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Effects of cholinergic system in antinociception induced by H_1 and H_2 receptor antagonists on somatic pain in Rats

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ABSTRACT

The present study was aimed to investigate the peripheral effects of chlorpheniramine and ranitidine and their relationship with cholinergic system on the somatic pain in rats. The somatic pain was induced by using formalin test. The effects of H1 and H2 receptor antagonists, chlorpheniramine and ranitidine, respectively, on formalin-induced pain was studied in rats. Physostigmine and atropine were subcutaneously injected alone and also in combination with chlorpheniramine and ranitidine. Formalin 1% produced biphasic pain response. Chlorpheniramine and ranitidine significantly reduced the second phase of pain ($p<0.05$). Physostigmine at doses 0.4mg/kg significantly reduced the second phase of pain. Atropine (2 mg/kg) had no significant effect in the first and second phases. Pre-treatment of chlorpheniramine (20mg/kg) before physostigmine (0.4mg/kg) prevented physostigmine induced antinociception. Ranitidine (40mg/kg) before physostigmine (0.4mg/kg) significantly suppressed the antinociceptive effects of physostigmine. Atropine before chlorpheniramine and ranitidine reversed the analgesic effects of chlorpheniramine and ranitidine. These results indicate that physostigmine has been able to inhibit the somatic pain through the cholinergic muscarinic receptors. Both of the histamine H1 and H2 receptor antagonists have analgesic effects and histamine H2 but not H1 antagonist probably is involved in the analgesic effects induced by physostigmine.

Keywords: Histamine H1 and H2 antagonists, Cholinergic System, Somatic pain.

INTRODUCTION

Pain is usually created due to destruction or damage to a tissue caused by chemical, thermal, mechanical and electrical triggers [1]. It usually appears in two intense acute and chronic forms and both of them create some problems in human and can prevent from doing the daily activities as a limiting or disabling factor. For the same reason since the human recognized pain, he was trying to find its reason and the way of eliminating it. In the studies done by American Pain Society, just in the US, 50 million people in different ages suffer from pain and more than 100 million dollars is spent in order to control their pain [2, 3]. New attitudes in discovering the analgesic drugs deal with cholinergic factors, cyclooxygenase 2 inhibitors, opioids influencing specific receptors, NMDA receptor antagonists, GABA agonists, tramadol-like agents and so on [4]. Recently, anti-histamines have been considered as the analgesic factors. The analgesic effects of chlorpheniramine (histamine H1 receptor antagonist) and ranitidine (histamine H2 receptor antagonist) have been reported in acetic acid-induced visceral pain [5]. Different reports have been presented for the role of histamine H2 receptor in pain responses. Zolantidine, H2 antagonist passing the blood-brain barrier, reduced the pain in the tail-flick and the hot-plate tests and also reduced the analgesic effect of

morphine in tail-flick test [6]. On the other hand, the role of acetylcholine as the cholinergic agonist and cholinesterase inhibitor which are totally called cholinomimetic has been confirmed in modulating pain and analgesia [7].

In the tail-flick and visceral pain induced by acetic acid tests in rats, the analgesic effects have been reported from the intraperitoneal injection of physostigmine [8]. In some cases, there is interaction between the histamine antagonists and cholinergic agents in the brain and peripheral tissues. For example, in the yawning behavior induced by physostigmine, the role of both histamine H1 and H2 receptors has been reported [9]. Therefore, the aim of this study, the investigation of the effects of histamine H1 and H2 receptors on the formalin-induced somatic pain and also its relationship with cholinergic system in rats in order to determine the mechanism of action and interaction of effects of these drugs in pain process.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats, weighing 200–250g were used in this experimental study. Rats were maintained in groups of six per cage in 12-hours light-dark cycle (light on at 07:00hr) at controlled ambient temperature (20–23 °C) with *ad libitum* food and water. All experiments on animals were performed with observing registered and international ethics for working with laboratory animals and according to the guideline of National Institute of Health.

Drugs

Drugs used in the present study included chlorpheniramine maleate, ranitidine hydrochloride, physostigmine (Eserin), atropine sulfate, formaldehyde solution (formalin 37%, Merck, Germany). All drugs were purchased from Sigma-Aldrich Company in Germany. The drugs were dissolved in normal saline.

