



Research Article

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Interference between histaminergic and cholinergic systems on the formalin plantar pain response in rats

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ABSTRACT

The purpose of this study was to investigate the interference of histaminergic and cholinergic systems on the pain response in rats. In this study, adult male Wistar rats were selected in the weigh range of 200-250 g. Rats were kept in groups of six rats in plastic cages and in a room with ambient conditions and the optimum temperature about 23 ± 2 °C and 12 hours of light; and the animals were fed with commercial pellet food. Stainless normal saline solution was formalin solution of 1, 2.5, and 5% which was prepared from commercial Formalin 37% with adding normal saline. Physostigmine solution of Eserine 0.01 Mg/Kg (Sigma-aldrich Co) was used as muscarinic receptors' agonist. Thioperamide solution of Thioperamide maleate salt 2.5 Mg/Kg (Sigma-aldrich Co) was as H3 receptors' antagonist solved with normal saline. Atropine solution of 2 Mg/Kg (Sigma-aldrich Co) was solved with normal saline as muscarinic receptor antagonist. The results showed that subcutaneous injection of atropine did not have significant effect on pain caused by plantar injection of formalin. Thioperamide intraperitoneal injection of dose 2.5 mg per kg of body weight caused a significant reduction ($P < 0.05$) of pain in both phases of pain. Subcutaneous injection of atropine was significantly prevented from the pain of thioperamide in the second phase of the pain response as licking foot ($P < 0.05$). Subcutaneous injection of physostigmine of 0.1 mg per kg of body weight caused a significant reduction of the pain response in both steps ($P < 0.05$). Thioperamide intraperitoneal injection alone decreased pain response to formalin injection in both periods significantly ($P < 0.05$). It (2.5 mg per kg of body weight) had no effect on reducing the pain of subcutaneous injection of physostigmine in both stages. It can be said that the environmental level, histaminergic and cholinergic systems have interaction in the regulation of pains originated plantar and muscarinic receptors and histamine H3 receptors are involved in the interaction between thioperamide and physostigmine.

Keywords: Atropine, Thioperamide, Physostigmine, rat

INTRODUCTION

Analgesic effects occur through the different systems and receptors. Among them the cholinergic system, nitric oxide system, and non-opioid receptors can be pointed. As well as there are some materials affecting by mechanisms other than the opioid mechanism and other cholinergic goals [1].

Studies on humans and animals have shown that cholinergic system, in particular, acetylcholine muscarinic receptors may have a role in memory. Visual Studies of brain activity shows an increased cholinergic activity and a decreased cholinergic activity, and reduced anticholinergic activity in subcortical brain regions (such as thalamus). These areas are responsible for continuing vigilance and attention. There are a certain correlation between the status of cholinergic neurons in former brain basal area and the severity of age-related cognitive impairment and also a lot of evidence has shown that as age increases, various aspects of learning and memory are impaired. Anticholinergic activity may also be involved in cognitive deficits and dementia. However, acetylcholine is a neurotransmitter involving in learning and memory processing a lot, a group of scientists highly hesitates in accuracy of the experimental data obtained from pharmacological and damage studies (which are interpreted as cholinergic mechanisms). Severe disorder of cholinergic system's activity is shown in dementia and particularly in decreasing the cognition depending on Alzheimer's disease and age. Though the loss of cholinergic activity plays a central role in the development of cognitive symptoms, it cannot clearly explain the whole process. No one of increased acetylcholine or cholinergic agonists' administration is able to compensate this type of cognitive disorders [2].

