

ARTICLE

THE EFFECT OF THE CHOLINERGIC SYSTEM ON PLANTAR PAIN RESPONSE IN RATS

Mohammad Radmehr<sup>1</sup>, Mohammad Pourahmadi<sup>2</sup>, Hossein Kargar Jahromi<sup>3\*</sup>, Nazanin Shafeie<sup>4</sup>, Saeid Mahmoudi Teimourabad<sup>5</sup>, Ali Eslamifar<sup>5</sup>, Navid Kalani<sup>1</sup>, Mojtaba Ghaedi<sup>2</sup>

<sup>1</sup>DepAnesthesiology and critical care and pain management research center, Jahrom University of Medical Sciences, Jahrom, IRAN

<sup>2</sup>Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom, IRAN

<sup>3</sup>Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, IRAN

<sup>4</sup>Veterinary Department (Ph.D), Islamic Azad University, Firozabad Branch, Firozabad, IRAN

<sup>5</sup>Department of Clinical Research, Pasteur Institute of Iran, Tehran, IRAN

ABSTRACT

The aim of this study was to investigate the effects of cholinergic system in the plantar pain of rats. In this study, adult male Wistar rats weighing 200-250 g were used, Mice were stored as groups of six rats in plastic cages in a room with ambient conditions and the optimum temperature about 23±2 ° C and 12 hours of light period, and the animals were fed with commercial pellet food while food and water was freely available for them. All tests were performed within 8 am to 3 pm. physostigmine solution 0.01 was used as muscarinic receptor agonist. In the control group (group one), the number of mice were 6, which were injected in plantar section with normal saline, and formalin pain response was investigated. In the second group, subcutaneous physostigmine (0.2 µg) was injected before formalin 1%. In the third group, subcutaneous physostigmine (0.1 µg) was injected before formalin 1%. In the fourth group, subcutaneous physostigmine (0.05 µg) was injected before formalin 1%. Formalin pain response was studied 20 minutes after subcutaneous injection of physostigmine in quantities 0.05, 0.1 and 0.2 mg per kg body weight. Physostigmine was scheduled to be injected 20 minutes before formalin injection. GLM ANOVA procedure of SAS software was used to check the data, and Tukey test was used to compare mean values. The results showed that subcutaneous injection of physostigmine in quantities 0.05, 0.1 and 0.2 mg per kg of body weight, reduced pain response in both the first and the second phases significantly (P<0.05). The duration of licking and biting the injected paw by subcutaneous injection of physostigmine by the amount of 0.1 mg per kg of body weight, had a significant reduction (P<0.05) than 0.05 mg. It can be concluded that the cholinergic receptors may have a role in regulating the pain.

INTRODUCTION

Pain and understanding it is one of the most important acts of the nervous system that provide required information about the existence of an injury or a lesion or threats they would create, and plans the appropriate response according to the type of stimulus. Pain is a complex phenomenon and includes both sensory and emotional components. In other words, pain is a sensory experience accompanied by motivational responses, which is made by autonomous motor coordination. From this perspective, understanding the pain is a necessary and prerequisite process for the survival of an organism [1].

Pain control is done through various systems and receptors. One of them is cholinergic system which is one of the few pain modulating systems that inhibits both constant and tonic pains in the cortex, the brain stem, and the spinal cord. Cholinergic system impacts applied by different receptors, mostly opioid analgesics, that induce their effect by binding to specific receptors coupled with G protein [2].

There is not enough information available on existence and importance of this core's muscarinic cholinergic receptors' neurons in pain control. Study was designed to determine the presence or absence of cholinergic muscarinic receptors in the dorsal (DPGI) and lateral (LPGI) paragigantocellularis core, and its role in controlling acute and chronic pain in young adult rats by formalin test.

Research results indicate that neurons in the dorsal and lateral parts of PGI core cholinergic receptors, have cholinergic muscarinic receptors which has a main role in understanding the pain, injection of Scopolamine which is an antagonist of this receptor, caused significant analgesia in both sub-sectors of DPG1 and LPGI in both acute and chronic phases of pain. Therefore, concerning that inhibition of cholinergic receptors of PGI core has reduced pain in rats, 1- it can be concluded that these neurons are cholinergic receptors, and 2- These receptors are involved in pain processing by this core.

On the other hand, the effect of scopolamine in lateral side of PGI core on pain intensity is more durable [3]. Physostigmine is an herbal alkaloid not only stimulates muscarinic and nicotinic sites in autonomous nervous system, but it stimulates nicotinic receptors in neural-muscle connection. Average duration time of it, is 2-4 hours. This drug increases tones in bowel or bladder, which makes it able to be used in obstruction incidents. Use of this drug in the eyes causes meiosis, and reduces the pressure inside the eyeball, and it is prescribed in glaucoma disease.

