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Research Article

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The Effect of the Histaminergic System on the Nociceptive Response to Plantar Injection of Formalin in Rats

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ABSTRACT

The present study aims to review the effect of the histaminergic system on the plantar nociception process in rats. In this study, adult male Wistar rats in the weight range of 200 to 250 grams were used. The rats were divided into groups of six and they were put in plastic cages in a room with desirable temperature of about 23±2ºC, desirable environmental conditions and 12 hours of light. They were fed with commercial pellet food. They had 24/7 access to food and water. All of the experiments were performed in the time interval of 8 to 15 hours. 5/2 Mg/Kg thioperamide solution was used as the H3 receptor antagonist along with normal saline. There were 6 rats in the first group, i.e. control group. In this group, the nociceptive response to plantar injection of normal saline was reviewed. In the second, third and fourth group the nociceptive response was reviewed 30 minutes after the intraperitoneal injection of 1, 2.5 and 5mg thioperamide per one kilogram of body weight was reviewed. There were 6 rats in each of these groups. At first, 2 Mg/Kg of atropine was subcutaneously injected and ten minutes later, there was an intraperitoneal injection of 15Mg/Kg of thioperamide. After 20 minutes, formalin was injected to the foot plantar of rats and their nociceptive response to formalin was studied. The results showed that intraperitoneal injection of thioperamide causes a significant decrease ($P<0.05$) in the nociception (licking and hitting the injected foot) in the second phase. Intraperitoneal injection of 1 and 2.5 Mg/Kg thioperamide reduces the nociception as hitting the foot in the first phase and in the second phase in comparison with 1 and 5 Mg/Kg thioperamide. It can be concluded that the effect of thioperamide seen in the present study is because of inhibiting the H_3 receptors.

Keywords: *Pain Receptors, Thioperamide, Histaminergic System, Rat*

INTRODUCTION

Sensing pain and perceiving it are some of the most important functions of the nervous system which provides the necessary information associated with a damaging or a potentially damaging stimuli. It designs the suitable reaction given the type of the stimulus. Pain is a complex phenomenon and includes both sensory and emotional components.

This means that pain is a sensory experience which is accompanies by incentive responses and also by somatic and motor adaptations. From this perspective, nociception is an essential process is a necessary process and it is a prerequisite for a living creature to survive (1). To put it simply, pain is a protective sensory experience to make the person aware of harms and damages to the body tissues. One of body's defense mechanisms that protects the tissues and organs against harm is the sense of pain which makes the central nerve system aware of the part of body that is being damaged. That is how the patient would think of a solution. Regulation of pain is a complex process which depends on many physiological, neural and hormonal factors. Sensitivity to pain and nociception might be increased or decreased because of some environmental occurrences along with changes in the chemical mediators that are released in the body. It is significantly important to know these chemical mediators in order to soothe the pain. Numerous parts of the central nervous system play roles in transferring and processing different types of pain. Some of the most important ones are Hypothalamus, thalamus, somatosensory cortex, cingulate cortex, hippocampal formation, amygdala, Sylvius periaqueductal gray matter, Habeluna, insular cortex, striatum and cerebellum (2). There are considerable evidences that suggest that sensory stimuli are even perceived without the cortex and this is true for pain in particular. These areas in the cortex are apparently responsible for an accurate and significant discriminating interpretation of pain and some of its emotional components. However, the cortex is not needed for the nociception alone (3).

The histaminergic system is a aminergic systems of the brain of mammals which interferes in regulate many of the brain functions including food intake, cardiovascular and respiratory functions, neuroendocrine responses, learning and memory through its four receptors (H_1, H_2, H_3 and H_4) (4, 5). Histamine is one of the aminergic neurotransmitters which plays a key role in regulating some of the physiologic and pathophysiologic occurrences. In mammals, brain histamine is made in a limited number of neurons which can be found in the tubermammillary nucleus of posterior hippothalamus. These neurons penetrate most of the parts of the brain and interfere in many of the functions of the brain including sleeping, being awake, hormonal secretion, cardiovascular control, regulating body temperature, food intake and memory formation (6).

