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# Central interaction between physostigmine and histamine during yawning in rats

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**Abstract:**
In this study, the effects of intraperitoneal (*ip*) injection of physostigmine, subcutaneous (*sc*) injection of atropine, and intracerebroventricular (*icv*) injections of histamine, chlorpheniramine ($H_1$-receptor antagonist), and ranitidine ($H_2$-receptor antagonist) in separate and combined treatments were investigated during yawning in rats. Physostigmine at a dose of 0.25 mg/kg produced the highest number of yawns. Atropine, used alone, was without effect, but physostigmine (0.25 mg/kg, *ip*)-induced yawning was blocked by pretreatment with atropine (1 mg/kg, *sc*). Histamine at the doses of 10, 20 and 40 μg produced yawning. Chlorpheniramine and ranitidine, used alone, had no effect, whereas pretreatments with chlorpheniramine and ranitidine at the same dose of 80 μg prevented histamine (40 μg, *icv*)-induced yawning. The suppressive effect of chlorpheniramine was more than that of ranitidine. Histamine (10 and 40 μg, *icv*) enhanced, whereas chlorpheniramine and ranitidine at the same dose of 80 μg suppressed, physostigmine (0.25 mg/kg, *ip*)-induced yawning. Atropine (1 mg/kg, *sc*) not only suppressed histamine-induced yawning, but also enhanced the inhibitory effect of chlorpheniramine, but not of ranitidine on yawning induced by histamine. These results indicate that muscarinic receptors mediate yawning induced by physostigmine. Histamine central $H_1$, and to a lesser extent $H_2$ receptors, may be involved in histamine-induced yawning. Cholinergic muscarinic receptors, as well as histaminergic $H_1$ and to a lesser extent $H_2$ receptors, may also be involved in the interaction between brain acetylcholine and histamine.

**Key words:**
physostigmine, atropine, histamine, chlorpheniramine, ranitidine, yawning, rats

**Abbreviations:** *icv* – intracerebroventricular, *ip* – intraperitoneal, *sc* – subcutaneous, PVN – paraventricular nucleus

## Introduction

Yawning, as a common physiological event, occurs with a low frequency in humans and animals and is under coordinated control of several neurotransmitters and neuropeptides such as dopamine, excitatory amino acids, serotonin, nitric oxide, noradrenaline, γ-aminobutyric acid (GABA), adrenocorticotropic hormone-related peptides, prolactin, urotensin, oxytocine and opioid peptides at the central nervous system level [2]. Several lines of pharmacological evidence suggest that acetylcholine is involved in the expression of the yawning. This evidence includes, for example, findings that cholinomimetic drugs (e.g., physostigmine and pilocarpine) induce yawning in rats and muscarinic receptor antagonists that cross the blood-brain barrier, such as atropine and scopolamine, but not the nicotinic receptor antagonist, mecamylamine, prevent yawning induced by dopamine $D_2$ receptor antagonists [15, 19, 23, 40]. On the other

hand, additional evidence suggests that the brain histamine may also be involved in the induction of yawning. It was found that microinjection of histamine into the paraventricular nucleus (PVN) of the brain produced yawning in the pentobarbital sodium anesthetized rats [33]. In the study of Seki et al. [33], the involvement of histamine $H_1$ receptors in histamine-induced yawning was clarified. In addition, Seki et al. [32] reported a suppressive effect of prylamine ($H_1$-receptor antagonist) in the light-induced yawning response in rats. Brain histamine, through its $H_1$, $H_2$ and $H_3$ receptors, influences the release and function of other neurotransmitters as well as neuropeptides such as dopamine, GABA, serotonin, acetylcholine, oxytocin and orexins [8, 17, 25]. In the higher functions of the brain such as arousal, learning and memory the interaction between histaminergic and cholinergic systems has been documented [6]. Moreover, it has been reported that brain histamine, through its central $H_1$, but not $H_2$ receptors mediates neostigmine-induced hyperglycemia in rats [20]. In addition, it was reported that central histamine-induced hyperglycemia was suppressed by *icv* injection of atropine [21]. Therefore, the present study was designed to investigate the interaction between physostigmine and brain histamine during yawning in rats. To clarify the involvement of histaminergic and cholinergic receptors in yawning, the effects of histamine $H_1$ and $H_2$ receptor antagonists, chlorpheniramine and ranitidine, respectively, and atropine (a muscarinic antagonist) were also examined.

## Materials and Methods

### Animals

Healthy adult male Wistar rats, weighing 220–250 g, were used in this study. Rats were maintained in polyethylene cages with food and water available *ad libitum* in a laboratory with controlled ambient temperature (22 ± 0.5°C) and under a 12 h light-dark cycle (lights on 07:00 h). Experiments were carried out between 13:00 h to 16:00 h. The experimental protocol was approved by the Laboratory Animal Care and Use Center of the Faculty of Veterinary Medicine of Urmia University.