Every moment our brain is faced with an influx of information that reaches from the body's internal and external environments. The brain not only acts as a passive repository to store this information, but it also processes them and creates appropriate responses to maintain homeostasis processes. The brain uses various neurotransmitters such as aminergic systems to process information. Histaminergic system is one of aminergic systems in the mammalian brain through which four types of receptors H₁, H₂, H₃ and H₄ in the regulation of many brain functions such as food intake, cardiovascular and respiratory functions, neuroendocrine responses, learning and memory [3, 4]. Histamine is one of the aminergic neurotransmitters and plays an important role in the regulation of physiological and pathophysiological events. In the mammalian, brain histamine is made in a limited number of neurons that are at the Tubero-mammillary core of the posterior hypothalamus. The redundancies of these neurons penetrate in more parts of the brain, and they interfere in many brain functions such as sleep and wakefulness, hormone secretion, cardiovascular control, body temperature regulation, food intake and memory formation [2].

Hippocampus is involved in various biological functions, including learning and memory, anxiety, and brain stimulation, using neurotransmitters such as muscarinic, GABA, serotonin and histamine [5, 6]. Histaminergic mechanisms may be related to the cholinergic system and have an important role in modulating some cholinergic behaviors. The effects of agonists and various histamine receptor antagonists in formalin pain in rats were examined in order to determine the possible role of histaminergic mechanisms in formalin pain. The findings of this study indicate that the cholinergic system may environmentally be involved in analgesia induced by inhibiting histamine H₃ receptors. As well as, Mobarakeh et al. (2009), using rats lacking histamine H₃ gene, reported that histamine H₃ receptors in the spinal cord has an inhibitory effect on the analgesic effects of morphine [7]. Physostigmine is a plant alkaloid that not only stimulates the muscarinic and nicotinic sites of the autonomic nervous system, but it also stimulates nicotinic receptors in the neural-muscle connecting location. Duration of its effect is about 2-4 hours. This medicine enhances intestine and bladder movement, which it is used in terms of the accumulation of these organs. The use of this medication in the eyes causes miosis and reduces inside pressure of the eyeball and treats glaucoma. Thioperamide H₃ receptor antagonist increases recycling histamine in the brain; and since none of the other drug classes has this ability, the drug is widely used for behavioral studies. Over the past seventy years, researches that were done on histamine fully focused on the role of histamine in allergic diseases [8]. Therefore, physostigmine (muscarinic receptor agonist) and atropine (muscarinic receptors' antagonist) alone and with histaminergic agents are used to investigate the role of cholinergic system in the histaminic effect mechanism.

Interaction between histamine and cholinergic system has been proved in the central nervous system as it modulates histamine cholinergic transmission. Histaminergic receptors in the brain are involved in the induction of thirst as histaminergic receptors are associated with cholinergic system and play an important role in the induction of drinking after central cholinergic pharmacological stimulation. Histamine is also released from mast cells peripherally and histamine is not able to cross the blood-brain barrier and on the other hand, interaction between

histamine and cholinergic system is unknown in the peripheral nervous system. Therefore, the objective of this study was to investigate the interaction of histaminergic and cholinergic systems on the formalin plantar pain response in rats.

Materials and Methods

In this study, adult male Wistar rats weighing 200-250 g were purchased from the veterinary faculty of Tehran; they were kept in groups of six rats in plastic cages in a room with ambient conditions and the optimum temperature for about 23 ± 2 °C and 12 h light and the animals were fed with commercial pellet food while they had water freely available. All tests were performed within 8 to 15 hours.

Stainless normal saline solution was formalin solution of 1%, 2.5% and 5% which was prepared of commercial formalin 37% by adding saline. Physostigmine solution of Eserine 0.01 Mg/Kg (Sigma-aldrich Co) was used as muscarinic receptor agonist. Thioperamide solution of Thioperamide maleate salt 2.5 Mg/Kg (Sigma-aldrich Co) was used as antagonist of H3 receptors that was solved with normal saline. Atropine solution of Atropine 2 Mg/Kg (Sigma-aldrich Co) was used as muscarinic receptor antagonist that was solved with normal saline.

