Effect of Long-Term Centrally Injected Histamine and Its Receptors Antagonist on The Hypothalamic Cyclooxygenase and Lipoxygenase Enzymes in Rats

Ayşenur BAŞ, Gökcen GÜVENÇ-BAYRAM, Burcin ALTINBAŞ, Ebru ÖZYURT, Ebru YALÇIN, Burcu ERBAYKENT-TEPEDELEN, Figen ERSOY,
Murat YALÇIN

a Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, b Department of Physiology, Faculty of Veterinary Medicine, Uludag University, Bursa, 16059, Turkey. c Department of Physiology, Faculty of Medicine, Sanko University, Gaziantep 27090, Turkey

Ayşenur BAŞ:
E-mail: ayserurbas16@gmail.com; Tel: 0 553 462 3087; Orcid: 0000-0002-7825-4823

Gökcen GÜVENÇ-BAYRAM:
E-mail: gokcenguvenc@hotmail.com; Tel: 0 506 937 7553; Orcid: 0000-0002-1413-3651

Burcin ALTINBAŞ:
E-mail: altinbasburcin@gmail.com; Tel: 0 506 230 5502; Orcid: 0000-0002-9534-736X

Ebru ÖZYURT:
E-mail: ebru.ozyurt@hotmail.com; Tel: 0 535 609 7030; Orcid: 0000-0003-1591-510X

Ebru YALÇIN:
E-mail: eprufaffle@gmail.com; Tel: 0 506 996 8635; Orcid: 0000-0003-1756-1288

Burcu ERBAYKENT:
E-mail: berbaykent@uludag.edu.tr; Tel: 0 505 255 7997; Orcid: 0000-0002-9565-6349

Figen ERSOY:
E-mail: figen@uludag.edu.tr; Tel: 0 533 415 0879; Orcid: 0000-0003-2267-069X

* Corresponding Author: Prof Murat Yalcin, DVM, PhD. Uludag Universitesi Veteriner Fakultesi Fizyoloji Anabilim Dali, Gorukle, 16059 Bursa Turkey

Tel: + 90 224 294 1228 Fax: + 90 224 294 1202 E-mail: murray@uludag.edu.tr; Orcid: 0000-0002-5600-8162
ABSTRACT

The current study was designed to determine the effect of centrally chronic-administrated histamine and histaminergic receptors antagonist on the level of hypothalamic cyclooxygenase (COX) and lipoxygenase (LOX) enzymes.

Studies were performed in male Sprague–Dawley rats. Histamine (100 nmol), histaminergic H1 receptor antagonist chlorpheniramine (100 nmol), histaminergic H2 receptor antagonist ranitidine (100 nmol) or histaminergic H3/H4 receptor antagonist thioperamide (100 nmol) was injected intracerebroventricularly for 7 days. Central chronic histamine treatment caused increases in the levels of all three enzymes in the hypothalamus. Central chronic treatments with all three histaminergic receptors antagonists reduced the hypothalamic COX-1 levels and raised the hypothalamic COX-2 and LOX levels.

In conclusion, our findings show that the central histamine has a possible role to affect the central COX and LOX pathways. This could be interpreted as that central histaminergic system might have a potential to activate central COX and LOX pathways to regulate central nervous system functions.

Keywords: Histamine, Histaminergic receptors, Hypothalamus, Cyclooxygenase, Lipoxygenase, Intracerebroventricular, Western blot.

Introduction
Histamine is found both in the nervous system as a neurotransmitter and non-nervous tissues as a signaling molecule.\(^1\) In the nervous system, there are two major sources of histamine, which are neurons and mast cells.\(^2\) Acting as a neurotransmitter via histaminergic receptors, central histamine has been shown to modulate many central nervous system functions, including energy balance, drinking, pain perception, learning, memory, and cardiovascular control.\(^2\)–\(^5\) Cell bodies of histaminergic neurons are located exclusively in the tuberomammillary nucleus of the posterior hypothalamus.\(^1,6\) Four histamine receptors have been cloned so far (H1-H4R) and their distribution shows distinctive patterns in the brain.\(^2\) Brain histamine holds a key role as a neurotransmitter in the hypothalamus and has been demonstrated to modulate the neuroendocrine regulation of key pituitary hormone indirectly via hypothalamic activation,\(^7\)–\(^9\) including adrenocorticotropic hormone,\(^10\) beta-endorphin,\(^11\) alpha-melanocyte stimulating hormone,\(^8\) and prolactin.\(^12\) Moreover, vasopressin and oxytocin secretions have also been demonstrated to be stimulated by histamine.\(^13\) It is also known that central histaminergic receptors, particularly via H1, contribute to histamine-regulated neuroendocrine control.\(^9,12,14\) Histamine has also been shown to play a substantial role in regulating hypothalamic–pituitary–gonadal axis.\(^15\) Central histaminergic system also has an important role in central control of the cardiovascular system. Indeed, centrally injected histamine caused pressor effects in normotensive\(^2,16\) and hemorrhaged hypotensive rats\(^3\)–\(^5,17,18\) by activating central histaminergic H1 receptors.

