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POSTISCHEMIC REGULATION OF CENTRAL HISTAMINE RECEPTORS

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Abstract—This study characterizes changes occurring in the central histaminergic system associated with ischemia–reperfusion pathology in the rat. Specifically, after a postocclusion time period of 48 h, we have analyzed histamine H_1 receptor mRNA expression, histamine H_2 receptor protein amount and binding densities, and histamine H_3 receptor mRNA expression and binding densities in brain regions that have been suggested to be selectively vulnerable to transient global ischemia, i.e. hippocampus, thalamus, caudate-putamen, and cerebral cortex.

We found an increase in H_1 receptor mRNA expression in the caudate-putamen: given that ischemia reduces glucose uptake and H_1 receptor activation has been shown to decrease this effect, an increase of expression levels may result in mitigating tissue damage due to energy failure observed in ischemia. A decrease in H_2 receptor binding densities in the caudate-putamen was also observed; the ischemia-induced decrease in H_2 receptor protein was also detectable by Western blot analysis. This phenomenon may underlie the previously reported ischemia induced striatal dopamine release. H_3 receptor mRNA expression was increased in the caudate putamen of the postischemic brain but was decreased in the globus pallidus and the thalamus; in association with this, H_3 receptor binding densities were increased in the cortex, caudate-putamen, globus pallidus, and hippocampus. The upregulation of H_3 receptor ligand binding may be involved in the previously reported continuous neuronal histamine release. Our data suggest that central histamine receptor expression and ligand binding are altered in brain ischemia in distinct areas, and may participate in neuroprotection and/or ischemia-associated neuronal damage.

Key words: striatum, hippocampus, thalamus, histamine H_1 receptor, histamine H_2 receptor, histamine H_3 receptor.

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Abbreviations: CA1, cornu ammonis 1; CBF, cerebral blood flow; LDF, laser-Doppler flowmetry; MAO, monoamine oxidase; MAO B, monoamine oxidase type B.

Histamine is regarded as a neurotransmitter and neuromodulator within the CNS (Haas and Panula, 2003; Wada et al., 1991). Cell bodies of histaminergic neurons are exclusively located in the tuberomamillary nuclei of the hypothalamus (Panula et al., 1984; Watanabe et al., 1984; Wouterlood et al., 1986) and give rise to widespread projections extending through the basal forebrain to the whole central CNS (Watanabe et al., 1984; Inagaki et al., 1988, 1990; Panula et al., 1989). This morphological feature results in the involvement of histamine in the regulation of numerous physiological functions and behaviors such as thermoregulation, circadian rhythms, neuroendocrine and cardiovascular function, catalepsy, locomotion, drinking and feeding, as well as learning and memory (Haas and Panula, 2003; Onodera et al., 1994).

There is evidence for the involvement of histamine in ischemia. For example, middle cerebral artery occlusion in rat induces a long-lasting increase in neuronal histamine release in the rat striatum (Adachi et al., 1992). Moreover, histamine depletion with α-fluoromethylhistidine, an inhibitor of histidine decarboxylase (the synthesizing enzyme for histamine), significantly increases the number of necrotic pyramidal cells in hippocampal CA1 region in rats subjected to cerebral ischemia (Adachi et al., 1993). On the other hand, postischemic loading with histidine, a precursor of histamine, decreases the amount of neuronal damage in the rat striatum (Adachi et al., 2004).

In the brain, the effects of histamine are mediated by three of the possible four different subtypes of receptors that have been demonstrated to exist by pharmacological studies, gene cloning and expression analysis: H_1 (Fujimoto et al., 1993; Palacios et al., 1981), H_2 (Gantz et al., 1991; Ruat et al., 1990), H_3 (Drutel et al., 2001; Lovenberg et al., 1999; Pollard et al., 1993), and H_4 (Coge et al., 2001; Liu et al., 2001; Zhu et al., 2001). Despite obvious involvement of histamine in both neurotransmission and regulation of brain blood flow and vascular permeability effects, of ischemia on histamine receptors have not been studied extensively.

For example, blockade of H_1 receptor-mediated function with an antagonist (mepyramine) significantly increases the number of necrotic pyramidal cells in hippocampal CA1 region in rats subjected to cerebral ischemia (Adachi et al., 1993). Blockade of central H_2 receptors results in aggravation of neuronal damage due to ischemia in gerbil hippocampus, presumably by enhancing ischemia-induced increase in extracellular glutamate (Adachi et al., 2001); moreover, H_2 receptor-mediated signaling has been shown to impart neuronal protection by prolonging the onset of anoxic depolarization resulting from induced transient forebrain ischemia in gerbils (Fuji-

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tani et al., 1996). In rat, H_2 receptor blockade aggravates ischemia-induced neuronal damage; the amount of ischemia-induced neuronal damage in the caudate-putamen is decreased when the substantia nigra is lesioned along with administration of an H_2 receptor antagonist (Otsuka et al., 2003). The foregoing study suggests that the control of dopamine release by H_2 receptors, shown previously by Philippu et al. (1984), in the striatum plays a role in determining the amount of neuronal cell death occurring as a result of ischemia-reperfusion injury. Finally, the aforementioned ischemia-induced long-lasting increase in neuronal histamine release in the rat striatum (Adachi et al., 1992) renders importance to the histamine H_3 receptor because of its ability to control histamine synthesis and release (Arrang et al., 1983, 1987). Moreover, the long-lasting increase in histamine release was shown to be modified by (*R*)-α-methyl-histamine, an H_3 receptor agonist, which suggests that there is a neuronal source for the released histamine (Adachi et al., 1992).