KEY WORDS

pain receptors, physostigmine, cholinergic system, Rat

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\*Corresponding Author  
Email:  
hossein.kargarjahromy@yahoo.com

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Thioperamide[H3 receptor antagonist] increases histamine recycling in the brain and because no other drug family is capable of that, it is used widely in the behavior studies. Over the past seventy years, all researches that was done on histamine, fully focused on the role of histamine in allergic diseases [4]. Therefore, to investigate the role of cholinergic system in the mechanism of the histamine effect, Physostigmine(muscarinic receptors agonist] and Atropine (muscarinic receptors antagonist] was used alone or along with histaminergic agents.

Tytgat (2009) examined hyoscine butyl bromide's function, as an anticholinergic compound on visceral pain and abdominal cramps. Emphasizing the good efficacy of the combination on these pains, he pointed that this ability is because of its high affinity to muscarinic receptors along the digestive tract and also binding affinity to nicotinic receptors which causes "ganglions block effect" [5].

The importance of studying the mechanisms involved in developing and feeling the pain process is to identify and manage pain, hence these are two major current practices in animal and human medicine. Up to date, a large number of studies concerning neuro transmitter systems' role in feeling pain, are focused on investigating opioid system, and role of other neuro transmitters, especially cholinergic system, has not been studied yet. However, the results of some studies with a focus on investigating potential relation between cholinergic system's performance and feeling pain, represents considerable evidence implicating the role of this system in processing pain. The present study was designed to determine the effects of muscarinic anticholinergic antagonists (atropine), on chronic pain, and mechanisms involved in it.

## MATERIALS AND METHODS

Adult male Wistar rats weighing 250-200 g were purchased from the School of Veterinary Medicine Tehran for the study. , Mice were stored as groups of six rats in plastic cages in a room with ambient conditions and optimum temperature about  $23\pm 2$  ° C and 12 hours of light period, and the animals were fed with commercial pellet food while food and water was freely available for them. All tests were performed within 8 am to 3 pm.

Eserine Physostigmine (0.01 Mg/Kg) solution (Sigma-Aldrich Co) used as an agonist of muscarinic receptor. In the control group (group one), the number of mice were 6, which were injected in plantar section with normal saline, and formalin pain response was investigated. In the second group, subcutaneous physostigmine (0.2 µg) was injected before formalin 1%. In the third group, subcutaneous physostigmine (0.1 µg) was injected before formalin 1%. In the fourth group, subcutaneous physostigmine (0.05 µg) was injected before formalin 1%. Formalin pain response was studied 20 minutes after subcutaneous injection of physostigmine in quantities 0.05, 0.1 and 0.2 mg per kg body weight. Physostigmine was scheduled to be injected 20 minutes before formalin injection.

To investigate pain in all groups, formalin test was used, which first was described by Dvubyson (1977) and now is a valid method in studying chronic pain. In order to induce and study pain and its responses in rats, formalin with 5 percent concentration was used in 50 microliter volume, and as mentioned earlier, using different concentrations of formalin causes a pain in feet of rats. On the other hand, pain responses was recorded by the method of measuring the duration of foot licking and biting (based on the experiences listed, this recording behavior in rats, is better than scoring method) [6]. In this method formalin was injected in the skin of the plantar section.

The animal was constrained calmly using a towel, and 50 microliter of formalin solution (1percent concentration) was injected to plantar area using needle 28. Plantar injection of diluted formalin to foot area, causes a rapid reaction in rat which includes stepping back the foot, trying to run, and groaning. Then the rat is put into the pain mirror device to investigate pain behavior. Plantar formalin pain behavior is a two-step sense. First step and second step of rat's pain behavior, was considered at intervals 0-5 minutes and 15-40 minutes, respectively.[ Fig. 4-3 ]shows licking of injection area behavior after injection of formalin in pain mirror device.



**Fig.1:** Licking of injection area, after plantar injection of formalin and placing the rat in the pain mirror device.

### Pain behavior

Pain mirror device was used in order to create and investigate the behavior of plantar formalin induced pain. Box was made of safety glass with dimensions of 25 × 30 × 30, which was placed on a framework, containing a mirror at an angle of 45 degrees. (Figure 2) placing a mirror with 45 degrees makes us able to see all animal's movements. Regarding that stress is induced by placing the rat in the case, waking it up forcedly, separating the animal from the group, moving it to another room, and by putting it in a new smell and light condition, hence, the stress should be minimized before starting the test [7]. In order to adapt to the environment, rats were transferred to the laboratory four hours before starting the experiment, and they were placed in the glass box of pain mirror device, half hours before the test. Rats were brought out of the case before injection, and after, they were put back in the glass case.