Arachidonic acid (AA) is a polyunsaturated fatty acid that exists in the phospholipids of cellular membranes and is found in abundant concentrations in the brain. AA forms prostaglandins (PGs) and leukotrienes (LTs) via the cyclooxygenase (COX) or lipoxygenase (LOX) pathway, respectively.\(^19,20\) Brain AA also has a crucial homeostatic role, including synaptic signaling, neuronal firing, neurotransmitter release, nociception, neuronal gene expression, cerebral blood flow, the sleep–wake cycle, appetite.\(^20\) It also modulates ion
channels and regulates the activity of many enzymes, including protein kinase A, protein kinase C, and NADPH oxidase. Recently we reported that intracerebroventricularly (ICV) administered AA, by activating the central COX pathway, leads to pressor cardiovascular effects in normotensive and hemorrhaged hypotensive rats, had a hyperventilation effect on respiratory system, and stimulation of male hypothalamic–pituitary–gonadal axis. We also reported that inhibition of central COX enzyme completely and blockage of central thromboxane A synthesis (TXA) partially prevented AA-evoked pressor, hyperventilation, and neuroendocrine effects.

Both the histaminergic system and COX or LOX metabolites of AA are active in the brain and control many central nervous system functions. Also, both the histaminergic system and COX or LOX metabolites of AA have similar function in controlling many central nervous system functions. Considering the above data, the primary aim of the current study was to show the effect of centrally administrated histamine and/or its receptor antagonists on the levels of hypothalamic COX and LOX enzymes.

**Materials and Methods**

**Animals and experimental protocol**

In the experiments, 25 adults, male Sprague–Dawley rats (3 months old, 270–300 g) (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used. Five rats were housed in individual cages under controlled conditions of temperature (20–22 °C), humidity (60–70%), lighting (12 h light/dark cycle) and nursed with food and water ad libitum.

Under sevoflurane (2–4%/100% O2) anesthesia, the rats were placed in a stereotaxic frame. According to coordinates are taken from the atlas of Paxinos and Watson, a burr hole was
drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to bregma for ICV treatment. Sterilized 22-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The guide cannula was lowered 4.5 mm below the surface of the skull and fixed to the skull with acrylic cement. A sterilized 28-gauge stainless steel obturator cannula was passed through the guide cannula to block the passage when the cannula was not in use. And then the rats were placed in individual cages and allowed to recover from anesthesia for 1 day. After surgery, the rats were daily treated with penicillin (0.3 mg/kg; intramuscular) as well as buprenorphine (25 µg/kg; subcutaneous) throughout the study. During the experiment, the rats remained calm without evidence of pain and freely moved in an individual cage.

To determine the chronic effect of histamine and histaminergic receptors on the hypothalamic cyclooxygenase and lipoxygenase enzymes, the animals divided into 5 groups. The groups were intracerebroventricularly (ICV) treated with saline (5 µL; n=5), histamine (100 nmol; n=5), histaminergic H1 receptor antagonist chlorpheniramine (100 nmol; n=5), histaminergic H2 receptor antagonist ranitidine (100 nmol; n=5) or histaminergic H3/H4 receptor antagonist thioperamide (100 nmol; n=5). All of the 5 groups were studied separately. The treatments were performed daily, between 09-10am, for 7 days. At the end of the experiment (at day 7), deeply anesthetized rats (2–4%/100% O₂) were decapitated and the brains were quickly removed from the skull. To dissect the hypothalamus of the rat curved forceps was used. The curved part of the forceps was pushed down around the hypothalamus from the brain, which was placed ventral side up, and gently “spoon” out the hypothalamus by pinching it out while pushing down with the forceps. Following that the spooned tissue was immediately placed into the bottom of a 15-ml Falcon tube and the tube was immediately snapped freeze on dry ice.
The Animal Care and Use Committee of Uludag University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, approved all experimental procedures (2019-06/06). Each animal was studied separately in a single experimental protocol, and each experimental group consisted of five rats.

