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Centrally injected histamine increases posterior hypothalamic acetylcholine release in hemorrhage-hypotensive rats

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ABSTRACT

Histamine, acting centrally as a neurotransmitter, evokes a reversal of hemorrhagic hypotension in rats due to the activation of the sympathetic and the renin–angiotensin systems as well as the release of arginine vasopressin and proopiomelanocortin-derived peptides. We demonstrated previously that central nicotinic cholinergic receptors are involved in the pressor effect of histamine. The aim of the present study was to examine influences of centrally administrated histamine on acetylcholine (ACh) release at the posterior hypothalamus—a region characterized by location of histaminergic and cholinergic neurons involved in the regulation of the sympathetic activity in the cardiovascular system—in hemorrhage-hypotensive anesthetized rats.

Hemodynamic and microdialysis studies were carried out in Sprague–Dawley rats. Hemorrhagic hypotension was induced by withdrawal of a volume of 1.5 ml blood/100 g body weight over a period of 10 min. Acute hemorrhage led to a severe and long-lasting decrease in mean arterial pressure (MAP), heart rate (HR), and an increase in extracellular posterior hypothalamic ACh and choline (Ch) levels by 56% and 59%, respectively. Intracerebroventricularly (i.c.v.) administered histamine (50, 100, and 200 nmol) dose- and time-dependently increased MAP and HR and caused an additional rise in extracellular posterior hypothalamic ACh and Ch levels at the most by 102%, as compared to the control saline-treated group. Histamine H1 receptor antagonist chlorpheniramine (50 nmol; i.c.v.) completely blocked histamine-evoked hemodynamic and extracellular posterior hypothalamic ACh and Ch changes, whereas H2 and H3/H4 receptor blockers ranitidine (50 nmol; i.c.v.) and thioperamide (50 nmol; i.c.v.) had no effect.

In conclusion, centrally administered histamine, acting via H1 receptors, increases ACh release at the posterior hypothalamus and causes a pressor and tachycardic response in hemorrhage-hypotensive anesthetized rats.

1. Introduction

In the mammalian central nervous system, histamine is present both in neurons and mast cells (Brown et al., 2001). Acting as a neurotransmitter via H1, H2, and H3 receptors, it affects many central nervous system functions, such as energy balance, drinking, pain perception, learning, and memory (Brown et al., 2001; Fogel et al., 2008). Histaminergic neurons are located in the tuberomammillary nucleus of the posterior hypothalamus (Schwartz et al., 1991) and send projections to many regions, including pontine and medullary centers responsible for the cardiovascular regulation (Bealer, 1999). Centrally injected histamine increases mean arterial pressure (MAP) and decreases heart rate (HR) in normotensive conscious rats and increases both parameters in normotensive anesthetized rats (Brown et al., 2001; Jochem, 2000). Interestingly, in hemorrhage-shocked rats, centrally injected histamine evokes a long-lasting survival with significantly higher pressor and tachycardic effects in comparison to normovolemic animals (Jochem, 2002, 2003). Moreover, our previous studies show interactions between the histaminergic and other neuronal systems, such as the noradrenergic (Jochem and Zwirska-Korczala, 2002), opioidergic (Jochem and Zwirska-Korczala, 2004), angiotensinergic (Jochem et al., 2006), serotonergic (Jochem et al., 2007, 2008), prostaglandinergic (Altinbas et al., 2012, 2014), and cholinergic systems (Yalcin et al., 2009; Jochem et al., 2010), in the central cardiovascular regulation.

The central cholinergic system also affects the cardiovascular regulation both in normotension and critical hypotension (Savci et al., 2002; Topuz et al., 2014). Interestingly, there are different cholinergic pathways responsible for blood pressure regulation, depending on the initial MAP. In normotensive animals the pressor effect of cholinomimetics is short-lasting and mediated through the central nicotinic mechanisms, which leads to the activation of the sympathetic nervous system (Savci et al., 2002; Topuz et al., 2014). In contrast, in hemorrhage-shocked rats centrally acting cholinomimetic drugs evoke a long-lasting resuscitating action mediated via central nicotinic receptors

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(Savci et al., 2002). Similarly to histamine-induced effects, peripheral mechanisms activated by the central cholinergic nicotinic pathway in hemorrhagic shock involve the sympathetic nervous system (Savci et al., 2002).

There are morphological and functional connections between the central histaminergic and the cholinergic systems (Bacciottini et al., 2001, 2002). Histaminergic neurons of the tuberomammillary nucleus provide differential direct and indirect projections to the mesopontine cholinergic complex (Hong and Lee, 2011). On the other hand, cholinergic neurons can modify histamine release from histaminergic neurons at the anterior hypothalamus by stimulating M1 muscarinic heteroreceptors (Prast et al., 1994). Since both systems demonstrate similar central cardiovascular effects in normotensive and hypotensive animals and are able to influence bidirectionally their actions, the goal of the present study was to evaluate effects of centrally administrated histamine on acetylcholine (ACh) release at the posterior hypothalamus of hemorrhage-hypotensive anesthetized rats using microdialysis and hemodynamic studies.

2. Methods

2.1. Animals

In the experiments, 84 adult, male Sprague–Dawley rats (280–340 g) (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used. Four to five rats were housed in individual cages under controlled conditions of temperature (20–22 °C), humidity (60–70%), and lighting (12 h light/dark cycle) and nursed with food and water *ad libitum*. The Animal Care and Use Committee of Uludag University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, approved all experimental procedures (http://oacu.od.nih.gov/regs/guide/guide1.htm).