[Fig.2] shows an example of the pain mirror device which was used in this study.



**Fig. 2:** The pain mirror device which was used in this study.

### Statistical analysis method

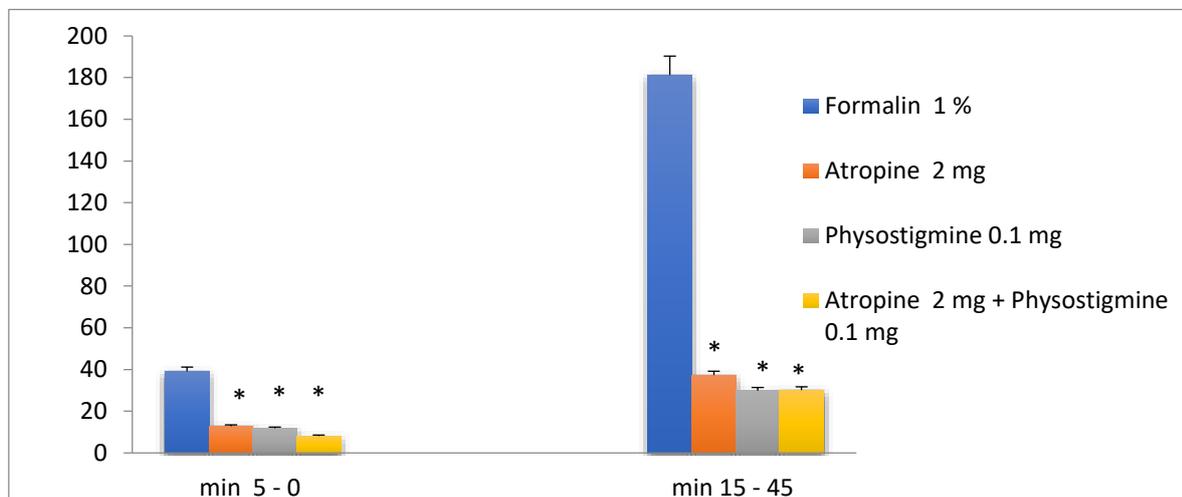
Based on the data from plantar injection of normal saline (control) and formalin in the foot paw with repeated factor measure statistical method (Factorial), and then Duncan test, and based on data's significant level, P was considered <0.05. In tests concerning to determine the appropriate dose response with different non-linear processing such as quadratic models, Broken Line, Line break with two defeats, exponential function, etc., the most suitable model is chosen by the coefficient of determination, and favorable response is achieved from it. GLM procedure of SAS software was used to analyze the variance, and Tukey test was used to compare mean values.

### RESULTS

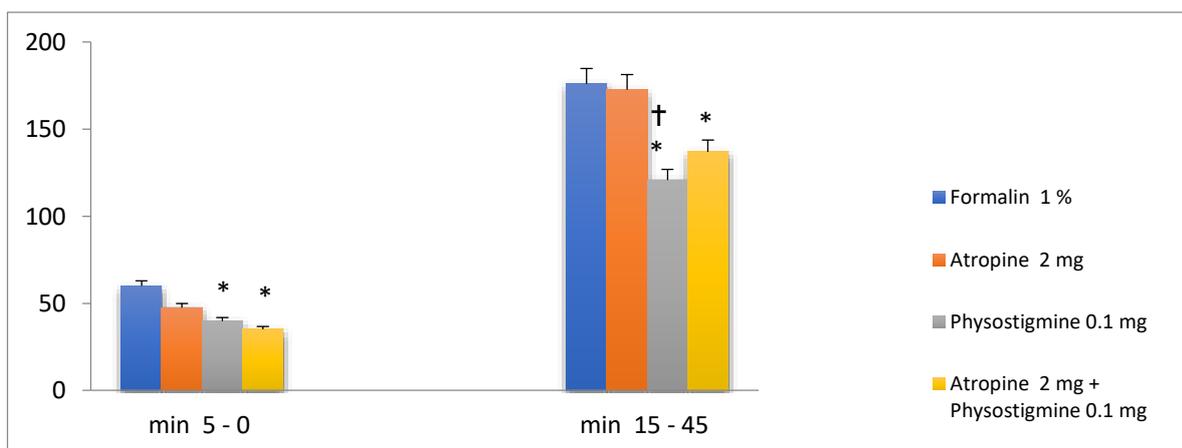
As shown on [Fig.3-4], subcutaneous injection of physostigmine in quantities 0.05, 0.1 and 0.2 mg per kg of body weight, reduced pain response in both the first and second phases significantly (P<0.05) . The

duration of licking and biting the injected paw by subcutaneous injection of physostigmine by the amount of 0.1 mg per kg of body weight, had a significant reduction ( $P < 0.05$ ) than 0.05 mg.

