



Investigating and comparing effects of atropine and physostigmine in peripheral pain examination due to formalin injection on rats

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ABSTRACT

There are several neurotransmitters that feel the pain and processing nervous system, and until now cholinergic system has not been well studied in this field. The purpose of this research is investigating effects of atropine and physostigmine on the response of formalin pain test. We divided 50 male wistar head rats into 5 groups, first group (saline normal injection 5 μ), second group (1% formalin injection into 50 μ), third group (physostigmine injection 0/1 mg/kg), fourth group (atropine injection 2 mg/kg), fifth group (atropine injection 2 mg/kg and physostigmine 0/1 mg/kg), after formalin injection, the animals were placed inside mirror pain machine and it was recorded pain response at the time ranges 0-5 and 15-45. Results investigated with spss software and ANOVA and Duncan's test. Formalin injection causes pain response in both time ranges. Atropine injection alone had no effect on pain response. Physostigmine effect alone, with a significant reduction ($p < 0/05$) in the number of foot motions in both stage and duration causes of licking and biting in the 15-45 minutes stage. Atropine and physostigmine injections in fifth group cause significant reduction in the number of foot motions and duration of licking and biting in the time range of 15-45 minutes. Perhaps there is a close relationship between cholinergic system and peripheral pain that can be taken through the action of muscarinic receptors.

INTRODUCTION

Understanding pain is one of the most important functions of the nervous system that provide required information that associated with an injury and design appropriate reaction depending on stimulus type [1]. Pain is a complicated phenomenon and it includes a sensory component and emotional- exciting component. It means that pain is a sensory experience that accompany with motivating responses and with body and moving autonomous dependences. From this view, understanding pain is a necessary process and it is a prerequisite for the preservation and continuing life for human being [2]. Pain regulation is a complicated process that depends on many factors such as physiological, nervous, and hormonal, some environmental events with change in chemical mediators that are secreted in the body causes decrease or increase pain sensibility and so it decrease or increase pain. It is important to understand chemical mediators that relief pain. Several areas of the central nervous system have an important role in transmission and processing of pain species that some of the most important include hypothalamus, thalamus, body sensory cortex, cingulate cortex, hippocampal formation, amygdala, gray matter around the aqueduct, sylvius, habenula, insular cortex, stratum, and cerebellum [3]. In this regard, it is used formalin widely, and behavior reactions is standard and it is known as formalin test. Formalin test is one of the standard tests for measuring responses to chemical painful stimuli that first introduced by Dobson and Dennis in 1977 [4].

Hippocampus, with using neural mediators such as muscarinic, GABA, serotonin, and histamine would interfere in diverse biological functions including memory and learning, anxiety, and brain arousal [5, 6]. Histaminergic mechanisms may be related to the cholinergic system and plays an important role in modulating some of the cholinergic behaviors. Our findings indicate that in peripheral level, cholinergic system may have an important role in analgesia induced by histamine H3 receptor inhibitors. Also, with using rats without histamine H3 gene, Mobarakeh and colleagues have reported that histamine in spinal cord level through H3 receptors has inhibitory effects on the analgesic effects of morphine [7]. Physostigmine is an herbal alkaloid that not only stimulates muscarinic and nicotinic sites in the autonomic nervous system, but also it stimulates nicotinic receptors in nerve-muscle binding. Duration of effect is about 2-4 hours. This drug increases bladder and bowel movements and its use in reposition of the organs. This drug causes miosis in the eye and it reduces pressure inside the eye and it used to treat glaucoma, over the past seventy years, the researches that were done on histamine, it is focused on the role of histamine on allergic diseases [8]. Thus, for investigating the role of the cholinergic system, it used physostigmine [muscarinic receptor agonist] and atropine [muscarinic receptor antagonist]. The purpose of this study is investigating and comparing effects of muscarinic receptors agonist such as physostigmine, and muscarinic receptors antagonist such as atropine in formalin pain on male rats.