First, atropine in the 2 mg/kg body weight was injected into a subcutaneous method, then physostigmine to the amount of 0.1 mg/kg body weight was injected into subcutaneous method after 10 minutes, then 10 minutes later thioperamide in the amount of 15 mg/kg body weight was injected intraperitoneally and 10 minutes later, formalin was injected in plantar and formalin pain responses were assessed.

The formalin test, which was described first by Dobison (1977) and now it is considered a reliable procedure in the study of chronic pain, was used to assess pain in all categories. In this study, formalin in concentration of 5% with a volume of 50 ml was used to create pain and evaluate reactions to pain in rats; and as mentioned, the use of different concentrations of formalin in rats' paw creates pain. On the other hand, pain responses were recorded by measuring the time of licking and biting the foot which on the basis of the mentioned experiences, the approach of recording behaviors in rats is better than scoring method [9]. In this method, the formalin was injected into the area under the skin of paw. The animal was lightly bound by a towel for injection and 50 ml of formalin solution with 1% concentration was injected to an area of rat paw by needle tip 28. Injection of diluted formalin into the plantar region of the foot causes an immediate reaction in the animal to pull back foot that is associated with trying to escape and groan. The animal immediately put into the pain mirror device to investigate the pain behavior. Formalin plantar pain behavior is in the form of a two-phase pain. In the present study, animal behavior was considered at intervals of 0-5 minutes and 15-40 minutes, respectively, as the first and second phases of pain. Figure 1 reveals licking behavior in the injected site after injection of formalin in the pain mirror device.



Figure 1. Licking the injected site after formalin injection to the animal's paw and putting it in the pain mirror device.

Evaluating pain behavior

Pain mirror device was used to create and investigate the behavior of plantar formalin injection. The device consists of a base and a box. Box made of toughened glass with dimensions of 25×30×30 was on a frame with a mirror at an angle of 45 degrees (Figure 2). Putting a mirror at an angle of 45 degrees makes all animal movements to be seen through it. Due to the fact that stress is not only created by putting the animal in the chamber, but it also is created by mandatory awakening the animal, animal isolation of groups, animal transfer to another room and new light and smell; therefore, before starting the test, these stresses should be minimized [10]. In order to adapt to the environment, animals were transferred to the laboratory four hours before the test and were placed inside a glass box of pain mirror device a half hours before the test. For injection, the animals removed from the box and were returned back into the glass container after injection. Figure 2 shows an example of the pain mirror device used in this study.



Figure 2. Pain mirror device that was used in this study.

Statistical analysis method

Data from plantar injection of normal saline (control) or formaldehyde in paw was considered to statistical method of repeated factor measure (factorial) and then Duncan test and data from injection of drug solutions using one-sided Analysis of Variance (ANOVA) and Duncan test and obtained data and significance level, $P < 0.05$. The best model was selected based on the coefficient of determination in tests related 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., and the favorable response will be achieved from it. GLM procedure in SAS software was used for the analysis of variance and the Tukey test to compare mean values.

Results and Discussion

As shown in Figures 3 and 4, subcutaneous injection of atropine (2 mg/kg of body weight) had significant effect on pain caused by plantar injection of formalin. Thioperamide intraperitoneal injection of 2.5 mg/kg of body weight caused a significant reduction ($P < 0.05$) pain response (licking the injected foot) in both phases of pain. Subcutaneous injection of atropine (2 mg/kg of body weight) prevented from the pain of thioperamide (2.5 mg/kg of body weight) and in the second phase, pain response is significantly licking leg ($P < 0.05$).

Physostigmine subcutaneous injection of 0.1 mg/kg of body weight dose caused a significant reduction ($P < 0.05$) pain response (licking the injected foot) both phases.

Thioperamide intraperitoneal injection (2.5 mg/kg of body weight) alone formalin pain response (licking the injected foot) significantly ($P < 0.05$) decreased in both phases.