### Drugs

Drugs used in the present study included histamine dihydrochloride (Merck, Darmstadt, Germany) chlorpheniramine maleate, ranitidine hydrochloride, physostigmine (Eserine) and atropine sulfate (Sigma – Aldrich, Steinheim, Germany). All drugs were dissolved in normal saline.

### Surgery

After a 15-day adaptation period, each rat was anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) injected *ip* and then placed in a stereotaxic apparatus (Stoelting, Wood Lane, IL, USA). The scalp was incised, and the skull was leveled off around the bregma. A 22 gauge, 12 mm stainless-steel guide cannula was inserted in the right lateral ventricle of the brain. The tip of the cannula was aimed at the following coordinates: 0.8 mm posterior to the bregma, 2 mm lateral to the midline and 4 mm below the top of the skull [26, 35]. The cannula was then fixed to the skull using three screws and dental acrylic. A 12.5 mm stylet was inserted in the cannula to keep it patent prior to injection. Animals were allowed a 10-day recovery period before experiments were initiated.

### Drug injection

For *icv* injections of normal saline (control), histamine, chlorpheniramine and ranitidine, a 28 gauge, 12.5 mm injection needle was attached to a 30 cm polyethylene tube fitted to a 5 μl Hamilton syringe. Then, the rat was restrained by hand, the stylet was withdrawn, and the injection needle was inserted into the guide cannula. The volume of the solutions to be injected into the lateral ventricle was 2 μl and the injection was made over a period of 60 s. *Ip* injections of normal saline and physostigmine were performed using a 25-gauge injection needle in a volume of 1 ml/kg. *Sc* injections of normal saline and atropine were performed using a 27-gauge injection needle in a constant volume of 0.2 ml/rat in the neck region. One specific group of rats was assigned to one specific drug treatment condition, and each group comprised six rats. Thus, each rat received two or three treatments, and four days was allowed between *icv* injections. *Sc* injections were performed 30 min and 20 min before *ip* and *icv* injections, respectively.

### Yawning

Yawning behavior was defined as a prolonged (more than 1 s), wide opening of the mouth followed by a rapid closure [13]. On the day of testing, rats were placed in clear plastic chambers (30 × 25 × 25 cm) and allowed to habituate to the chamber for a period of 30 min. *Sc*, *icv* and *ip* injections were performed at the end of the habituation period. Thirty minutes after sc injections of normal saline and atropine, 10 min after *icv* injections of normal saline, histamine, chlorpheniramine and ranitidine and immediately after *ip* injections of normal saline and physostigmine, the number of yawns was counted for a period of 45 min by blinded observers.

### Cannula verification

During the surgery and before *icv* injections, a rise in the cerebrospinal fluid (*csf*) through the cannula was observed. For additional confirmation of the placement of the cannula in the lateral ventricle of the brain, at the end of experiments the rats were *icv* injected with 10 µl methylene blue, and they were then deeply anesthetized with a high dose of ether and decapitated. The brains were removed and placed in a formaldehyde (10%) solution. After 24 h, the brains were sliced into 1 mm slices and the placement of the tip of the cannula and distribution of the dye in the lateral ventricle were visually controlled. Data from rats with an incorrect placement of the cannula were excluded from the analysis.

### Statistical analysis

To evaluate significance differences among treated groups, factorial analysis of variance (ANOVA) and Duncans test were applied. All values are expressed as the mean ± SEM. Statistical significance was set at $p < 0.05$.

## Results

Physostigmine injected *ip* at doses of 0.625 and 1 mg/kg did not produce yawning, whereas at doses of 0.125, 0.25 and 0.5 mg/kg, physostigmine produced yawning (Fig. 1).

No significant differences were observed between the numbers of yawns obtained from *sc* injection of atropine at the doses of 0.25, 1 and 4 mg/kg (1.0 ± 0.5, 1.2 ± 0.4 and 0.7 ± 0.3) *vs.* those obtained with normal saline (0.7 ± 0.3).

*Sc* injection of atropine at doses of 1 and 4 mg/kg, but not at the dose of 0.25 mg/kg, significantly ($p < 0.05$)