MATERIALS AND METHODS

In this research, it used 50 adult male wistar rats with the age range 120-130 days, with an approximate weight of 200-220 gr, that accidentally divided into 5 groups as follow: first group (saline normal injection of 50 ml), second group (50 ml of 1% formalin injection into the paw), third group (subcutaneous injection of physostigmine as 0/1 mg/kg), fourth group (subcutaneous injection of atropine as 2 mg/kg), fifth group (subcutaneous injection of atropine as 2 mg/kg and subcutaneous injection of physostigmine as 0/1 mg/kg). In the third group, physostigmine for 20 minutes and in the fourth group atropine for 40 before formalin injection was injected, in fifth group, first injected atropine and after 10 minutes physostigmine were injected, and finally after 20 minutes, it was injected formalin into animal's paw.

Method of pain in animals: To assess pain for all groups it was used formalin test that first time Dobson described it in 1977 (9). According to this method, 50 ml of formalin 0/1% by insulin syringe were injected into the animal's foot.

Investigating the pain reaction: Pain response recorded by measuring time duration of foot licking and biting (10). Plantar injection of formalin in the foot region causes pulling up foot rapidly that is associated with escaping and shouting. Immediately after formalin injection, the animal is placed inside mirror pain machine, of course the animals were placed inside mirror pain machine for 30 minutes before starting test to adapt the conditions. Plantar formalin pain behavior, is a two-step procedure that was the intervals 0-5 minutes and 15-45 minutes respectively as the first and second phases of the pain, thus, after formalin injection, it was recorded amount of foot licking and foot hit in the time blocks of 0-5, 15-20, 20-25, 25-30, 30-35, and 35-40 minutes.

Methods of statistical analysis

Data of normal saline and formalin injection by using repeated measures factor (factorial) and thus SPSS software version 20 and ANOVA test were analyzed. It was considered Significant level of $P < 0/05$. In the factorial designed examination, that is as 2^3 , we used the following statistical model:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ij}$$

μ = Average population, A_i = effect of i^{th} level of atropine, B_j = effect of j^{th} of physostigmine, AB_{ij} = Atropine and physostigmine interaction, ϵ_{ij} = effect of random error with zero average and variance σ^2 .

RESULTS

Investigating pain response after formalin plantar injection:

According to figures 1 and 2, time duration of licking and hit after formalin plantar injection in time intervals 0-5 and 15-40 minutes, it shows a significant difference compared to saline normal group.

