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Histamine up-regulates astrocytic glutamate transporter 1 and protects neurons against ischemic injury

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ABSTRACT

Astrocytic glutamate transporter 1 (GLT-1) is responsible for the majority of extracellular glutamate clearance and is essential for preventing excitotoxicity in the brain. Up-regulation of GLT-1 shows benefit effect on ischemia-induced neuronal damage. In present study, we examined the effect of histamine, a neurotransmitter or neuromodulator, on GLT-1 expression and function. In acute hippocampal slices, histamine selectively increased GLT-1 expression independent of neuronal activities. Similar up-regulation of GLT-1 was also observed after histamine treatment in pure cultured astrocytes, which was abolished by H1 receptor antagonist or PKC inhibitor. Cell surface biotinylation and whole-cell patch recordings of glutamate transporter current confirmed the up-regulation of functional GLT-1 following histamine exposure. Histamine treatment decreased the extracellular glutamate content and alleviated neuronal cell death induced by exogenous glutamate challenge. Moreover, we found a significant neuroprotective effect of histamine in brain slices after oxygen-glucose deprivation (OGD). In addition, histidine, the precursor of histamine, also showed neuroprotection against ischemic injury, which was accompanied by reversion of declined expression of GLT-1 in adult rats subjected to middle cerebral artery occlusion (MCAO). These neuroprotective effects of histamine/histidine were blocked by GLT-1 specific inhibitor dihydrokainate or H1 receptor antagonist. In summary, our results suggest that histamine up-regulates GLT-1 expression and function via astrocytic H1 receptors, thus resulting in neuroprotection against excitotoxicity and ischemic injury.

1. Introduction

In the mammalian CNS, glutamate is the major excitatory neurotransmitter. The low glutamate level in the extracellular space is maintained by a family of high-affinity, Na^+-dependent glutamate transporters, which finely control the integrity of excitatory synaptic transmission and prevent glutamate-induced excitotoxicity (Maragakis and Rothstein, 2004; Shigeri et al., 2004; Tzingounis and Wadiche, 2007). To date, five subtypes of glutamate transporters (EAAT1-EAAT5) have been identified, and these subtypes have different regional and cellular distributions (Gegelashvili and Schousboe, 1998; Robinson and Dowd, 1997). In particular, GLAST (also known as EAAT1) and GLT-1 (also known as EAAT2) are mainly expressed in astrocytes, and evidences support that GLT-1 is the most abundant and responsible for the majority of glutamate uptake (up to 90%) in the CNS (Danbolt, 2001; Robinson, 1998).

The important roles of glutamate transporters especially GLT-1 in excitotoxicity and ischemia-induced neuronal damage have been revealed by transgenic ablation or antisense down-regulation

Abbreviations: ACSF, artificial cerebrospinal fluid; CNS, central nervous system; DHK, dihydrokainate; EAAT, excitatory amino acid transporter; GABA, gamma-aminobutyric acid; GLAST, glutamate aspartate transporter; GLT-1, glutamate transporter 1; GS, glutamine synthetase; HTMT, histamine trifluoromethyl toluidide; L-TBOA, L-threo-β-benzyloxyaspartate; MCAO, middle cerebral artery occlusion; OGD, oxygen-glucose deprivation; RIPA, radioimmune precipitation assay; SD, Sprague-Dawley; TTX, tetrodotoxin.

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(Rao et al., 2001; Rothstein et al., 1996; Tanaka et al., 1997). On the other hand, accumulating evidences support that genetic over-expression or pharmacological up-regulation of GLT-1 markedly decreased glutamate level and protected neurons against exogenous glutamate toxicity (Guo et al., 2003; Liu et al., 2013; Rothstein et al., 2005). Moreover, a novel chemical compound, ONO-2506, increased glutamate transporter expression and consequently ameliorated the delayed infarct expansion and neurological deficits in adult rats subjected to transient middle cerebral artery occlusion (Mori et al., 2004). Therefore, GLT-1 may be a potential therapeutic target for ischemic injury, and more selective agents or endogenous modulators need to be discovered.