Nociceptive testing

Formalin test was used for induction of pain. Before performing the test, rats were placed individually in Plexiglas observation chamber (25×30×30 cm) for three successive days and each day for 30 min in order for them to become compatible the work method and to minimize the effects of the pain reducer factors such as the stress of new environment [10]. In this experiment, intraplantar injection of formalin was used in order to create and investigate the somatic pain; this is known as formalin test and has been innovated by Dennis & Dubuisson. Formalin test is the important animal model in the study of the acute long-term inflammatory pain [10]. For evaluating the formalin pain was used mirror pain apparatus. This device is consisted from the following parts: a steel frame which contains a slot for putting the glass, a rectangular steel chamber from Plexiglas in dimensions of 25×30×30 cm in order for putting the animal, a mirror which is put in the frame of device at an angle of 45 degrees. Mirror makes us able to observe the animal's ventral surface. After 30 minutes of adaptation period in the chamber, the animal was slowly brought out and after tying it with hands, the formalin solution 1 % in a volume of 50 microliter was injected subcutaneously in the ventral surface of the right hind paw by using the needle gauge 29[11,12]. The pain behavior including licking and biting the injected paw was recorded in 5-minutes intervals for one hour.

The obtained results were assessed by using single-way analysis of variance (ANOVA) and then they were analyzed by Duncan test and all data was expressed mean ± standard error of mean and evaluated in the significance level of $P < 0.05$[13].

RESULTS

The results obtained from the present study indicated that the intraplantar injection of formalin 1 %induced biphasic pain response (the first phase 0–5 min and the second phase 20–45 min after injection of formalin). The intraperitoneal injection of chlorpheniramine at doses of 5, 10 and 20mg/kg and ranitidine at doses of 20, 40 and 80mg/kg did not have a significant effect in the first phase of pain. The intraperitoneal injection of chlorpheniramine at doses of 5mg/kg did not have a significant effect, while the doses of 10 and 20mg/kg significantly reduced the pain response in the second phase ($p<0.05$). Ranitidine at doses of 20mg/kg did not have a significant effect in the second phase of pain, but the doses of 40 and 80mg/kg significantly reduced the pain response in the second phase ($p<0.05$) (Figure 1).

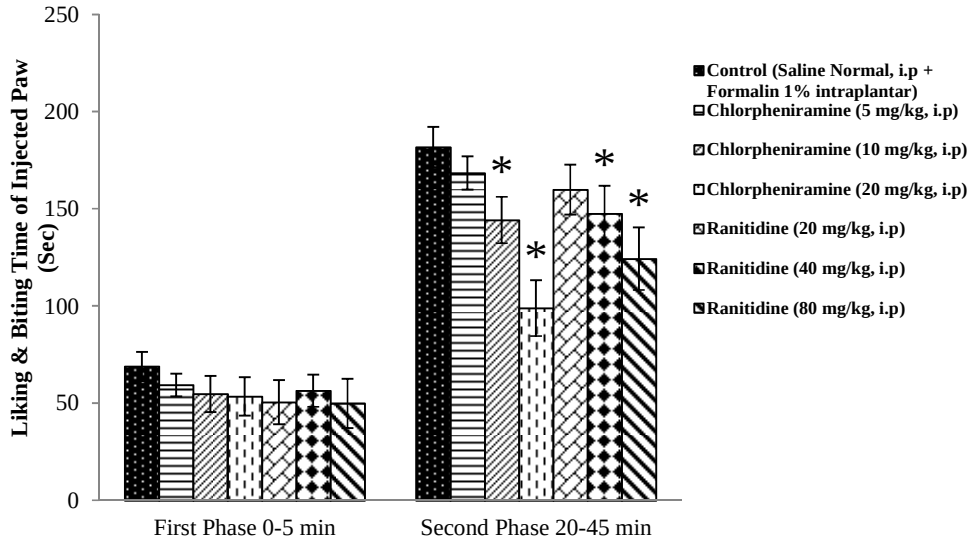

Figure 1: Effects of intraperitoneal (i.p) injection of chlorpheniramine and ranitidine on formalin induced somatic pain
Values are means ±SEM (n=6). P<0.05 significant vs. control group*

The subcutaneous injection of physostigmine at doses of 0.2, 0.4 and 0.8mg/kg suppressed the pain response in the second phase significantly ($p<0.05$). Atropine in dose of 2mg/kg had no significant effect in the first and second phases of pain. The pre-treatment of atropine before physostigmine at dose of 0.4 mg/kg prevented physostigmine induced analgesia (Figure 2).

