu M, Kontinen VK, et al. Systemic physostigmine shows antiallostatic effects in neuropathic pain. *Anesth Analg* 1999; 89: 428-433.
  32. Panneton MW, Hsu H, Gan Q. Distinct central representations for sensory fibers innervating either the conjunctiva or cornea of the rat. *Exp Neurol* 2010; 90: 388-396.
  33. Guy N, Chalus M, Dallel R, et al. Both oral and caudal parts of the spinal trigeminal nucleus project to the somatosensory thalamus in the rat. *Eur J Neurosci* 2005; 21: 741-754.
  34. Hayashi O. Localization and response characteristics of thalamic corneal units in the cat. *Nippon Ganka Gakkai Zasshi* 1995; 99: 586-594.
  35. Dupouy V, Zajac M. Neuropeptide FF receptors control morphine-induced analgesia in the parafascicular nucleus and the dorsal raphe nucleus. *Eur J Pharmacol* 1997; 330: 129-137.