Hippocampus uses nervous mediators such as muscarinic, gaba, serotonin and histamine to interfere in various biological functions including memory, learning, anxiety and arousal (7, 8). Histaminergic mechanisms might have an important role in moderating many cholinergic behaviors and be associated with the cholinergic system. In order to determine the probable effect of histaminergic mechanisms in the formalin-induced pain, the effect of various agonists and antagonists of histamine receptor on the formalin-induced formalin in rats has been reviewed. The findings show that the cholinergic system, at a peripheral level, might play a role in the analgesia caused by inhibiting histamine H_3 receptors. In addition, Mobarakeh et al (2009) used rats lacking the histamine H_3 gene and reported that the histamine H_3 receptors in the spinal cord has an inhibitory effect on the morphine-induced antinociception (9). Therefore, by taking into consideration that there haven't been many studies that focused on the effect of the histaminergic system on the responses to the formalin-induced pain, the present study aims to clarify this point in rats.

Materials and Methods

In this study, adult male Wistar rats were used. These rats weighed 200 to 250 grams and they were bought from Faculty of Veterinary Medicine of Tehran University. The rats were divided into groups of six and they were put in plastic cages in a room with desirable temperature of about 23±2ºC, desirable environmental conditions and 12 hours of light. They were fed with commercial pellet food. They had 24/7 access to food and water. All of the experiments were performed in the time interval of 8 to 15 hours. 5/2 Mg/Kg thioperamide solution (Sigma-aldrich Co) Thioperamide maleate salt) was used as the H3 receptor antagonist along with normal saline. There were 6 rats in the first group, i.e. control group. In this group, the nociceptive response to plantar injection of normal saline was reviewed. In the second, third and fourth group the nociceptive response was reviewed 30 minutes after the intraperitoneal injection of 1, 2.5 and 5mg thioperamide per one kilogram of body weight was reviewed. There were 6 rats in each of these groups. At first, 2 Mg/Kg of atropine was subcutaneously injected and ten minutes later, there was an intraperitoneal injection of 15Mg/Kg of thioperamide. After 20 minutes, formalin was injected to the foot plantar of rats and their nociceptive response to formalin was studied.

In order to review the sense of pain in all groups, the formalin test was used. This text was first presented by Dubisson (1977) and it is now a valid method used for reviewing chronic pain. In the present study, the somatic pain was created through a plantar injection with 5% formalin with a 50µL volume and then it was reviewed. As it was already mentioned, using various concentrations of formalin creates pain in the rats' plantar. On the other hand, Responses to pain were recorded through measuring the duration of licking and biting the injected foot. According to the cited experiences, it is way better to record the behaviors of the rats than to use the scoring method (10). In this method, we have a plantar subcutaneous injection of formalin.

The rats were lightly kept with a towel and they are injected with 50 µL formalin solution with the concentration of 1% on the foot plantar using the needle number 28. When the foot plantar is injected with diluted formalin, the animal immediately reacts by pulling back the foot and whines and tries to run. The rats are instantly put inside the pain mirror so that their behavioral response to pain would be reviewed. The response to pain caused by plantar injection of formalin has two phases. In this present, the animal's behavior in the first five minutes and in the time interval of 15 to 40 minutes were considered as the first and second phase of pain. Figure 3-4 shows the animal licking the injected body part after it was injected with formalin in the pain mirror device.

Figure 1 – licking the injected part of the body after plantar injection of formalin in the pain mirror device

Review of pain behavior

The pain mirror device was used to create the behavior caused by plantar injection of formalin which was then reviewed. This device has one basis and one box. The box is made of shatterproof glass with the dimensions of 25×30×30 on a framework and it has a mirror with a 45-degree angle (figure 2). It is because of the 45-degree angle that every move the animals make can be monitored. There are various factors that might make the animals stressed such as putting them in a cage, keeping them awake, separating one from the group, moving them from one room to another with different lighting and smell. Since there are numerous stressful factors, thus the researchers must try to minimize these factors (11). In order create a compliance between the animals and the environment, they are transferred to the laboratory four hours before the experiments begin and they are placed inside the glass box in the pain mirror device half an hour before the beginning of the test. The animals are taken out of the box to be injected with formalin and are put back in it after the injection. Figure no. 2 illustrates the pain mirror box used in this study.