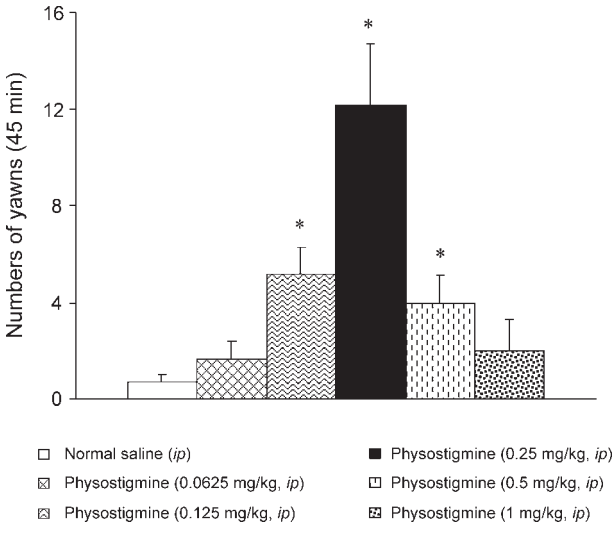


**Fig. 1.** Yawning response after *ip* injection of normal saline and physostigmine. Values are the means ± SEM (n = 6 for normal saline and physostigmine 0.0625 and 0.125 mg/kg and n = 6 for physostigmine 0.25, 0.5 and 1 mg/kg). n= 16 rats for all treatments. * $p < 0.05$ as compared to other treatments

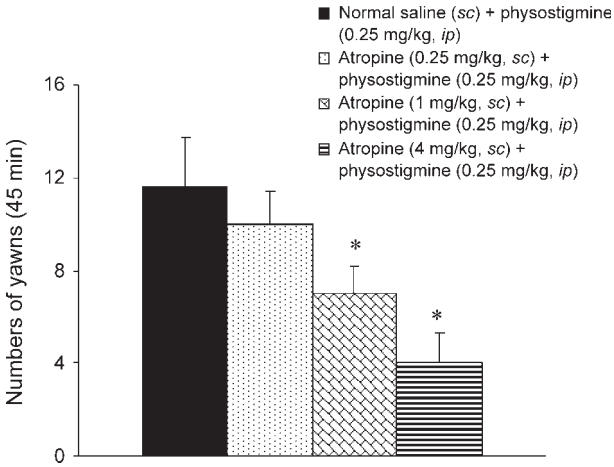


**Fig. 2.** Effect of *sc* pretreatment with atropine on physostigmine-induced yawning. Values are the means ± SEM (n = 6 for saline plus physostigmine and atropine 0.25 mg/kg plus physostigmine and n = 6 for atropine 1 and 4 mg/kg plus physostigmine). * $p < 0.05$ as compared to the other treatments

prevented the physostigmine (0.25 mg/kg, *ip*)-induced yawning (Fig. 2).

Normal saline and histamine (5 μg) injected *icv* produced a negligible yawning response, whereas histamine at doses of 10, 20 and 40 μg dose-dependently induced a yawning response (Fig. 3).

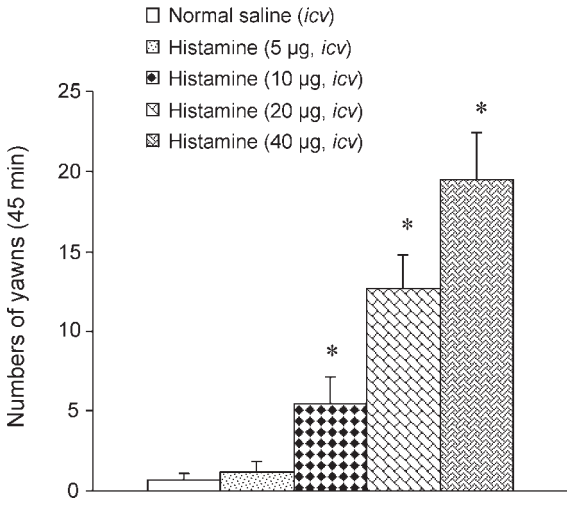


**Fig. 3.** Yawning response after *icv* injection of histamine. Values are the means ± SEM (n = 6 for saline and histamine 5 and 10 μg and n = 6 for histamine 20 and 40 μg). * $p < 0.05$ as compared to normal saline and histamine 5 μg

No significant differences were observed among the numbers of yawns obtained from *icv* injections of chlorpheniramine (1.2 ± 0.5, 1.4 ± 0.7 and 1.1 ± 0.5) and ranitidine (0.8 ± 0.5, 1.3 ± 0.6 and 1.2 ± 0.5) used alone at the same doses of 20, 40 and 80 μg as compared to that obtained from normal saline (0.8 ± 0.4).

Pre-treatments (*icv*) with histamine $H_1$ and $H_2$ receptor blockers, chlorpheniramine and ranitidine, respectively, at the same dose of 80 μg, significantly ($p < 0.05$) reduced the yawning response induced by *icv* injection of histamine at a dose of 40 μg. The inhibitory effect of chlorpheniramine was significantly ($p < 0.05$) higher than that of ranitidine (Fig. 4).

Histamine injected *icv* at doses of 10 and 40 μg before *ip* injection of physostigmine at a dose of 0.25 mg/kg significantly ($p < 0.05$) increased the number of yawns as compared to physostigmine used alone (Fig. 5).