Thioperamide intraperitoneal injection (2.5 mg/kg of body weight) had no effect on reducing the pain of subcutaneous injection of physostigmine (0.1 mg/kg of body weight) in both phases.

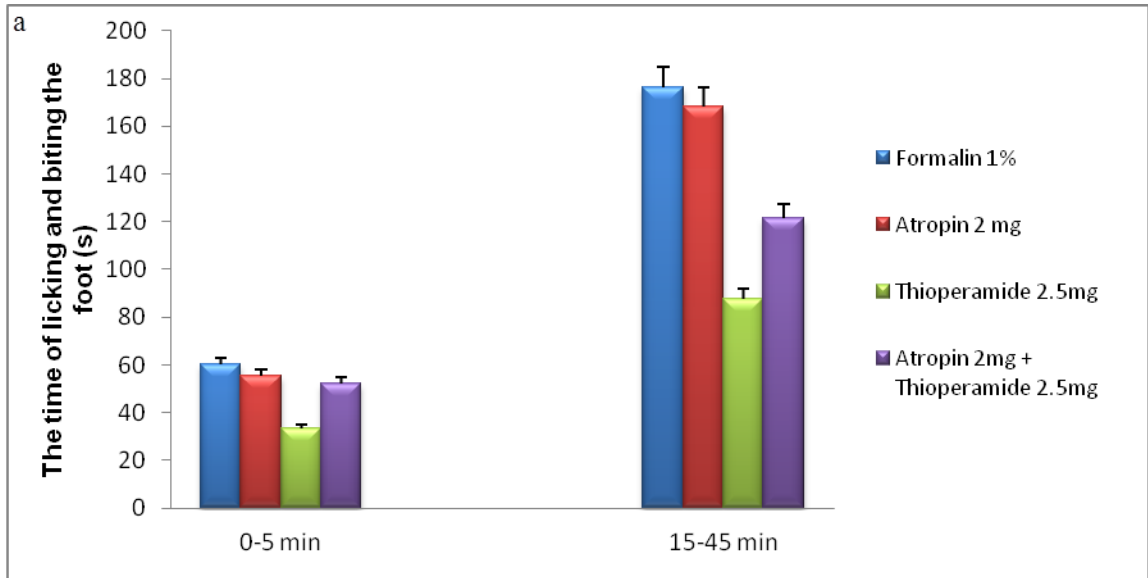


Figure 3. The time of licking and biting foot (subcutaneous injection of atropine and intraperitoneal thioperamide).