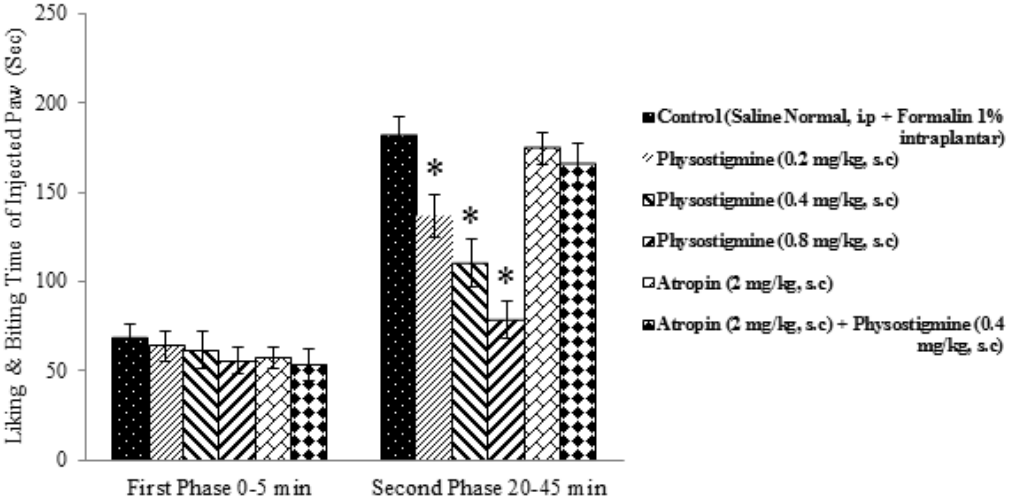

Figure 2: Effects of subcutaneous (s.c) injection of physostigmine and atropin on formalin induced somatic pain
Values are means ±SEM (n=6). P<0.05 significant vs. control group.*

Chlorpheniramine at dose of 20mg/kg before the subcutaneous injection of physostigmine at dose of 0.4mg/kg insignificantly inhibited the antinociceptive effects of physostigmine. The intraperitoneal injection of ranitidine at dose of 40mg/kg before physostigmine at dose of 0.4mg/kg significantly prevented by the antinociceptive effects induced by physostigmine ($p<0.05$). Pre-treatment with atropine before chlorpheniramine and ranitidine insignificantly reversed the antinociceptive effects of chlorpheniramine and ranitidine in the response of pain induced by formalin (Figure 3).

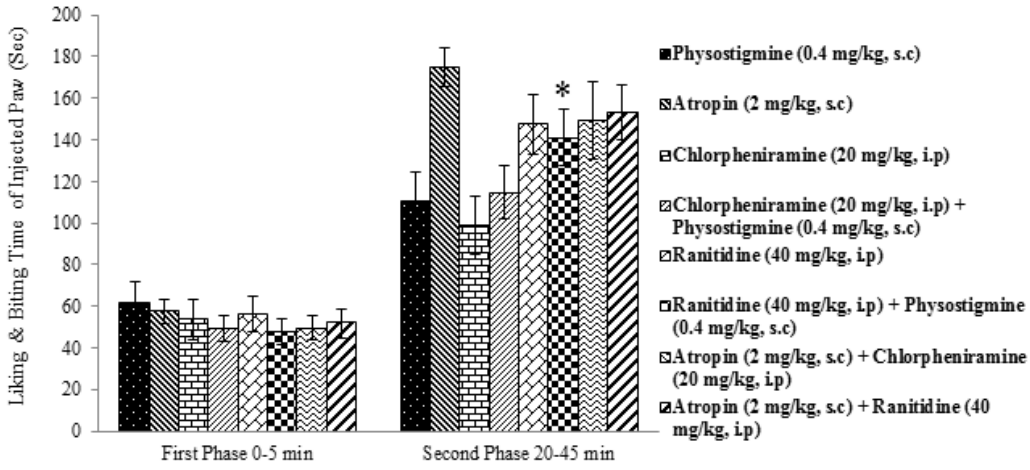

Figure 3: Effects of cholorpheniramine and ranitidine interaction with physostigmine and atropin on formalin induced somatic pain
Values are means ±SEM (n=6). P<0.05 significant vs. control group. physostigmine (0.4mg/kg).*