Figure 2 – the pain mirror device used in this study

The statistical analysis method

The data obtained from plantar injection with normal saline (control group) and with formalin (experimental group) was analyzed using the statistical method factor repeated measures (factorial) and then the Duncan. On the other hand, the data obtained from the experiment where the solution was injected was analyzed by one-way analysis of variance method (ANOVA) and then the Duncan test was used. The significance level has been $P<0.05$. In the experiments associated with determining the response, the proper dose of the substance has been specified by processing different nonlinear models such as second-pseudo, broken line, broken line with two breaks and exponential function, etc. The rate of determination coefficient has been selected as the best model and the desirable response is obtained from it. The GLM procedure of the SAS software was used for the analysis of variance and the Tukey method was used to compare the means.

Figures 3 and 4 illustrate the duration of licking and hitting the injected foot after the plantar injection with normal saline and formalin at the concentrations of 1, 2.5 and 5%. Figure 5 and 6 show them in 5-minute intervals (0 – 5 and 15 – 40). intraperitoneal injection of thioperamide causes a significant decrease ($P<0.05$) in the nociception (licking and hitting the injected foot) in the second phase. Intraperitoneal injection of 1 and 2.5 Mg/Kg thioperamide reduces the nociception as hitting the foot in the first phase and in the second phase in comparison with 1 and 5 Mg/Kg thioperamide.

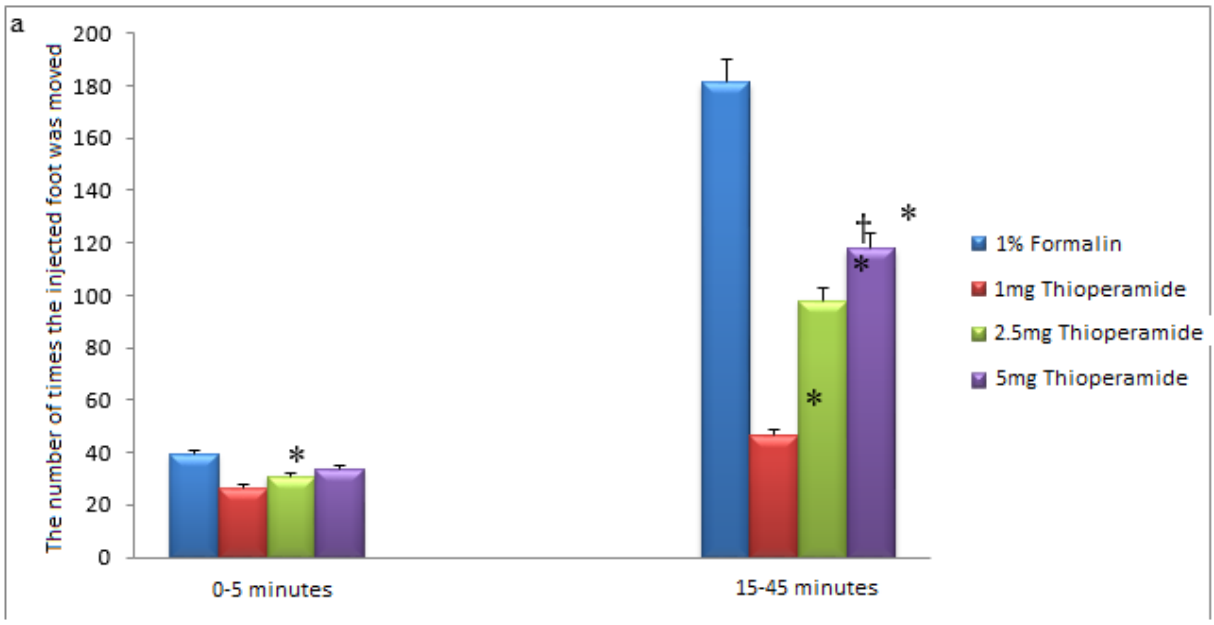

Figure 3 – the number of times the injected foot was moved (intraperitoneal injection of Thioperamide)

* Shows that there is a significant difference at the level of P<0.05 compared with plantar injection with normal saline and other time intervals take 5 minutes.