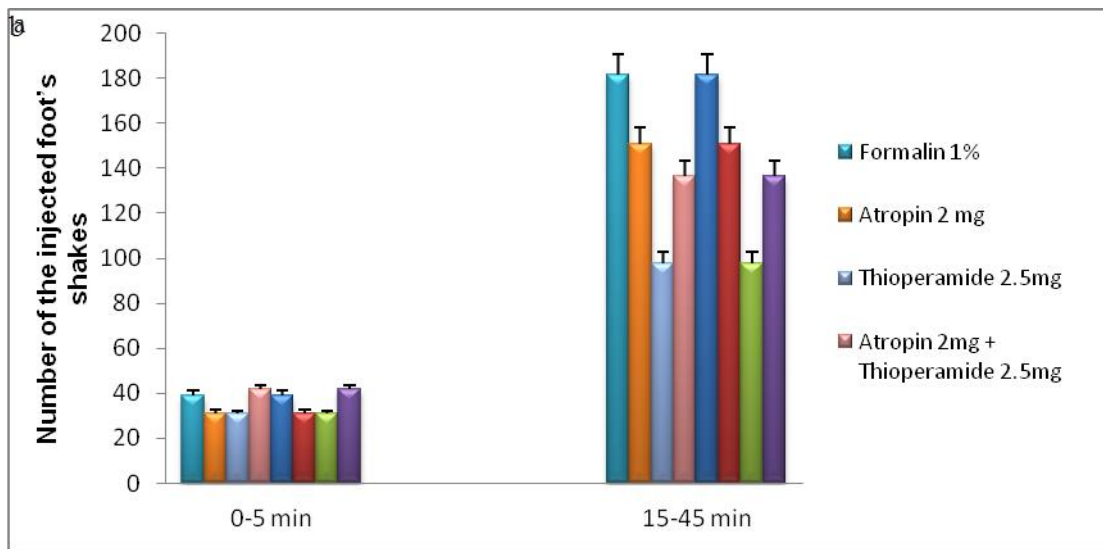


Figure 4. Number of the injected foot's shakes (subcutaneous injection of atropine and intraperitoneal thioperamide).

*) Indicating a significant difference ($P < 0.05$) with group of formalin 1%.

†) indicating a significant difference ($P < 0.05$) with groups receiving 2 mg atropine.

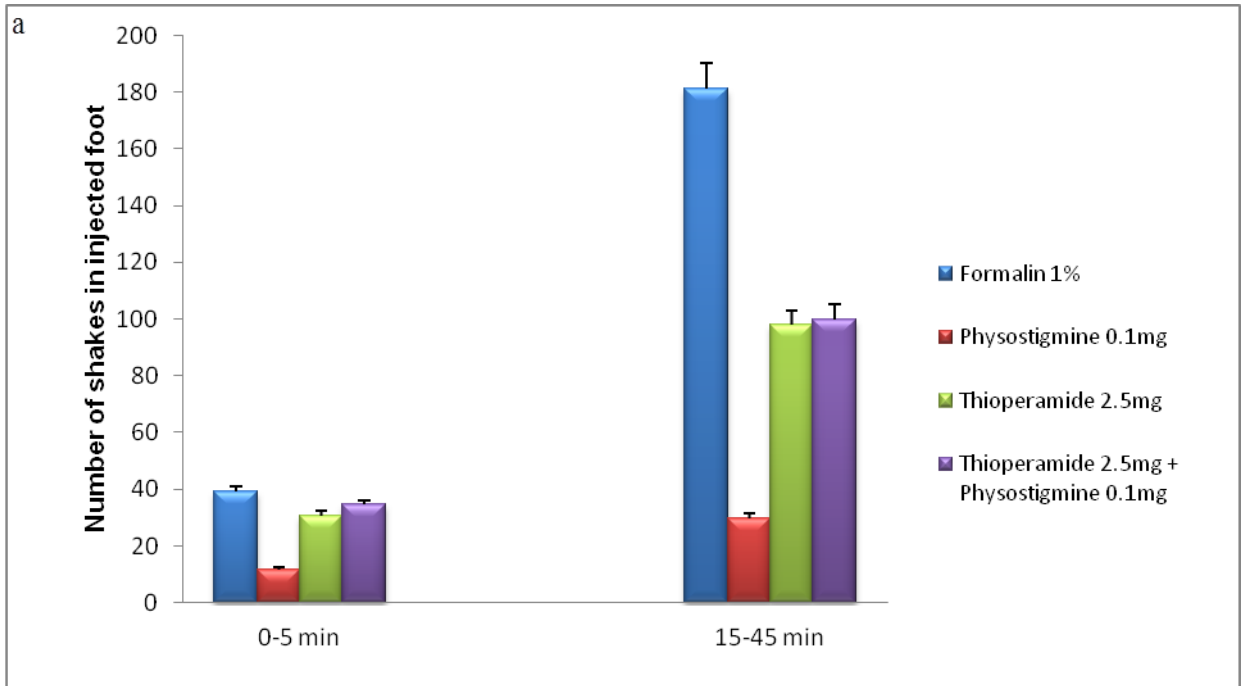


Figure 5. The number of shakes in injected foot (subcutaneous injection of physostigmine and intraperitoneal thioperamide).