Chlorpheniramine, and ranitidine injected *icv* at the same dose of 80 μg before *ip* injection of physostigmine (0.25mg/kg) significantly ($p < 0.05$) reduced physostigmine-induced yawning (Fig. 6).

Atropine (1 mg/kg, *sc*) significantly ($p < 0.05$) reduced yawning induced by histamine (40 μg, *icv*). The suppressive effect of chlorpheniramine, but not ranitidine on histamine-induced yawning was significantly ($p < 0.05$) increased by atropine (Fig. 7).

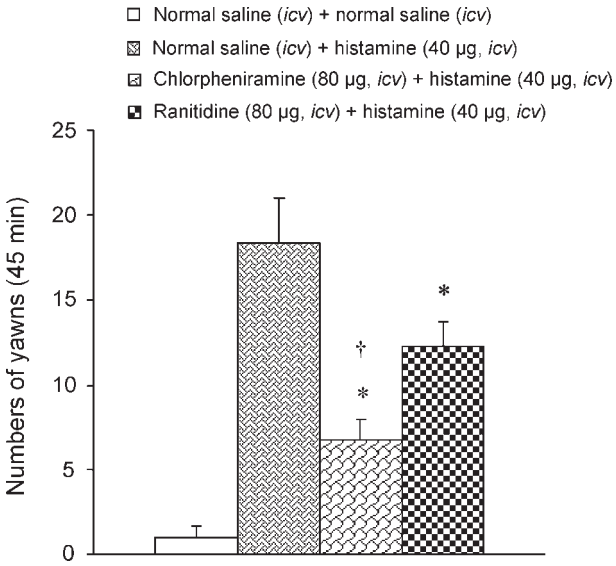


**Fig. 4.** Effect of *icv* pretreatments with chlorpheniramine and ranitidine on yawning induced by histamine. Values are the means ± SEM (n = 6 for saline and histamine plus saline and n = 6 for chlorpheniramine plus and ranitidine plus histamine). * $p < 0.05$ as compared to histamine plus normal saline, † $p < 0.05$ as compared to ranitidine plus histamine

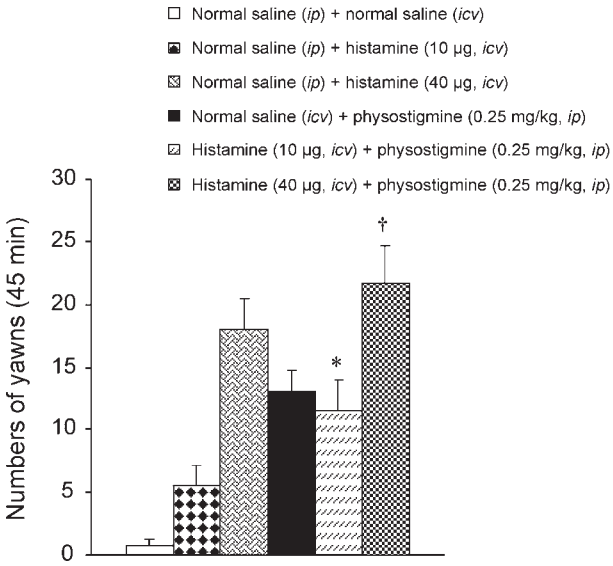


**Fig. 5.** Effect of *icv* pretreatments with histamine on yawning induced by physostigmine. Values are the means ± SEM (n = 6 for saline and saline plus histamine 10 and 40 μg and n = 6 for histamine 10 and 40 μg plus physostigmine). * $p < 0.05$ as compared to normal saline plus histamine 10 μg, † $p < 0.05$ as compared to normal saline plus histamine 40 μg

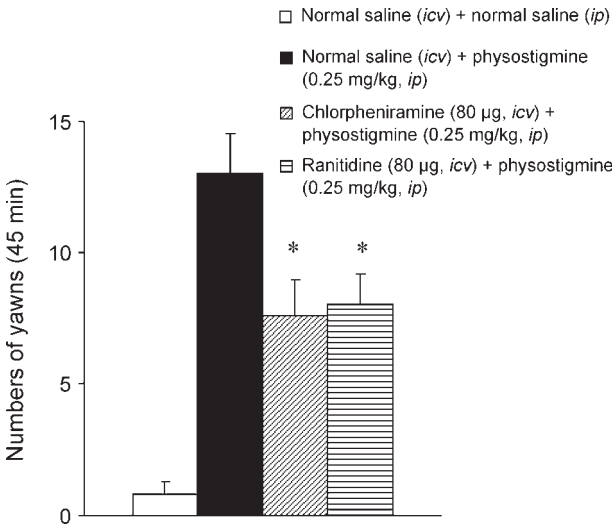


**Fig. 6.** Effect of *icv* pretreatments with chlorpheniramine and ranitidine on physostigmine-induced yawning. Values are the means ± SEM (n = 6 for saline and saline plus physostigmine and n = 6 for chlorpheniramine and ranitidine plus physostigmine). * $p < 0.05$ as compared to saline plus physostigmine