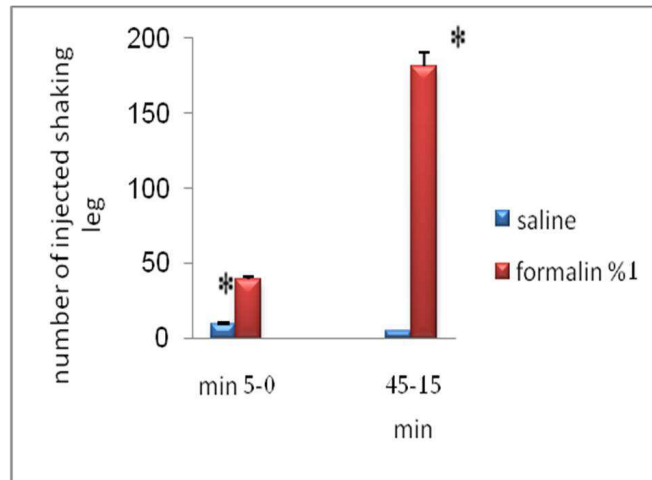


Figure 1 : Number of shaking leg after formalin injection 1%

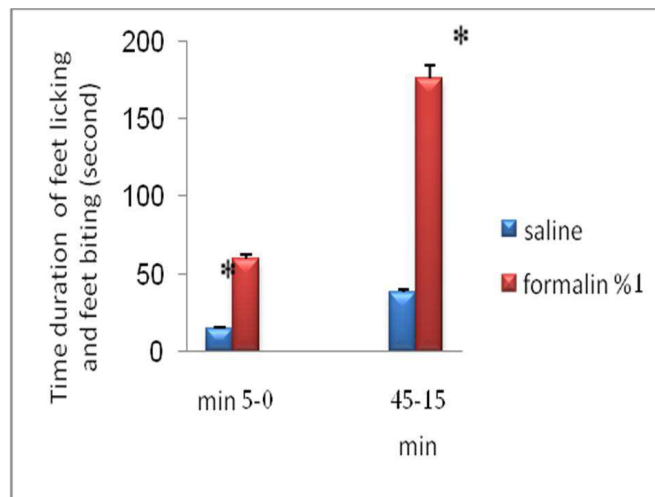


Figure2: Time of foot licking and biting after formalin injection 1%
It shows significant difference at (p< 0/05) level

Investigating effect of subcutaneous injection of atropine to pain response after formalin plantar injection.

According to Figure 3 and 4, atropine injection did not produce significant effect on pain response.

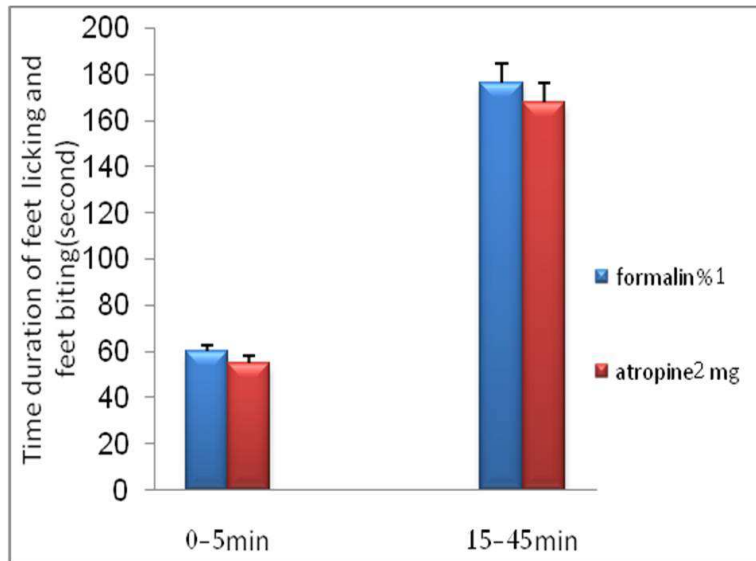


Figure3: number of foot shaking after atropine injection

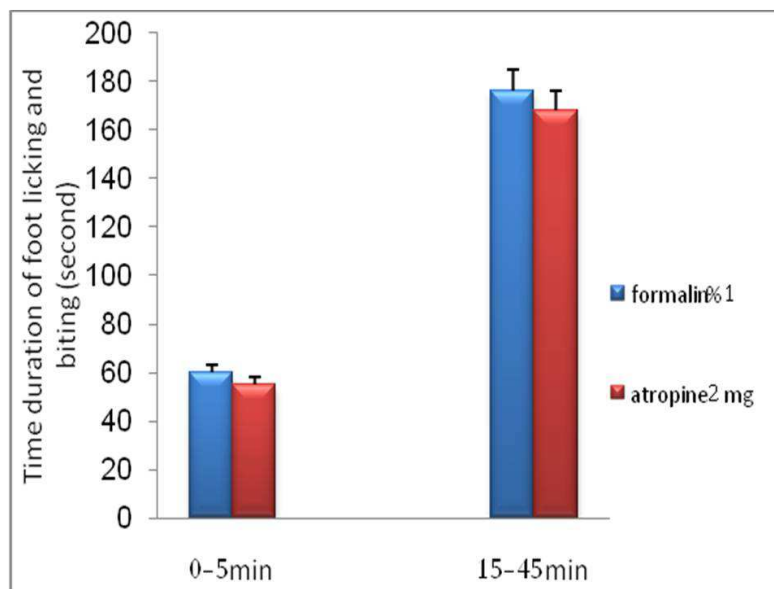


Figure4: Time of foot licking and biting after atropine injection

Investigating effect of subcutaneous injection of physostigmine on pain response after formalin plantar injection

According to Figures 5 and 6, subcutaneous injection of physostigmine reduced pain response on both first and second phases significantly.