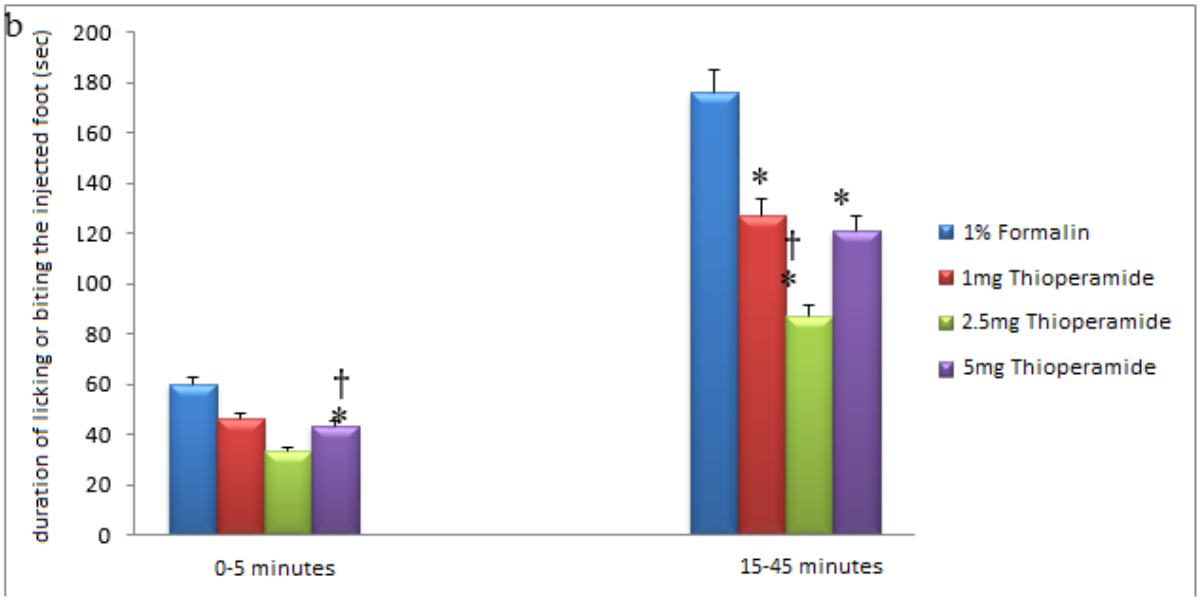

Figure 4 – duration of licking and biting the foot (intraperitoneal injection of Thioperamide)

* Shows that there is a significant difference at the level of P<0.05 with the groups with 1% formalin.

* Shows that there is a significant difference at the level of P<0.05 with the groups with 1mg and 5mg thioperamide receptors.

Results and Discussion

In the present study, intraperitoneal injection of thioperamide causes a significant decrease in the pain caused by plantar injection of formalin in the first and the second phase. Thioperamide tends to function better with H_3 and H_4 receptors especially with H_3. However, the H_4 receptor and the gene expressing it are released in Hippocampus and plays a role in learning, memory and brain spark.

Intraperitoneal injection of thioperamide decreases the pain caused by physostigmine. This shows that histamine H_3 receptor can practically reduce the peripheral histamine pain.

Histamine H_3 receptor functions as the autoreceptor in the end of an axon of histaminergic neurons. The release of histamine in the histaminergic synapses can be decreased by activating this receptor using histamine H_3 receptor antagonists. Conversely, it can be increased by inhibiting this receptor with thioperamide (histamine H_3 receptor antagonists).

Histamine H_3 receptor functions as a heteroreceptor at the nerve ending of other neurotransmitter systems such as cholinergic, gabaergic, serotonergic and glutamatergic systems and interfere in stimulating or inhibiting the release of other nervous mediators such as acetylcholine and serotonin. Accordingly, it is possible that the reduction of pain caused by the intraperitoneal injection of thioperamide is associated with increasing histamine or other mediators in the present study; because by activating the gabaergic and cholinergic systems, pain is decreased.