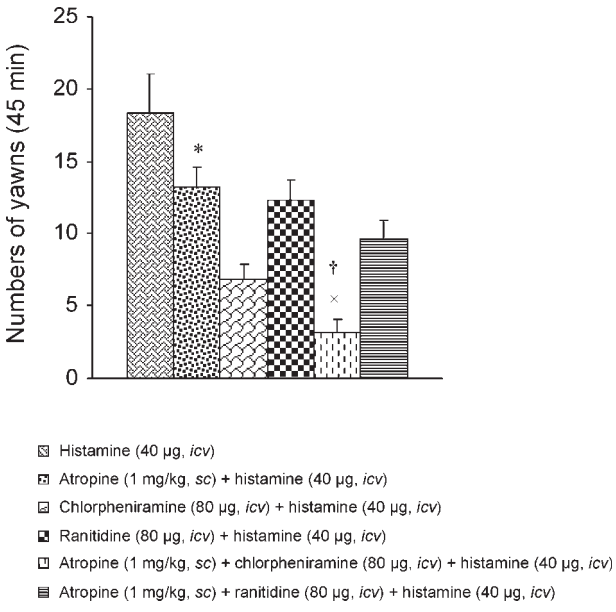


**Fig. 7.** Effect of *sc* pretreatments with atropine on histamine-induced yawning and on the suppressive effects of chlorpheniramine and ranitidine on yawning induced by histamine. Values are the means ± SEM (n = 6 for histamine and atropine plus histamine, n = 6 for chlorpheniramine and ranitidine plus histamine and n = 6 for atropine plus chlorpheniramine plus histamine and atropine plus ranitidine plus histamine). * $p < 0.05$ as compared to histamine 40 µg, × $p < 0.05$ as compared to chlorpheniramine plus histamine, † $p < 0.05$ as compared to atropine plus ranitidine plus histamine

## Discussion

The results of the present study show that physostigmine at the low (0.625 mg/kg) and high (1 mg/kg) doses was without effect, whereas at the doses of 0.125, 0.25 and 0.5 mg/kg, with the highest response at the dose of 0.25 mg/kg, physostigmine produced yawning. Physostigmine is a major alkaloid found in the seeds of the fabaceous plant *Physostigma venenosum*, and it is a powerful and reversible acetylcholine esterase inhibitor that effectively increases the concentration of acetylcholine in the sites of cholinergic transmission [43]. Physostigmine, as a cholinomimetic agent, at the dose range of 0.05–1 mg/kg has been frequently used in the study of the involvement of the cholinergic system in behavioral and physiological events. Most research has reported that physostigmine at the doses of 0.1–0.3 mg/kg, especially at the dose of 0.2 mg/kg, produces an increased yawning response [15, 24, 36, 41, 42]. Physostigmine at higher doses (0.5–2 mg/kg) produces tremor, fasciculation, salivation, tongue protruding, hypothermia and defecation [7, 22, 23, 29, 36, 37]. In the present study, atropine prevented physostigmine-induced yawning. This finding is in agreement with those from other investigations [7, 15, 34]. It would appear that physostigmine-induced yawning and the inhibitory effect of atropine on the yawning induced by physostigmine may occur at a central level. Distigmine, a peripherally acting cholinesterase inhibitor, did not produce any yawning, whereas E2030 and donepezil, with marked preferential central cholinergic activities, relative to peripheral activities, produced more yawning and scopolamine, a centrally acting antimuscarinic drug, inhibited E2030-iduced yawning [23].

The results presented here indicate that *icv* injection of histamine produced yawning. Histamine $H_1$ and $H_2$ blockers, chlorpheniramine and ranitidine, respectively, produced no yawning response in the absence of histamine, but in the presence of histamine, chlorpheniramine and to a lesser extent ranitidine prevented histamine-induced yawning. This indicates that both histamine central $H_1$ and $H_2$ receptors are involved in yawning induced by brain histamine. The cell bodies of the histaminergic neuron system are concentrated in the tuberomammillary nucleus of the hypothalamus and send out axons to innervate the entire central nervous system [17, 31]. The areas known to be involved in the induction of yawning (e.g., hip-