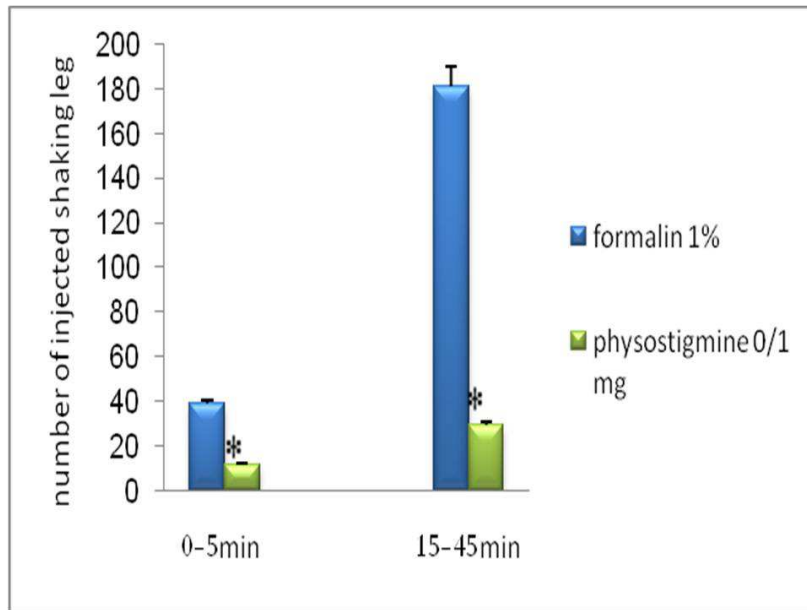


Figure 5 : Number of foot shaking after physostigmine injection

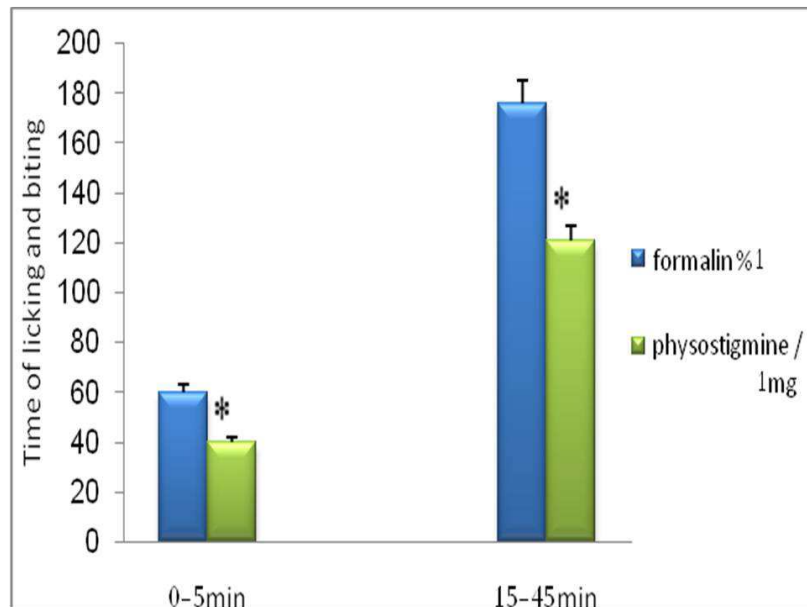


Figure6: Time of foot licking and biting after physostigmine injection
It shows significant difference at ($p < 0.05$) level