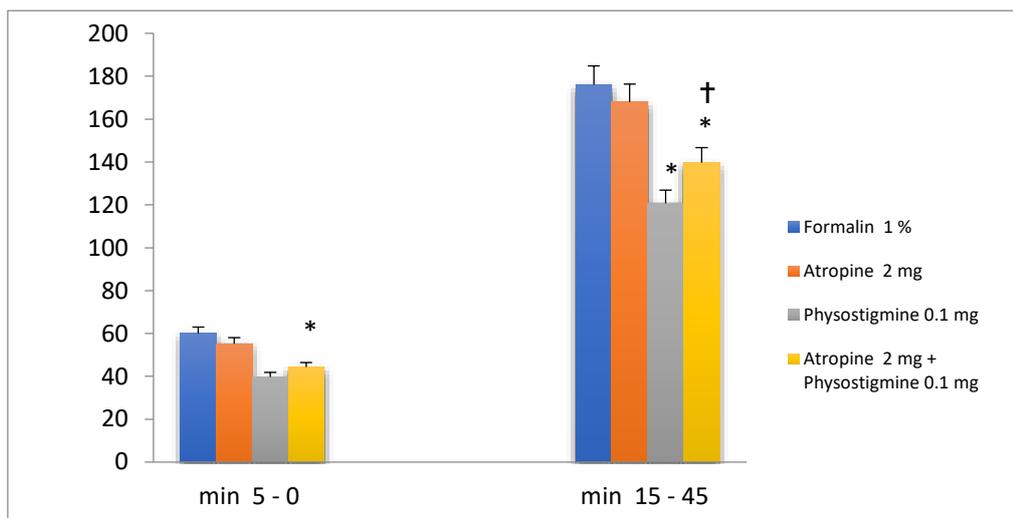
Subcutaneous injection of atropine (2 mg per kg body weight) didn't cause significant effects on formalin pain, but pre-injection of atropine (2 mg per kg body weight) prevented a significant reduction ( $P < 0.05$ ) for physostigmine (0.1 mg per kg body weight) derived pain in the second step of pain response (as foot kicking).



**Fig. 1:** The number of injected foot shock (Physostigmine subcutaneous injection).  
\* Indicate a significant difference in ( $P < 0.05$ ) level with formalin 1% group



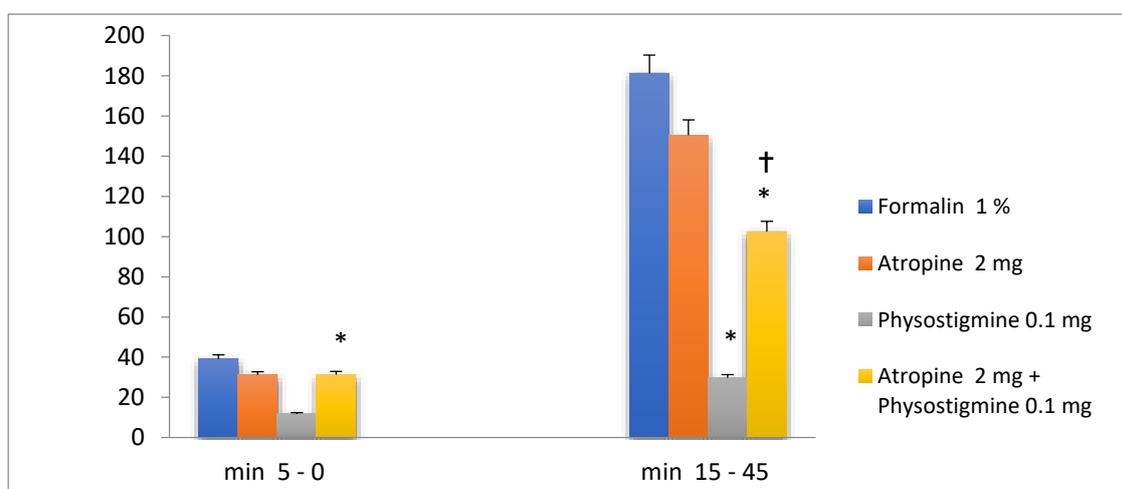
**Fig. 2:** Duration of licking and biting the leg (Physostigmine subcutaneous injection)  
\* Indicate a significant difference in ( $P < 0.05$ ) level with formalin 1% group  
† Indicate a significant difference in ( $P < 0.05$ ) level with Physostigmine 0.05 mg group