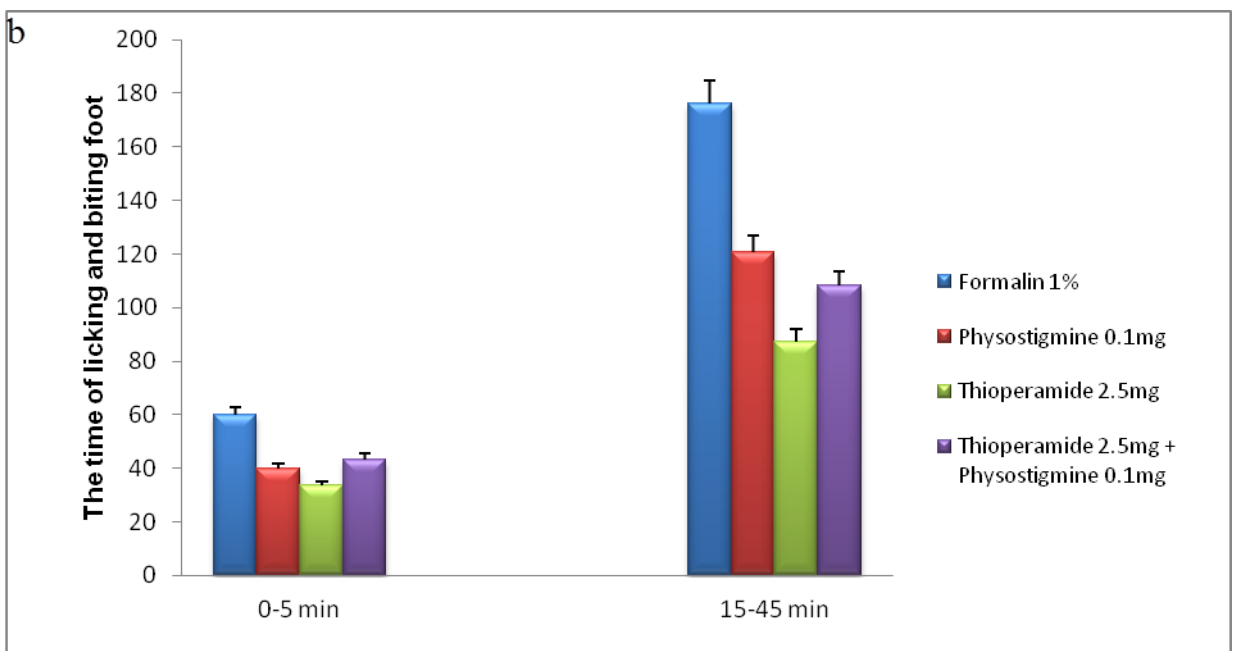


Figure 6. The time of licking and biting foot (subcutaneous injection of physostigmine and intraperitoneal thioperamide).

*) Indicates a significant difference ($P < 0.05$) with formalin 1%.

†) indicates a significant difference ($P < 0.05$) with the group receiving 0.1 mg of physostigmine.