pocampus, PVN, pituitary gland, nigrostriatal system, locus cereleus, nucleus of solitary tract, the dorsal motor nucleus of the vagus nerve, ventrolateral medulla and spinal cord) [2, 30], are also innervated by the brain histamine system [31]. It has been found that microinjection of histamine into the medial, but not into the lateral and caudal parts of the PVN produces a yawning response in anesthetized rats [33]. It was reported that after microinjection of trifluoromethyl toluidide dimaleate, an $H_1$-receptor agonist, into the PVN, yawning was produced, while pyrilamine ($H_1$-receptor antagonist) prevented histamine-induced yawning [33]. In addition, dimaprit, an $H_2$-receptor agonist, produced yawning after microinjection into the PVN, but cimetidine ($H_2$-receptor antagonist) did not prevent histamine-induced yawning [33]. *Icv* injection of pyrilamine blocked the yawning induced by light stimulation, suggesting a role for brain histamine $H_1$ receptors in modulating light-induced yawning [32]. In this regard, Gower et al. [16] reported that the $H_1$-receptor antagonist, dexchlorpheniramine, inhibited apomorphine-induced yawning. Moreover, Ferrari and Baggio [14] found that cimetidine but not ranitidine, injected *ip* antagonized the yawning induced by apomorphine. In our study, the route of ranitidine injection, the yawning inducing agent, and the conscious level of the animal may affect the results obtained from ranitidine. However, further studies are required to identify the histamine $H_2$-receptor action on the yawning response. Yawning is a complex arousal defense reflex located in the reticular brainstem with a peripheral and central arch, whose aim is to reverse brain hypoxia [38]. On the other hand, the involvement of the brain histamine in the arousal regulation has been documented [8]. Seki et al. [33] reported an arousal shift in the electrocorticogram after microinjection of histamine into the PVN. Therefore, it seems that the histamine-induced yawning observed in the present study may be associated with the action of histamine on the area involved in the activation of yawning/arousal mechanisms.

The results of the present study indicate that brain histamine produced an additive effect on physostigmine-induced yawning, and histamine $H_1$ and $H_2$ blockers suppressed the yawning induced by physostigmine. On the other hand, atropine suppressed histamine-induced yawning and enhanced the suppressive effects of chlopheniramine but not for ranitidine on the yawning induced by histamine. These data therefore indicate that brain histamine, through its $H_1$ and $H_2$ receptors, might have an excitatory effect on acetylcholine function, and that cholinergic muscarinic receptors might interact with central $H_1$ but not $H_2$ histaminergic stimulation of yawning. Microinjection of histamine into the basolateral amygdala increased the escape latency in acquisition and avoidance tasks [1]. The basolateral amygdala receives abundant cholinergic innervation from the nucleus basalis mangocellularis [10]. Moreover, in the ventral striatal neurons, histamine increased while α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, decreased the release of acetylcholine [27]. By whole-cell current-clamp recording, it was shown that histamine excited the septohippocampal cholinergic neurons [39]. It was reported that in the ventral striatal neurons, thiazolylethylamine (TEA, $H_1$-receptor agonist) and dimaprit ($H_2$-receptor agonist) enhanced the release of acetylcholine, while blockade of histamine $H_1$ and $H_2$-receptors with triprolidine ($H_1$-receptor antagonist) and impromidine ($H_2$-receptor antagonist) produced opposite effects [28]. On the other hand, cholinergic nucleus basalis neurons projecting to the cortex were excited by histamine mostly *via* $H_1$ but also *via* $H_2$ receptors [18]. In addition, blockade of medial septum-diagonal band $H_2$ receptors with cimetidine was found to antagonize the release of acetylcholine produced by $H_3$ receptor antagonist, whereas triprolidine, an $H_1$ receptor antagonist was without effect [5]. Betahistine, as a partial histamine $H_1$ receptor agonist, was reported to increase acetylcholine from the cortex of freely moving rats [12]. It has been reported that mepyramine and cimetidine failed to alter acetylcholine spontaneous release from the cortex: however, $H_1$ but not $H_2$ receptor antagonists antagonized the releases of acetylcholine elicited by histamine [11]. In freely moving rats, intraseptal administrations of thioperamide and ciproxifan, $H_3$ receptor antagonists increased the release of acetylcholine from the hippocampus and cimetidine inhibited the effects of thioperamide and ciproxifan [3, 4]. Central pretreatments with pyrilamine and atropine suppressed central histamine-induced hyperglycemia and suggested that histamine $H_1$ receptors and muscarinic cholinergic neurons are involved in histamine-induced hyperglycemia [21]. Bugajski and Gądek, [9] have suggested that cholinergic muscarinic receptors interact with central $H_1$ and $H_2$ histaminergic stimulation during the increased pituitary-adrenocortical response in stressed rats. The

discrepancies between findings of $H_2$ receptors involvement in acetylcholine release and function may be associated with the fact that histamine, *via* $H_2$ receptors, inhibits activity of dopaminergic and GABAergic neurons, thus indirectly modulating the release of acetylcholine. Moreover, histamine also slightly increases acetylcholine release through $H_2$ receptors, possibly located on cholinergic neurons [27, 28].

In conclusion, the results of the present study suggest that the central histaminergic and cholinergic systems interact with each other in the modulation of yawning. Cholinergic muscarinic receptors as well as histaminergic $H_1$, and and to a lesser extent $H_2$ receptors, are involved in the interaction between brain acetylcholine and histamine.