**Fig. 3:** Duration of licking and biting the leg (Atropine and Physostigmine subcutaneous injection).

\* Indicate a significant difference in (P<0.05) level with formalin 1% group

† Indicate a significant difference in (P<0.05) level with atropine 2 mg group



**Fig.4:**The number of injected foot shock (Atropine and Physostigmine subcutaneous injection).

\* Indicate a significant difference in (P<0.05) level with formalin 1% group

† Indicate a significant difference in (P<0.05) level with atropine 2 mg group

## CONCLUSION

In the present study, plantar subcutaneous injection of physostigmine created an analgesic effect in formalin induced pain test. In addition, subcutaneous injection of atropine alone did not change the pain intensity, but pre injection of atropine, inhibited the analgesia induced by physostigmine. These findings showed that cholinergic receptors may have a role in regulating pain. Several neurotransmitters play a role in processing and feeling the pain in the nervous system, but the role of cholinergic system in this context has not been investigated as well. In order to review anticholinergic effects on pain, the impact of atropine subcutaneous administration (2 mg per kg) on chronic pain sensation in male rats was studied by the formalin test. The

results obtained in this experiment, showed that atropine receiving male rats had a higher pain threshold than the control group.

Pain relief effect of atropine showed no significant difference in male rats' test. The findings of this study indicate that anticholinergic agents such as atropine can reduce chronic pain. This study aimed to draw a clear picture of anticholinergic muscarinic antagonists (atropine) impact on chronic pain and the mechanisms involved in it.

Geraldine et al. (1999) investigated pain relief effect of atropine by In vivo and In vitro methods.

During in vivo study, low concentration of atropine increased electrical stimulated and nicotine derived contraction in the ileum of Guinea pigs, but it inhibited electrical stimulated and drug (such as nicotine) derived contraction.

During In vitro study, Intracerebroventricular administration of atropine, reduced acute and visceral pain in mice and rats. Based on these results, the probable mechanism of atropine pain relief property is attributed to its effect on muscarinic receptors, and muscarinic M1 receptor is assumed to have an effect in this process. In summary, based on the results of this study, atropine can have pain relief properties as a para sympathetic combination.

The importance of studying the mechanisms involved in developing and feeling the pain process is to identify and manage pain, hence these are two of the major current practices in animal and human medicine. Up to date, a large number of studies concerning neuro transmitter systems role in feeling pain, are focused on investigating opioid system, and role of other neuro transmitters especially cholinergic system has not been studied yet. However, the results of some studies with a focus on investigating potential relation between cholinergic system performance and feeling pain, represents considerable evidence implicating the role of this system in processing pain. The present study was designed to determine the effects of muscarinic anticholinergic antagonists (atropine) on chronic pain and mechanisms involved in it.

The results of this study show that the average chronic pain threshold is higher in the male intervention group than the male control group. It means that male rats receiving atropine, felt significantly lower chronic pain than normal saline receiving mice. Five types of receptors are described for cholinergic system named M<sub>1</sub> .M<sub>2</sub> .M<sub>3</sub> .M<sub>4</sub> .M<sub>5</sub>. These five types are involved in major activities of the cholinergic system including pain and analgesia, stress, tolerance and addiction, learning and memory, neurological disorders and movement [8]. Physostigmine acts through M1 receptors and atropine is a competitive muscarinic receptors' antagonist with high affinity to M1 receptors. Physostigmine and atropine are frequently used to investigate the role of cholinergic system in peripheral, spinal, and spinal trigeminal pain-analgesia mechanisms. Administration of Physostigmine in formalin pain, reduced first and second step of pain in rats, and local injection of atropine completely inhibited analgesic effect of Physostigmine.

Therefore, it can be expected that the cholinergic system would regulate the mechanisms of pain in peripheral surfaces, and the study showed that the activation and inhibition of the peripheral cholinergic system in surface level, impacts on feeling pain with plantar origin.

#### CONFLICT OF INTEREST

There is no conflict of interest.

#### ACKNOWLEDGEMENTS

None

#### FINANCIAL DISCLOSURE

None

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