**Western blot analysis**

The hypothalamus samples were used for experiments and total protein was extracted using protein extraction kit (BioVision BCA protein assay kit Cat K813-2500) according to the manufacturer’s instructions. Protein samples (50 µg) were separated on 8% SDS polyacrylamide gels, afterwards transferred to nitrocellulose (NC) membranes and membranes were blocked using TBS-T (Tris-Base-Saline containing 0.1% Tween 20) containing either 5% skim milk (w/v). The membranes were incubated with a rabbit polyclonal antibody against COX-1 (1:1000, Cell Signaling, Leiden, Netherlands), COX-2 (1:1000, Abcam, Burlingame, California, US) or LOX (1:1000, Cell Signaling, Leiden, Netherlands), followed by incubation with anti-mouse or anti-rabbit HRP-conjugated antibodies (1:2000, Cell Signaling, Leiden, Netherlands). The primary and secondary antibody incubations were carried out in TBS-T containing 0.5% dry milk at RT for 1 h or at +4°C overnight. β-actin antibody (1:5000, Sigma-Aldrich, UK) was used as normalization and protein loading control.

Protein expression was detected using 2 mL Clarity Western ECL Substrate (Bio-Rad, US) for 5 min and bound antibodies were visualized on Fusion FX-7 imaging device (Vilber Lourmat, Torcy, France). The optical densities of protein bands on the images were quantitatively analyzed with Image J software.

**Drugs and ICV injections**
The following drugs were used: histamine, chlorpheniramine, ranitidine, and thioperamide (Sigma-Aldrich Co., Deisenhofen, Germany). The drug solution was prepared freshly in saline on the day of the experiment. The dose of drugs was chosen from our previous studies. \textsuperscript{3-5,15,31}

Intracerebroventricular injections were made by using hand-made injection cannula (28 gauge stainless steel tubing). The injection cannula was connected to polyethylene tubing, which was filled with saline or saline solution of the studied agent in a 10 µL microsyringe. Injection cannula, polyethylene tubing and microsyringe were daily sterilized. For the ICV injection, 5 µL volume of the solution was infused within 60 s. During the injection, an air bubble moving in the polyethylene tubing was closely watched to ensure the drug was delivered in its entirety. After ICV injection, sterile obturator cannula was inserted in guide cannula.

Data and statistical analysis

All values are given as mean ± standard error of the mean (SEM) with p < 0.05 considered as the level of significance. Statistical evaluation was performed by repeated-measures analysis of variance (ANOVA; two-way) and the post-ANOVA test of Bonferroni by using Sigma Stat 3.5 software (CA, USA).

Results

Effects of intracerebroventricular injection of histamine and histaminergic receptor antagonists on the hypothalamic COX-1, COX-2 and LOX enzymes

The amount of hypothalamic COX-1 increased by 4 % with histamine treatment, decreased by 27%, 17% and 22% with histaminergic H1 receptor antagonist chlorpheniramine,
histaminergic H2 receptor antagonist ranitidine and histaminergic H3/H4 receptor antagonist thioperamide, respectively (Fig 1A, 2).

Central chronic-treatment with histamine, histaminergic H1 receptor antagonist chlorpheniramine, histaminergic H2 receptor antagonist ranitidine and histaminergic H3/H4 receptor antagonist thioperamide caused to increase in 29%, 37%, 45% and 27% in hypothalamic COX-2 amount (Fig 1B, 2), respectively.

Moreover, the amount of hypothalamic LOX increased by 8%, 9%, 4% and 1% with histamine, histaminergic H1 receptor antagonist chlorpheniramine, histaminergic H2 receptor antagonist ranitidine and histaminergic H3/H4 receptor antagonist thioperamide, respectively (Fig 1C, 2).

Through the study, the food and water intake, and body weight of the animals in each group were recorded daily. Histamine and ranitidine treatments caused slightly decreases in the body weights of the animals by reducing food intake, but not significantly (data not shown).

**Discussion and Conclusion**

These data demonstrate that ICV chronically administered histamine produced to increase in hypothalamic COX-1, COX-2 and LOX levels. Moreover, central chronic-treatments with chlorpheniramine, ranitidine and thioperamide led to decrease in hypothalamic COX-1 level and to increases in hypothalamic COX-2 and LOX levels.