DISCUSSION

In this study, after the intraplantar injection of formalin 1 %, the behaviors of licking and biting the injected paw were created in the intervals of 0–5 min and the total time of 20–45 min after the injection. Regarding to the fact that in these 5 minutes intervals, the pain reaction was very much more intensive than the other 5 minutes intervals, we can concluded that the pain has been created in two-phase (the first phase 0–5 min and the second phase 20–45 min after injection) and the pain response has reduced between the two mentioned phases. After injection of formalin in concentrations of 1, 2.5 and 5 % and in volumes of 20 and 50 microliter in the rats, the incidence of pain responses has been reported in two-phase [14,15]. However, in animals for example rabbits and sheep, the response caused by the injection of formalin at doses of 5 and 10 % has been reported in a one-phase [16]. Therefore, the two-phase pain order created in the present study is compatible with the mentioned studies. However, the obtained results from this study about the pain responses induced by formalin are completely consistent with our previous findings and the findings of the others.

The results of present study indicated that physostigmine induced antinociceptive effects and atropine inhibited the analgesic responses induced by physostigmine. This shows that physostigmine probably plays a role in modulating pain through the muscarinic cholinergic receptors. There are several evidences that confirm the interference of physostigmine in modulation of pain. The subcutaneous injection of physostigmine has produced the antinociceptive effects in the neuropathic pain model in rats. As the muscarinic receptor antagonist, atropine has reversed the analgesic effects induced by physostigmine [17]. Also in the tail-flick tests and the visceral pain induced by acetic acid in mice, the antinociceptive effects have been reported cause by intraperitoneal injection of physostigmine [8]. In a study, the intrathecal injection of physostigmine has been able to suppress the pain response induced by formalin in both phases [18]. According to these findings, also the results of this study are consistent with the previous studies. In the present study, both histamine H1 and H2 receptors induced antinociceptive effects. In some reports, it has been referred that some of the histamine H1 and H2 receptors have created in laboratory animal models [19]. In the formalin test, the intraperitoneal injection of chlorpheniramine and cimetidine has reduced the pain response induced by formalin in mice [14, 15]. In a study, it has been reported that the intraperitoneal injection of chlorpheniramine and ranitidine has induced analgesic effects in the visceral pain model in rats [5]. In another study, the subcutaneously injection of dex-chlorpheniramine and ranitidine has suppressed the pain behavior induced by formalin in mice [20]. The results obtained from this study are also in accordance with the mentioned findings.

In this study, ranitidine but not chlorpheniramine inhibited the antinociceptive effects induced by physostigmine, and atropine could not reverse the suppressing effects of both histamine antagonists. These results show that there is probably an interaction between the histamine H2 receptors but not H1 receptors with the physostigmine in modulation of pain and analgesic effects. In a study, it was determined that ranitidine, but not famotidine has increased acetylcholine in the myenteric neural network in guina pigs [21]. Also nizatidine and ranitidine but not

famotidine (histamine H2 receptors antagonists) have had stimulation effects in the acetyl cholinesterase activity and bicarbonate secretion in the duodenum of rats [22]. This difference in findings is probably related to the type of the used test and also the kind of the used histamine antagonists. Positive and negative interactions have also been reported in the other physiologic actions such as memory and learning, yawing, secretion of gastric acid [9, 23, and 24].

CONCLUSION

In conclusion, according to the findings of this study, it can be concluded that physostigmine has been able to inhibit the nociception induced by formalin through the muscarinic cholinergic receptors. On the other hand, both of the histamine H1 and H2 receptors antagonists have induced the antinociceptive effects through reducing the pain intensity in the second phase; and the histamine H2 receptors antagonist but not H1 probably interfere in the analgesic effects caused by physostigmine. In order for clarifying the effect mechanism, it is necessary to investigate the other histamine receptor antagonists in the other models, too.

Conflict of Interest
No conflict of interest associated with this work.

Contribution of Authors
I declare that this work was done by the author named in this article and all liabilities pertaining to claim relating to the content of this article will be borne by the authors.

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