Histamine is recognized as an important neurotransmitter or neuromodulator in the CNS. Histaminergic neurons are located in the hypothalamus tuberomammillary (TM) nucleus, and project widely throughout the brain (Wada et al., 1991). Four histamine receptors have been identified (H1–H4), and three of them (H1–H3) are prominently expressed in the brain (Haas and Panula, 2003; Nguyen et al., 2001). Previous works suggest that central histamine via its receptors may be an endogenous protective factor for cerebral ischemia (Adachi, 2005; Hu and Chen, 2012). Either intracerebroventricular administration of histamine or intraperitoneal injection of histidine, a precursor of histamine, markedly alleviates the neuronal injury in ischemic animal models (Adachi et al., 2005; Fujitani et al., 1996). In addition, preischemic treatment of histamine suppresses the increased glutamate content during ischemia (Hamami et al., 2004), whereas blockade of histamine H1 receptors by terfenadine, chlorpheniramine or triprolidine enhances NMDA receptor-dependent excitotoxicity (Diaz-Trelles et al., 2000). Our previous work revealed that in histidine decarboxylase knockout (HDC-KO) mice, which are lack in endogenous histamine, the glutamate content in the hippocampus is significantly higher following contextual fear conditioning compared with that in wild-type mice (Liu et al., 2007). Although these evidences implicate that the neuroprotection by histamine against ischemic injury may originate from interfering glutamate levels, the mechanisms are largely unclear.

In the present study, we examined whether histamine regulates the astrocytic glutamate transporter GLT-1, and thereby exerts neuronal protection against excitotoxicity and brain ischemia.

2. Materials and methods

All experiments were carried out in accordance with the ethical guidelines of the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.1. Hippocampal slice preparation and drug treatment

Two to three weeks old male Sprague-Dawley (SD) rats were used to prepare hippocampal slices. After decapitation, the brains were removed and submerged in artificial cerebrospinal fluid (ACSF) at 4 °C and saturated with 95% O_2/5% CO_2. The hippocampal slices (400 μm thick) containing hippocampus and parts of adjacent cortex were cut using a vibratome (VT1000S, Leica instruments Ltd., Germany) and equilibrated in an incubation chamber with oxygenated ACSF at 25 °C. The ACSF used in cutting and incubation contained (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1 NaH_2PO_4, 1.3 $MgSO_4$, 26.2 $NaHCO_3$, and 11 D-glucose (pH 7.4).

The brain slices were treated with histamine (10^{-5} M) or together with tetrodotoxin (TTX, 1 μM) and nimodipine (5 μM) for 2 h. Then the brain slices were lysed for Western blotting analysis (cortical and hippocampal region) or transferred to oxygen–glucose deprivation (OGD) condition. The histamine receptor antagonists diphenhydramine (10 μM), cimetidine (10 μM) and thioperamide (10 μM) were added 30 min before histamine treatment. All the drugs used above were purchased from Sigma, USA.

2.2. OGD model in hippocampal slice

After drug treatment in normal ACSF for 2 h, slices were carefully transferred into glucose-free ACSF bubbled with 5% CO_2 and 95% N_2 for 20 min at 37 °C to induce oxygen–glucose deprivation (OGD), while control slices were kept in oxygenated normal ACSF. Following OGD, the slices were incubated in normal ACSF again for 1 h (reperfusion). Dihydrokainate (DHK, 300 μM, sigma) was added 10 min before OGD and maintained throughout the entire OGD/reperfusion. Cell viability was detected with 0.25% 2,3,5-triphenyltetrazolium hydrochloride (TTC) staining. Formazan was dissolved in ethanol/dimethylsulfoxide (DMSO) (1:1) with its content measured at 490 nm. Cell viability was normalized to the dry weight of the slices and then normalized to control slices as percentage values. Alternatively, the neuronal degeneration after OGD was measured by incubating the slices for additional 1 h in ACSF with 0.01 mg/ml propidium iodide (PI, MP Biomedicals, France). Then the image of PI uptake was captured by a digital camera (Andor, Northern Ireland) mounted on the microscope (Nikon FN1, Japan).