However, it is important to note that as we inhibit or activate the H_3 receptor in the peripheral tissues and in the spinal cord, the obtained results do not comply with those of the present study. This contrast between the results is because of the fact that the histamine released because of inhibiting the peripheral H_3 receptor of histamine stimulates pain receptors; because it has become evident that local histamine plays a role in creating pain after plantar injection of formalin and injecting it to mouth or face.

Moreover, the present study showed that the physostigmine response is diversed by injecting atropine and therefore, the muscarinic receptors play a mediatory role in the physostigmine effect.

On the other hand, injection of thioperamide alone reduces the pain and injecting thioperamide before physostigmine helps the effect of physostigmine in reducing pain. Accordingly, it can be argued that the histaminergic system plays a role in regulating formalin-induced pain through the H_3 receptor. Histamine is one of the local mediators of inflammation which plays a role in both of the phases of formalin-induced pain (12). On the other hand, the histaminergic system is one of the four aminergic system of the brain of mammals which has an important role in processing the input data. Histamine can be found in two main parts of the brain: neurons and mast cells. Mast cells are rare in the brain (5). The histaminergic system uses 4 of its receptors in the physiologic processes such as fluid balance, appetite control, temperature regulation, cardiovascular responses, motivation, anxiety, being awake, learning, memory and gaping (5, 13). The cell body of the neurons of the histaminergic system is accumulated in the tumeromammillary nucleus of hypothalamus and sent their axons all around the nervous system (4, 5). Hippocampal formation of low to moderate receive histaminergic processes and the distribution of H_1, H_2, H_3 and H_4 receptors in various parts of the limbic system has been proven (14). The role of the histaminergic system as the regulator of pain through injecting it to cerebral ventricles and brain nucleuses has been reviewed. Intracerebroventricular injection of histamine in small laboratory white mice has been effective, in such a way that it reduces pain in testing the pain induced by electrically stimulating their tail, hot plate and intraperitoneal injection of acetic acid and phenylalanine (15).

There are different neurotransmitters and neuromodulators that are effective on the role that hippocampus plays in reducing pain, analgesia and regulating the pain processes. Using formalin-induced pain in rats has specified the role of glutamatregic and serotonergic systems in the hippocampus and dentate gyrus in regulation of pain (16, 17). Four types of specific receptors have come to be known for the functions of histamine (H_1, H_2, H_3 and H_4), all four of which can be found in the brain. The H_3 receptor, as the pre-synapsis autoreceptor, prevents the release of histramine from the nerve ending of the histaminergic neurons in the brain of rats (4, 5). As the empirical experiments show, three of the histamine receptors (H_1, H_2 and H_3) can play a role in the pain processes. Other neurotransmitter

systems such as cholinergic, gabaergic, serotonergic and glutamatergic systems and interfere in stimulating or inhibiting the release of other nervous mediators such as acetylcholine and serotonin (4, 5). Accordingly, it is possible that the reduction of pain caused by the intraperitoneal injection of thioperamide is associated with increasing histamine or other mediators in the present study; because by activating the gabaergic and cholinergic systems, pain is decreased (18).

However, it is important to note that as we inhibit or activate the H_3 receptor in the peripheral tissues and in the spinal cord, the role of these receptors in the peripheral and spinal regulation of pain has been specified to some extent (19, 20). On the other hand, the intracerebroventricular injection of thioperamide reduces pain and the intracerebroventricular injection of R-alpha methylhistamine increases pain (21). According to the recent reports, GSK189254, which is one of the selected and strong antagonists of H_3 receptors in a few empirical models, has been able to reduce the pain felt by rats (22). In this regard, injecting thioperamide in the dentate bumps leads to the reduction of the pain induced by plantar injection of formalin in rats in the first and the second phase and it helps reduce the pain induced by injecting histamine in the dentate bumps (17).

Thioperamide tends to function better with H_3 and H_4 receptors especially with H_3(23). However, the H_4 receptor and the gene expressing it are released in the hippocampus of rats (24). It can be argued that the effect of thioperamide seen in this study is because of inhibiting the H_3 receptor.

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