Each animal was studied separately in a single experimental protocol, and each experimental group consisted of seven rats.

2.2. Surgical procedures

Under ketamine (70 mg/kg; i.m.) and xylazine (10 mg/kg; i.m.) anesthesia, the left femoral artery was cannulated with PE 50 tubing filled with heparinized saline (100 U/ml). The tip of tubing was covered and exteriorized at the neck of the rat. For intracerebroventricular (i.c.v.) treatment, a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma. A 22-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The cannula was lowered 4.5 mm below the surface of the skull and fixed to the skull with acrylic cement. For microdialysis study, anesthetized and catheterized rats were placed in a stereotaxic frame. The skull was exposed and drilled over the posterior hypothalamus (coordinates: 3.6 mm posterior to bregma, 0.5 mm lateral (left) to the midline and 9.0 mm vertical to the skull). Handmade microdialysis probes (by Burcin Altinbas) were used for microdialysis study. Probes (molecular weight cutoff dialysis membrane was 18 kDa and length was 2.0 mm) were implanted and then fixed with acrylic cement to the skull.

2.3. Cardiovascular measurements and hemorrhage protocol

In order to measure MAP and HR the arterial cannula was connected to a volumetric pressure transducer BPT 300 (BIOPAC Systems Inc., CA, USA) attached to MP36 system (BIOPAC Systems Inc., CA, USA). After connection, baseline MAP and HR measurements were recorded. Rats were allowed to stabilize for 30 min before experiments. The blood pressure was reported as MAP (mmHg) and HR was expressed as beats per minute (bpm).

Following recording of baseline MAP and HR, acute hypotensive hemorrhage was performed by withdrawing a total volume of 1.5 ml of blood/100 g body weight over a period of 10 min with a constant rate via arterial catheter. The arterial catheter was then flushed with 0.1 ml of heparinized saline (50 U/ml) and reconnected to the transducer. After a 10-min stabilization period, the drugs were given to the rats via guide cannulas and cardiovascular parameters were monitored for the next 60 min.

2.4. Microdialysis study and measurement of ACh and choline (Ch) levels

Microdialysis probe was attached to the perfusion pump. The dialysis probe was perfused with artificial cerebrospinal fluid (pH 7.4) of the following composition: 120 mmol/l NaCl, 1.3 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l NaH_2PO_4, 3.5 mmol/l KCl, 25 mmol/l $NaHCO_3$, 10 mmol/l glucose, and 20 μmol/l neostigmine (a reversible acetylcholinesterase (AChE) inhibitor). The perfusion rate was 2 μl/min. Dialysis probe was perfused for the first 40 min of the stabilization period and samples were collected in 10 min intervals. After this period, one more sample was collected and measured for basal ACh and Ch levels. After that, hemorrhage was initiated, and the collection of the samples was continued. After the termination of bleeding, the drugs or vehicle was injected i.c.v., and samples were collected over the next 60 min. At the end of the experiments, animals were killed by using an overdose of pentobarbital sodium (200 mg/kg; i.v.), and brains were removed and fixed in 10% formalin. Serial coronal sections were sliced at 40 μm in cryostat and stained with toluidine blue to verify the location of the tip of the dialysis probe (Fig. 1).

Equipment for measurements of ACh and Ch levels consisted of an online membrane degasser, isocratic pump, and pulse dampener (Hitachi L2130, Japan). Injections (10 μl) were made through a Rheodyne 7725i valve fitted with a 20 μl loop. Online electrochemical detection was performed by an Amor detector fitted with a platinum electrode (Antec Leyden Ltd., The Netherlands). The columns and detector cell were kept at 24 °C in a column oven. Chromatograms were collected and analyzed using Agilent EZChrom Elite (Agilent Technologies, Inc., CA, USA) software. Results were calculated from peak area readings. Levels of neurotransmitter (pmol) were analyzed for posterior hypothalamus.

ACh and Ch are electrically inert and so need to be converted to H_2O_2 prior to detection. To do this, a dedicated column arrangement consisting of guard, separation, and immobilized-enzyme reactor (IMER) columns, containing acetylcholinesterase and choline oxidase,

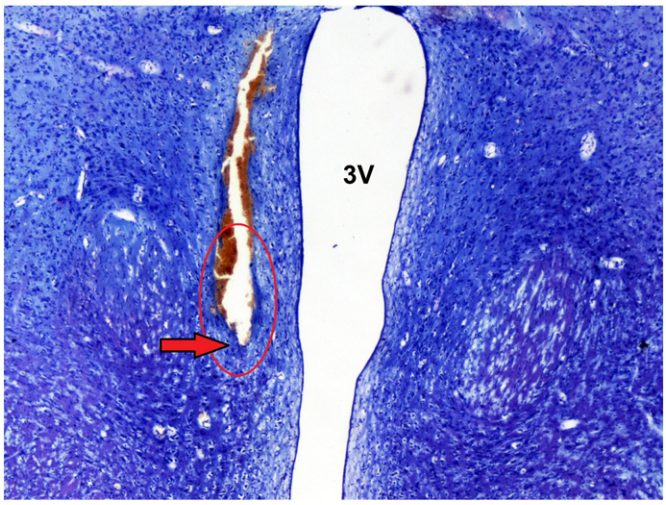

Fig. 1. Photomicrograph of a typical placement of a unilateral microdialysis probe in the posterior hypothalamus. The arrow indicates the tip of the microdialysis probe. The circle shows area of posterior hypothalamus. 3 V: third ventricle.r