## References:


1. Alvarez E, Ruarte M: Histaminergic neurons of the ventral hippocampus and the baso-lateral amygdala of the rat: functional interaction on memory and learning mechanisms. Behav Brain Res, 2002, 128, 81–90.
2. Argiolas A, Melis MR: The neuropharmacology of yawning. Eur J Pharmacol, 1998, 343, 1–16.
3. Bacciottini L, Giovanelli L, Passani MB, Schunack W, Mannaioni P F, Blandina P: Ciproxifan and cimetidine modulate *c-fos* expression in septal neurons, and acetylcholine release from hippocampus of freely moving rats. Inflam Res, 2000, 49, S41–S42.
4. Bacciottini L, Mannaioni PF, Chiappetta M, Giovannini MG, Blandina P: Acetylcholine release from hippocampus of freely moving rats is modulated by thioperamide and cimetidine. Inflam Res, 1999, 48, S63–S64.
5. Bacciottini L, Passani MB, Giovannelli L, Cangioli I, Mannaioni PF, Schunack W, Blandina P: Endogenous histamine in the medial septum-diagonal band complex increases the release of acetylcholine from the hippocampus: a dual-probe microdialysis study in the freely moving rat. Eur J Neurosci, 2002, 15, 1669–1680.
6. Blandina P, Efoudebe M, Cenni G, Mannaioni P, Passani MB: Acetylcholine, histamine and cognition: two sides of the same coin. Learn Mem, 2004, 11, 1–8.
7. Bourson A, Moser OC: Yawning induced by apomorphine, physostigmine or pilocarpine is potentiated by dihydropyridine calcium channel blockers. Psychopharmacology (Berl), 1990, 100, 168–172.
8. Brown RE, Steven DR, Haas HL: The physiology of brain histamine. Prog Neurobiol, 2001, 63, 637–672.
9. Bugajski J, Gądek A: The effect of adrenergic and cholinergic antagonists on central histaminergic stimulation of pituitary-adrenocortical response under stress in rats. Neuroendocrinology, 1984, 38, 447–452.
10. Carlsen J, Zaborszky L, Heimer L: Cholinergic projections from the basal forebrain to the basolateral amygdaloid complex: a combined retrograde fluoresent and immunohistochemical study. J Comp Neurol, 1985, 234, 155–167.
11. Cecchi M, Passani MB, Bacciottini L, Mannaioni PF, Blandina P: Cortical acetylcholine release elicited by stimulation of histamine H1 receptors in the nucleus basalis magnocellularis: a dual-probe microdialysis study in the freely moving rats. Eur J Neurosci, 2001, 13, 68–78.
12. Cenni G, Passani MB, Mannaioni PF, Blandina P: Betahistine increases Ach release from the cortex, but not histamine release from the nucleus basalis magnocellularis of freely moving rats. 2. Histaminergic mechanism in the CNS. Inflam Res, 2006, Suppl 1, S28–S29.
13. Collins GT, Witkin GM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH: Dopamine-agonist induced yawning in rats: a dopamine $D_3$ receptor-mediated behavior. J Pharmacol Exp Ther, 2005, 314, 310–319.
14. Ferrari F, Baggio G: Influence of cimetidine, ranitidine and imidazole on the behavioral effects of (+/–) N-n-propylnorapomorphine in male rats. Psychopharmacology (Berl), 1985, 85, 197–200.
15. Gower AJ: Effects of acetylcholine agonists and antagonists on yawning and analgesia in the rat. Eur J Pharmacol, 1987, 139, 79–89.
16. Gower AJ, Berendsen HH, Broekkamp CL: Antagonism of drug-induced yawning and penile erection in rats. Eur J Pharmacol, 1986, 122, 239–244.
17. Haas HL, Panula P: The role of histamine and the tuberomammillary nucleus in the nervous system. Nat Rev Neurosci, 2003, 4, 121–130.
18. Khateb A, Fort P, Pegna A, Jones BE, Muhlethaler M: Cholinergic nucleus basalis neurons are excited by histamine in vitro. Neurosci, 1995, 69, 495–506.
19. Kimura H, Yamada K, Nagashima M, Furukawa T: Involvement of catecholamine receptor activities in modulating the incidence of yawning in rats. Pharmacol Biochem Behav, 1996, 53, 1017–1021.
20. Nonogaki K, Iguchi A, Li X, Tamagawa T, Watanabe G, Hiyoshi Y, Sakamoto N: Role of brain histamine $H_1$- and $H_2$- receptors in neostigmine-induced hyperglycemia in rats. Life Sci, 1992, 51, PL131–PL134.
21. Nonogaki K, Li X, Tamagawa T, , Watanabe G, Hiyoshi Y, Ozawa K, Sakamoto N, Iguchi A.: Histamine-induced, central nervous system-mediated hyperglycemia is suppressed by atropine in the brain. Life Sci, 1993, 52, PL107–PL110.
22. Ogren So, Carlsson S, Bartfai T: Serotonergic potentiation of muscarinic agonist evoked tremor and fasciculation in rat and mouse. Psychopharmacology (Berl), 1985, 86, 258–264.
23. Ogura H, Kosasa T, Kuriya Y, Yamanishi Y: Central and peripheral activity of cholinesterase inhibitors as revealed by yawning and fasciculation in rats. Eur J Pharmacol, 2001, 415, 157–164.
24. Okuyama S, Shimamura H, Hashimoto S, Aihara H: Relation between yawning behavior and central serotonergic neuronal system in rats. Naunyn Schmiedebergs Arch Pharmacol, 1987, 335, 667–672.