COX and LOX metabolites play roles in physiological and pathophysiological processes in the central nervous system, such as synaptic signaling, neuronal firing, neurotransmitter release, nociception, neuronal gene expression, cerebral blood flow, the sleep–wake cycle, appetite,20 modulation of ion channels, and regulation of the activity of many enzymes,
including protein kinase A, protein kinase C, and NADPH oxidase. In addition, previous reports have demonstrated that the brain COX and LOX products of AA is involved in central sympathoadrenomedullary activation. In our previous study, the cardiovascular effects observed after ICV AA injection reflect the central to peripheral effects of AA on activating the sympathoadrenergic, vasopressinergic, and renin–angiotensin systems. Phospholipase A₂ (PLA₂) activation via ICV administered melittin and PG activation, including TXA₂, PGE₂, PGD₂, and PGF₂ increases MAP. Previous reports have indicated that TXA₂, PGE₂, and PGF₂ production in the brain increases promptly after hemorrhage or cerebral hypoperfusion. We reported that centrally injected TXA₂, one of the COX products of AA, increases MAP under normal conditions and reverses hypotension under hemorrhagic shock conditions. The activation of the catecholaminergic, vasopressinergic, and renin–angiotensinergic systems mediates these cardiovascular responses to TXA₂ as peripheral mechanism. Additionally, our recent report demonstrated that ICV administered melittin affects the cardiovascular system via PLA₂ activation and increases MAP under normal and hypotensive conditions.

Histamine serves an important function as a neurotransmitter in the central nervous system. The histaminergic neurons originate from the tuberomammillary nucleus of the posterior hypothalamus and send projections to most regions of the brain. The central histamine system is involved in many brain functions, such as arousal, control of pituitary hormone secretion, body temperature, circadian rhythms, and the suppression of eating and cognitive functions. The effects of neuronal histamine are mediated via G-protein-coupled H₁–H₄ receptors. The histaminergic system in the brain is also known to stimulate the hypothalamic–pituitary–adrenal axis via central histaminergic H₁, H₂, and H₃ receptor activation and the central COX-1 signaling pathway, but not COX-2. The H₁ receptors are present at high density in
the limbic system, including many nuclei of the hypothalamus. Similar to the histamine H1 receptor, the H2 receptor is widely expressed in the brain.  

Previously, it was reported that the centrally administered histamine-induced elevation of plasma catecholamines was attenuated by central pretreatment with ketoprofen, an inhibitor of COX-1, and furegrelate, an inhibitor of TXA2 synthase, and also was abolished by bilateral adrenalectomy. These results suggest that the brain AA generated by histamine may be metabolized to TXA2 by brain COX-1 and TXA2 synthase, thereby evoking the secretion of noradrenaline and adrenaline from adrenal noradrenaline- and adrenaline-containing cells respectively in rats. Also it was reported that the centrally administered histamine activates the central adrenomedullary outflow by brain phosphatidylinositol-specific PLC- and diacylglycerol lipase-dependent mechanisms, thereby increasing plasma levels of noradrenaline and adrenaline in rats. Histaminergic H1-receptor stimulation releases inositol phosphate and can promote the formation of AA, most likely through the activation of PLA2 and the formation of cGMP. A single H2 receptor may be linked not only to adenylyl cyclase activation but also to reduction of PLA2 activity. In addition, histamine H1 receptor-mediated relaxation in rabbit cerebral arteries partly involves the release of prostaglandins. Moreover, we recently reported that H2 histamine receptor activation completely blocked the PLA2 activator melittin-induced pressor response. The histamine H2 receptor behaves as a complete agonist that is equipotent to or slightly more active than histamine in the central regulation of the cardiovascular system. Those studies clearly show that there is an interaction between central histaminergic system and central COX and/or LOX pathways. Those reports also support our current findings.

In conclusion, current data demonstrate that central histaminergic system has a potential role on central COX and LOX pathways. This could be interpreted that central histaminergic and
central COX and LOX pathways have an interaction to regulate in many central nervous system functions.

References


Figure 1. Effects of intracerebroventricular injection of histamine and histaminergic receptor antagonists on hypothalamic COX-1 (A), COX-2 (B), and LOX (C) amount. The rats were chronically treated with saline (S; 5 µL; ICV; n=5), histamine (H; 100 nmol; ICV; n=5),
chlorpheniramine (CP; 100 nmol; ICV; n=5), ranitidine (R; 100 nmol; ICV; n=5) or thioperamide (TP; 100 nmol; ICV; n=5) for 7 days. Data are given as mean ± SEM of five measurements. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *p<0.05, significantly different from the value of the saline-treated group.

![Western blots](image)

**Figure 2.** Western blots—levels of COX-1, COX-2 and LOX. The rats were centrally injected with saline (S; 5 µL), histamine (H; 100 nmol), chlorpheniramine (CP; 100 nmol), ranitidine (R; 100 nmol) or thioperamide (TP; 100 nmol) for 7 days. At the end of the study, levels of COX-1, COX-2 and LOX were measured by using Western blot. Two samples for each injection were showed in the figure.