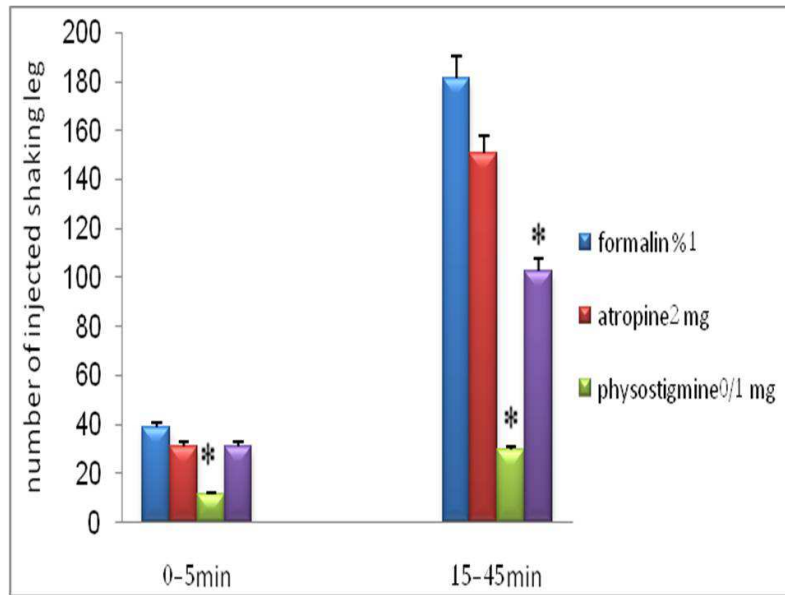


Figure 7 : number of leg shaking after atropine and physostigmine injection

-Interference function between atropine and phsostigmine on pain response after formalin plantar injection

According to Figure 7 and 8, atropine injection alone has no effect on pain response, but if it injected before physostigmine, it can reduce pain response significantly at time range of 15-45 minutes.

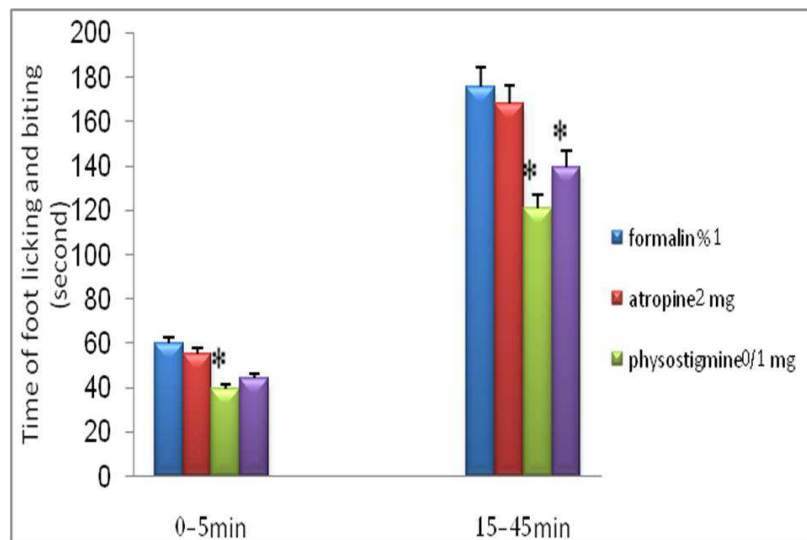


Figure8: Time of foot licking and biting after atropine and physostigmine injection
It shows significant difference at (p< 0/05)level.

DISCUSSION AND CONCLUSION

The results show that normal saline injection with the volume of 50 ml under inner skin surface of rat paw, it takes very mild reaction behavior for just 5 minutes after injection. Normal saline injection in different volume depending on research method used as control in most studies and perhaps the most important reason to use is that the solution is isotonic that does not create Tonus and pressure in injection site . Weak reactions of pain in the first five minutes after the injection of normal saline can be due to entering needle under the skin. Despite subcutaneous injection in this study is carried outwith needle number 28, but entering needle in tissue with every number can be due to