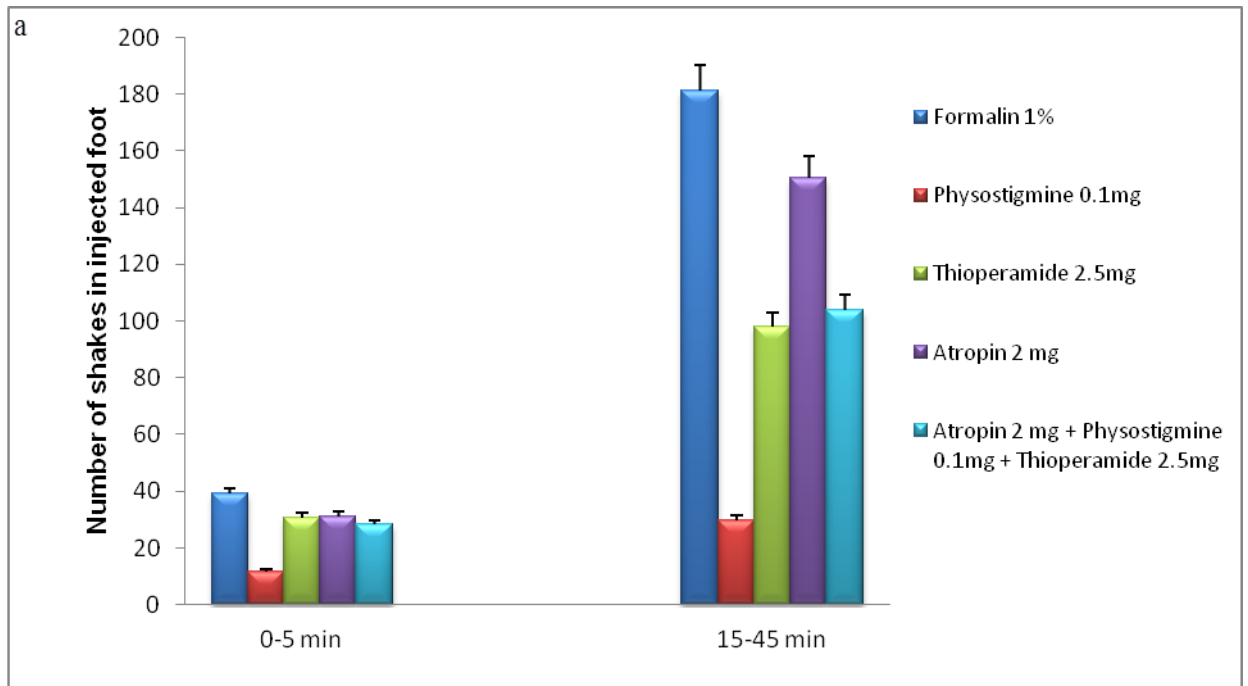


Figure 7. The number of shakes in injected foot (subcutaneous injection of atropine, physostigmine and intraperitoneal thioperamide).