25. Passani MB, Giannoni P, Buchrelli C, Baldi E, Blandina P: Histamine in the brain: beyond sleep and memory. Biochem Pharmacol, 2007, 73, 1113–1122.
26. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. Compact Third Edition, Academic Press, San Diego, 1997.
27. Prast H, Tran MH, Fischer H, Kraus M, Lamberti C, Grass K, Philippu A: Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of histamine $H_3$ receptors. Naunyn Schmiedebergs Arch Pharmacol, 1999, 360, 558–564.
28. Prast H, Tran MH, Lamberti C, Fischer H, Kraus M, Grass K, Philippu A: Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of $H_1$ and $H_2$ histamine receptors. Naunyn Schmiedebergs Arch Pharmacol, 1999, 360, 552–557.
29. Sarkar S, Thomas B, Muralikrishanan D, Mohanakumar KP: Effects of serotoninergic drugs on tremor induced by physostigmine in rats. Behav Brain Res, 2000, 109, 187–193.
30. Sato-Suzuki I, Kita I, Oguri M, Arita H: Stereotyped yawning response induced by electrical and chemical stimulation of paraventricular nucleus of the rat. J Neurophysiol, 1998, 80, 2765–2775.
31. Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M: Histaminergic transmission in the mammalian brain. Physiol Rev, 1991, 71, 1–51.
32. Seki Y, Nakatani Y, Kita I, Sato-Suzuki I, Oguri M, Arita H: Light induces cortical activation and yawning in rats. Behav Brain Res, 2003, 140, 65–73.
33. Seki Y, Sato-Suzuki I, Kita I, Oguri M, Arita H: Yawning/cortical activation induced by microinjection of histamine into the paraventricular nucleus of the rat. Behav Brain Res, 2002, 134, 75–82.
34. Sharifzadeh M, Abdollahi M, Dehpour AR, Kebriaeezadeh A, Samini M, Mohammad M: Alternation of physostigmine-induced yawning by chronic lithium administration in rats. Pharmacol Toxicol, 1997, 81, 159–163.
35. Tamaddonfard E, Khalilzadeh E, Hamzeh-Gooshchi N, Seiednejhad-Yamchi S: Central effect of histamine in a rat model of acute trigeminal pain. Pharmacol Rep, 2008, 60, 219–224.
36. Ushijima I, Mizuki Y, Imaizumi J, Yamada M, Noda Y, Yamada K, Furukawa T: Characteristics of yawning behavior induced by apomorphine, physostigmine and pilocarpine. Arch Int Pharmacodyn Ther, 1985, 273, 196–201.
37. Ushijima I, Yamada K, Inoue T, Tokunaga T, Furukawa T, Nada Y: Muscarinic and nicotinic effects on yawning and tongue protruding in the rat. Pharmacol Biochem Behav, 1984, 21, 297–300.
38. Walusinski O: Yawning: unsuspected avenue for a better understanding of arousal and interoception. Med Hypotheses, 2006, 67, 6–14.
39. Xu C, Michelson KA, Wu M, Morozova E, Panula P, Alreja M: Histamine innervation and activation of septohippocampal GABAergic neurons: involvement of local Ach release. J Physiol (Lond.), 2004, 561, 657–670.
40. Yamada K, Furukawa T: Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology (Berl), 1980, 67, 39–43.
41. Zarrindast MR, Fazli-Tabai S, Semnanian S, Fathollahi Y: Influence of different adrenoceptors agonists and antagonists on physostigmine-induced yawning in rats. Pharmacol Biochem Behav, 1999, 62, 1–5.
42. Zarrindast MR, Toloui V, Hashemi B: Effects of GABAergic drugs on physostigmine-induced yawning in rats. Psychopharmacology (Berl), 1995, 122, 297–300.
43. Zhao B, Moochhala SM, Tham SY: Biologically active components of *Physostigma venenosum*. J Chromatogr B, 2004, 812, 183–192.

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