2.3. Pure primary astrocyte culture and neuron–astrocyte co-culture

Pure primary astrocyte cultures and neuron–astrocyte co-cultures were prepared from P0 SD rats. Briefly, the cerebral cortices and hippocampus were digested with 0.25% (0.125% for co-culture) trypsin (Gibco) for 10 min at 37 °C, and then the dissociated cells were seeded onto poly-D-lysine-coated flasks or coverslips in 24-well plates. The astrocytes were cultured in DMEM (Gibco, USA) medium supplemented with 2 mM L-glutamine and 10% (vol/vol) fetal bovine serum (FBS). To obtain neuron-astrocyte co-cultures, the culture medium was replaced by Neurobasal with L-glutamine (1:400, Gibco, USA) and B27 (1:50, Gibco, USA) 24 h after cell seeding. The medium was changed every 2–3 days, and the flasks were shaken overnight to minimize neurons and microglia, when astrocytes reached confluence. Two or three days after subculture, astrocytes were treated with different concentrations of histamine (10^{-7}–10^{-5} M) or histamine trifluoromethyl toluidide (HTMT, 10^{-6} M, Tocris) for 24 h. Diphenhydramine (10^{-5} M), cimetidine (10^{-5} M), thioperamide (10^{-5} M), Rp-cAMP (10^{-5} M, sigma), Bisindolylmaleimide II (Bis, 10^{-5} M, sigma) or DHK (300 μM, sigma) was added 30 min before histamine treatment. The supernatant medium was then collected to determine glutamate and GABA content by high-performance liquid chromatography (HPLC), and the astrocytes were used for glutamate transporter current recording, cell surface biotinylation or lysed for Western blotting analysis. For prolonged H1 receptor antagonists exposure, cultured astrocytes were treated with diphenhydramine (10^{-5} M or 10^{-4} M) or pyrilamine (10^{-5} M or 10^{-4} M) for 48 h, 72 h or 48 h followed by removing the drug and incubation for another 24 h. Then the supernatant was used for HPLC analysis, and astrocytes were lysed for Western blotting analysis.

2.4. Analysis of glutamate and GABA concentrations by HPLC

As previously described (Jin et al., 2005; Shen et al., 2008), culture medium after drug treatment was deproteinized with 0.4 mol/L perchloric acid and centrifuged at 15 000 × *g* for 20 min at 4 °C. Then the supernatant was filtered through a 0.22 μm polyvinylidene difluoride membrane. The glutamate and GABA content in the supernatant was measured by HPLC (ESA, Chelmsford, MA, USA) which was controlled by CoulArray® software. The system consists of model 582 pump, model 540 autosampler and four channel CoulArray electrochemical detector. After reacting with the derivate o-phthalaldehyde, analysates were separated on a reversed-phase column (3 μm, 3 × 50 mm Capcell Pak MG C18 column; Shiseido, Japan). A two-component gradient elution system was used, component A of the mobile phase being 100 mM Na_2HPO_4, 13% acetonitrile, and 22% methanol, pH 6.8, and component B being similar to A except with 5.6% acetonitrile and 9.4% methanol. A gradient elution profile was used as follows: 0–3.5 min, isocratic 100% B; 3.5–20 min, linear ramp to 0% B; 20–22 min, isocratic 0% B; 22–23 min, linear ramp to 100% B; 23–30 min, isocratic 100% B. The temperature of the column was maintained at 38 °C. The flow rate was set to 0.75 ml/min. The first cell was set at 250 mV, whereas the second cell was set at 550 mV for glutamate or 350 mV for GABA determination. Under these conditions, the retention time of glutamate and GABA is 8.5 and 21.9 min, respectively. The detection limits (signal/noise ≥ 3) were 10 ng for glutamate, 10 ng for GABA. The amount of glutamate/GABA per mg protein was calculated, then normalized to the values in control group.

2.5. Whole-cell patch recording in cultured astrocytes

The cultured astrocytes were used for glutamate transporter current analysis by whole-cell patch recording, including transporter GLT-1 and GLAST mediated current. The standard external solution contained (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 D-glucose and 10 HEPES, adjusted to pH 7.4. The patch pipette solution contained (in mM): 140 KSCN, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, 3 MgATP and 10 HEPES, adjusted to pH 7.2. The osmolarity of the solutions was 310–320 mOsm/L. Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm on a two-stage puller (PP-830, Narishige). The resistance between the recording electrode filled with the pipette solution and the reference electrode was 3–5 MΩ. The membrane potential was held at −70 mV throughout the experiment. Signals were acquired using a Multiclamp 700A amplifier (Molecular Devices, CA) controlled by Clampex 9.0 software via a Digidata 1320A interface (MolecularDevices). L-threo-β-benzyloxyaspartate (L-TBOA, Tocris, UK), DL-2-amino-5-phosphonovaleric acid (APV, Sigma), and 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt hydrate