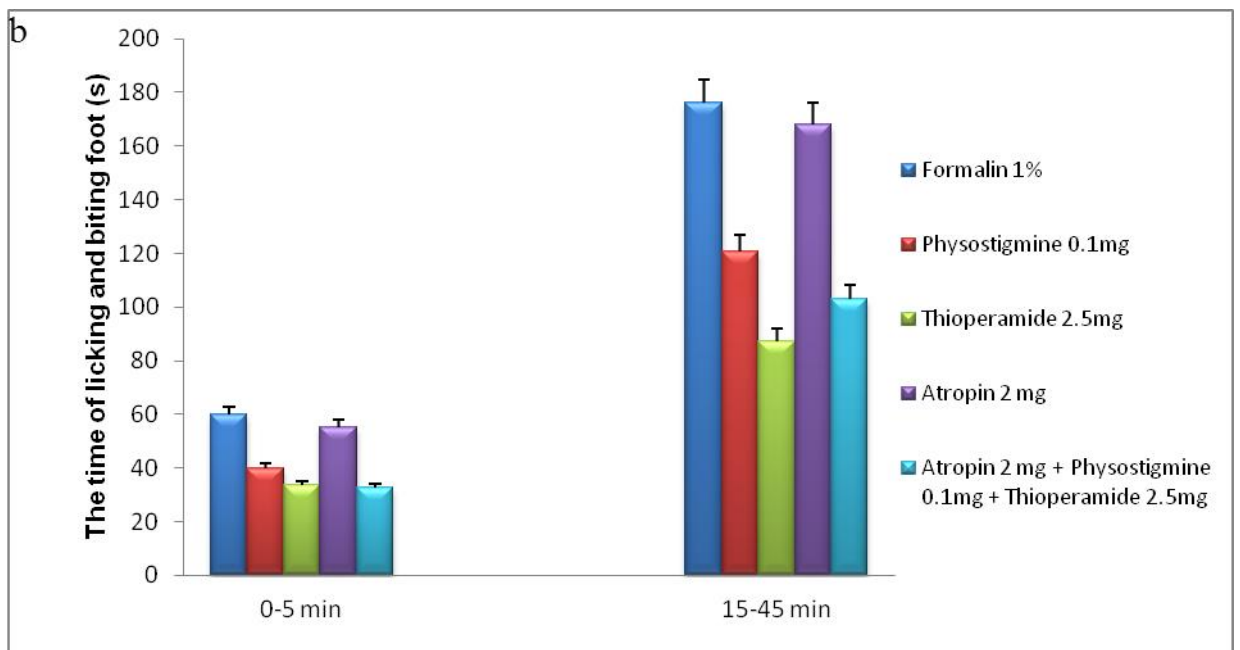


Figure 8. The time of licking and biting foot (subcutaneous injection of atropine, physostigmine and intraperitoneal thioperamide).

*) Indicates a significant difference ($P < 0.05$) with formalin 1%.

†) indicates a significant difference ($P < 0.05$) with the recipient groups of 2mg atropine.

Discussion and conclusion

In the present study, thioperamide had a synergistic effect in analgesia at the perimeter level on physostigmine. In addition, the antagonist of histamine H3 receptors inhibited analgesic effect of physostigmine. Atropine injection before thioperamide prevented the effect of reduction of the pain from thioperamide. In addition, atropine also prevented the analgesic effect of thioperamide. Thus, these data show that thioperamide through H3 receptors may have catalytic role on physostigmine action, and muscarinic receptors may interfere with the histaminergic inhibitory effect on pain. The biochemical, pharmacological, and behavioral findings have revealed that physostigmine affects histamine release and performance in the central nervous system through muscarinic receptors, but there is not any report to indicate interfering cholinergic and histaminic system in formalin-induced plantar pain. In TMN nucleus that the cell body of neurons is located in the histaminergic system, physostigmine depolarized histaminergic neurons and increased their irritability [11].

The findings of this study indicate that the cholinergic system may environmentally involve in analgesia induced by histamine H3 receptors. Also, Mobarakeh et al. (2009) by using Syrians without histamine H3 gene reported that histamine has an inhibitory effect on the analgesic impacts of morphine in the spinal cord through H3 receptors [7]. The reasons for this paradox may be that histamine released from the inhibition of peripheral histamine H3 receptor is led to stimulation of receptors for pain, because the involvement of local histamine has specified in creating the plantar formalin pain [12] and also released histamine can involve in increasing the pain by activating the peptides causing pain, such as substance P [13]. However, other actions of the brain, including hormone secretion, conditioned place preference and feeding, interaction between histamine H3 receptors and physostigmine have been reported [14, 15].

In this study, atropine prevented from the induced pain by physostigmine. Atropine is a competitive antagonist of muscarinic receptors with high affinity for the M_1 receptors.

As a result, this study suggests that on the environmental level, histaminergic and cholinergic systems have the interfering in the regulation of pains originated by plantar and muscarinic receptors and histamine H3 receptors are involved in the interaction between thioperamide and physostigmine.

References

1. Hejazian, S. (2007). Investigation of analgesic opioid receptors from extract of hops (*Humulus lupulus* L.) in Syrian rats. *Medicinal and Aromatic Plants of Iran research*. 23 (2): 73-166.
2. S.R. M, H. J., E. A., and F. D. Effect of age on cholinergic and adrenergic muscarinic system response of isolated atrium of rat's heart.
3. E. Tf, A. Ap. Effect of intraperitoneal injection of vitamin C on formalin- and histamine-induced pain and edema in rats.
4. Mirhadi, Kh.N., M. N., and S.H., T. Comparison of formalin pain behavior in normal and cholestatic male rats.