stimulation of pain receptors that is often associated with pain responses. In previous studies, normal saline injection in a volume of 50 microliters in paw rats, weak pain response has been reported in 5 minutes after injection. According to Figures 1 and 2, time of foot licking and hit after formalin plantar injection indicates in concentration of 1%. After formalin plantar injection in concentration of 1%, in the first 5 minutes of the third to eight time blocks, a significant difference [$p < 0/05$] was observed. In other words, two phases of formalin pain identified [firststage: 0-5 minutes and second stage: 15-45 minutes]. As a result, two phases of formalin pain in present study, there is no conflict with previous reports and clearly explicit that formalin is two phases pain. The first phase of formalin pain is a neurogenic pain and it created with stimulation of pain receptors directly and in this phase there is no chemical mediator. Second phase of formalin pain is a inflammatory pain such as prostaglandins, bradykynin, histamine and enzymes. Results of this study shows that formalin plantar injection to rat's paw creates a two phase's behavior that associated with foot hit and licking injected area. It is reported that subcutaneous injection of 5% formalin into rat upper lip create a two-stage scrub [11], also Klavoy and colleagues have investigated effect of different concentrations of formalin on pain behavior in rats and reported that formalin in higher concentration of 0/5 creates a two phases pain and for creating plantar pain in rats, it should select concentration between 0/5 to 5 [12]. And also subcutaneous formalin injection in rabbits and sheep to animal's ear creates a one-stage pain for 10 minutes after injection [13].

Effect of cholinergic system on plantar formalin pain response

In this study, physostigmine subcutaneous injection in plantar pain test of formalin, it was created anti pain. In addition, atropine subcutaneous injection alone had no change in pain, after atropine injection, it blocked analgesia induced by physostigmine. These findings indicate that cholinergic receptors may be involved in pain regulation. Several nervous transporters are involved in pain processing, and process nervous system. But the role of cholinergic system in this area has not been well studied. To evaluate effect of anti cholinergic on pain, effect of atropine subcutaneous injection [2 mg/kg] in male rats, it was studied chronic pain by formalin test. The result of this examination showed that rats receiving atropine had higher pain threshold than control. The pain relieving effect of atropine in experimental rats was not significantly different, the finding of this study indicate that anti cholinergic agents such as atropine can reduce chronic pain. This study in order to better understand antagonists effect of muscarinic anti cholinergic [atropine] on chronic pain and its mechanism. Jeraldini and colleagues were investigated effect of pain relieving of atropine by IN VITRO and IN VIVO methods. In IN VIVO study low concentration of atropine increased contraction due to electrical and nicotine stimulation in indian pigileum but in high concentration inhibit contraction due to electrical and nicotine stimulation. In IN VIVO study, prescribing inner brain venter of atropine reduced acute visceral pain in mice and rats. Based on these results, a possible mechanism of pain relief of atropine has been related to muscarinic post synaps receptors and muscarinic M1 receptor has been involved in this process. In addition, based on the result of this study, effect of atropine as a parasympathetic combination can have pain relief property [14]. Emphasis Study and investigating mechanism involved in onset of pain and pain management is that it is the main section of medicine activities of animals and human. Much of the study of neurotransmitter systems involved in pain perception has been directed to investigate opioid system and it is not involved the role of other neurotransmitters especially cholinergic system. However, in recent years, the results of some studies has been concentrated on investigating possible relationship between cholinergic system function and feeling pain or controlling based on the role of this system in processing pain. This study is to understanding muscarinic antagonists and anticholinergic [atropine] on chronic pain and its relating mechanisms. The results obtained in this study suggest that average threshold of chronic pain in male test group is higher than male control group. It means that male rats that receiving atropine in ratio with rats that just received physiologic serum has significantly lower chronic pain. Five types of receptors have been described in the cholinergic system: receptors M₁, M₂, M₃, M₄, M₅. This five recipients have the main role in cholinergic system including pain behavior and analgesia, stress, tolerance and addiction, learning and memory, and neurological and movement disorders [15]. Physostigmine act via M₁ receptors and competitive atropine antagonist of muscarinic receptors of cholinergic system are with high affinity for M₁ receptors. Physostigmine and atropine frequently to examine the role of cholinergic system in peripheral mechanisms of pain and analgesia, spinal cord, and spinal trigeminal are used. The use of physostigmine in formalin pain, it reduced pain in rats in first and second stages and atropine focal injection inhibited the analgesia effect of physostigmine completely. Thus, it can be expected that cholinergic system regulate receptor mechanisms in pain peripheral levels, and this study showed that activating and inhibiting cholinergic system in peripheral level effects on pain receipt with plantar source.

Appreciating

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