This study was undertaken to characterize changes occurring in H_1 receptor mRNA expression in comparison to H_3 receptor mRNA expression and H_2 receptor binding densities in comparison to H_3 receptor binding densities in brain regions that are selectively vulnerable to transient global ischemia, e.g. hippocampal formation, thalamus, caudate-putamen, and neocortex (Kirino and Sano, 1984; Siesjö, 1988). The goal of this study is to determine if there is evidence suggesting that the central histaminergic system imparts neuroprotection to the ischemic brain.

EXPERIMENTAL PROCEDURES

Animals

The Animal Research Committee of the Helsinki University Central Hospital and the governmental animal health authorities had approved the study protocol which is in accordance with the European Convention (1986) guidelines. All efforts were made to minimize the number of animals used and their suffering. We used adult male Wistar rats (Harlan Nederland, Horst, The Netherlands) weighing 250–350 g. Rats were housed under diurnal lighting conditions and allowed free access to food and water.

Surgery

Rats were anesthetized by an i.p. injection of ketamine hydrochloride (50 mg/kg, Ketalar, Parke-Davis, Täby, Sweden) and a s.c. injection of medetomidine hydrochloride (0.5 mg/kg, Domitor, Orion, Finland). A PE-50 polyethylene tube was placed into the left femoral artery for continuous monitoring of blood pressure (Olli Blood Pressure Meter 533, Kone Oy, Espoo, Finland) and for periodic monitoring of arterial pH and blood gases. Rectal temperature was monitored and maintained at 37 °C during the operation with a heating blanket and a thermoregulated heating lamp.

The model of four-vessel occlusion of global ischemia was used in male Wistar rats (Pulsinelli and Buchan, 1988). Briefly, one day prior to the ischemia protocol, the second cervical vertebrae had been exposed to permit electrocoagulation of both vertebral arteries. For continuous on-line monitoring of cerebral blood flow (CBF) with laser-Doppler flowmetry (LDF) (Oxyflo, Optronix, Oxford, UK), the skull was exposed through a midline incision and thinned with a handheld drill anterior of the bregma locally to permit placing of the tip of the fiberoptic monitoring device mounted to a stereotactic device. To produce global cerebral ischemia, both carotid arteries were exposed through a ventral midline neck incision and vascular occluders custom-made from polyethylene tubings were placed around both common carotid arteries. While the rat was immobilized in a stereotactic device and baseline CBF recording had stabilized, the vascular occluders were used simultaneously to start the cerebral ischemia for a period of 10 min. The severity of ischemia i.e. reduction of CBF by at least 90% from the baseline, as well as reperfusion were verified on-line with LDF in each case. The pH and arterial blood gases of the animals were sampled to ascertain that they were kept within physiological range. Sham animals received the same surgery except for producing the common carotid artery occlusions. Following recovery from anesthesia, the animals survived for 48 h, after which the animals were killed with an overdose of pentobarbital (Mebunat, Orion, Esbo, Finland) administered intraperitoneally and cardioperfused with buffered saline.

Tissue preparation

For *in situ* hybridization and receptor binding experiments (global ischemia, $n=5$; sham control, $n=5$), the animals were decapitated, brains were removed, frozen in isopentane (−25 °C), and stored at −70 °C. Tissues were then cut to 20 μm cryosections, thaw mounted onto Polysine slides (Menzel-Gläser, Germany), and stored at −70 °C until used.

In situ hybridization histochemistry

The oligonucleotide probes used for *in situ* hybridization were designed so that they specifically recognized mRNAs of histamine H_1 and H_3 receptors. Sequences for both oligonucleotide probes used in this study have been published previously: H_1 receptor (Lintunen et al., 1998) and H_3 receptor (the oligonucleotide probe detecting all isoforms) (Drutel et al., 2001). As a control, we used a normal hybridization mixture with a 100-fold excess of unlabeled specific probes. As an additional control, we used a *Staphylococcus aureus* chloramphenicol acetyltransferase-specific oligonucleotide probe of the same size. The hybridization procedure used has been described before and was used with minor modifications (Lintunen et al., 1998; Dagerlind et al., 1992). All probes were labeled with [^{35}S]deoxyadenosine-5′-alpha(-thio)-triphosphate (New England Nuclear, Boston, USA) at their 3′ ends using terminal deoxynucleotide transferase (Promega, Madison, WI, USA). Nonincorporated nucleotides were removed by purification through Sephadex G-50 columns.

Before hybridization, cryosections were taken from the −70 °C environment and kept at room temperature for 10 min and treated with UV light for 5 min. The hybridization (10^7 cpm/ml) was carried out at 50 °C for 16–20 h in a humidified chamber. Post-hybridization washes were carried out as described previously (Lintunen et al., 1998). Sections and carbon-14 standards were exposed for 10 days to Kodak BioMax X-ray films (Kodak, Rochester, NY, USA).

Receptor binding autoradiography

The method for determining histamine H_2 receptor binding densities on slide-mounted tissue sections has been published previously (Ruat et al., 1990; Traiffort et al., 1992; Honrubia et al., 2000; Sallmen et al., 2003). Briefly, slide-mounted sections were incubated for 3 h at room temperature in 50 mM Na_2/K phosphate buffer pH 7.5 containing 0.1 nM [^{125}I]iodoaminopotentidine. Non-specific binding was defined with the use of 10 μM tiotidine on adjacent tissue sections. After three rinses (each 10 min) in ice-cold buffer followed by a brief immersion in ice-cold water, sections were air-dried and apposed to Kodak BioMax X-ray films (Kodak) for a period of one day.