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Full paper

Histamine elicits glutamate release from cultured astrocytes

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ABSTRACT

Astrocytes play key roles in regulating brain homeostasis and neuronal activity. This is, in part, accomplished by the ability of neurotransmitters in the synaptic cleft to bind astrocyte membrane receptors, activating signalling cascades that regulate concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and gliotransmitter release, including ATP and glutamate. Gliotransmitters contribute to dendrite formation and synaptic plasticity, and in some cases, exacerbate neurodegeneration. The neurotransmitter histamine participates in several physiological processes, such as the sleep-wake cycle and learning and memory. Previous studies have demonstrated the expression of histamine receptors on astrocytes, but until now, only a few studies have examined the effects of histamine on astrocyte intracellular signalling and gliotransmitter release. Here, we used the human astrocytoma cell line 1321N1 to study the role of histamine in astrocyte intracellular signalling and gliotransmitter release. We found that histamine activated astrocyte signalling through histamine H_1 and H_2 receptors, leading to distinct cellular responses. Activation of histamine H_1 receptors caused concentration-dependent release of $[Ca^{2+}]_i$ from internal stores and concentration-dependent increase in glutamate release. Histamine H_2 receptor activation increased cyclic adenosine monophosphate (cAMP) levels and phosphorylation of transcription factor cAMP response-element binding protein. Taken together, these data emphasize a role for histamine in neuron-glia communication.

1. Introduction

Astrocytes are the most abundant cell type in the central nervous system. They are involved in a wide range of physiological processes, including blood flow regulation,[1] energy metabolism,[2] ionic homoeostasis,[3] and synaptic function.[4,5] The ability of astrocytes to modulate synaptic function is, in part, mediated by their ability to bind locally released neurotransmitters, and respond with the release of gliotransmitters, such as adenosine triphosphate (ATP), glutamate, and D-serine.[6,7] Gliotransmitter release appears to be disturbed under pathological conditions, and is associated with neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.[8,9]

Released neurotransmitters bind to various subclasses of G protein-coupled receptors (GPCRs) on astrocytes to activate intracellular signalling and regulate intracellular second messengers.[10] Signalling through G_q-coupled GPCRs can increase intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration through the activation of phospholipase C (PLC); PLC cleaves the membrane phospholipid phosphatidylinositol 4,5-bis-phos-phate to yield 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate ($InsP_3$), the latter of which can bind to $InsP_3$ receptors leading to Ca^{2+} release from intracellular stores.[11] In contrast, signalling through G_s-coupled GPCRs activates adenylyl cyclase, which increases the formation of cyclic AMP (cAMP). The importance of G_q-linked intracellular Ca^{2+} signalling for gliotransmitter release has been highlighted in several studies.[8,9,12] Furthermore, a connection between cAMP treatment and enhanced glutamate release from astrocytes was reported.[13]

The neurotransmitter histamine plays a key role in the sleep-wake cycle and in learning and memory.[14] Alterations in brain histamine levels are closely connected with central nervous system dysfunction, and are thought to contribute to neurological disorders, including Alzheimer's disease and depression.[15,16] Histamine binds to four distinct GPCRs (histamine H_1–H_4 receptors); of these, previous studies have reported the expression of H_1–H_3 receptors on astrocytes.[17] In an early study, Inagaki et al. showed comparable

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H_1 receptor binding capacities in primary astrocyte cultures and brain tissue, where besides astrocytes neurons also express H_1 receptors.[18] Additionally, histamine stimulation increases $[Ca^{2+}]_i$ in astrocytes.[19–21] Taken together, previous findings indicate a significant role for histamine in astrocyte signalling. Yet, only a few studies to date have examined the importance of histamine in astrocyte signalling, and the effect of histamine on gliotransmitter release is unknown. In the present study, we examined the role of histamine in astrocyte intracellular signalling and gliotransmitter release in the human astrocytoma cell line 1321N1.

2. Methods

2.1. Cell culture

The 1321N1 human astrocytoma cell line was kindly donated by the late Professor Norimichi Nakahata, Tohoku University. Cells were cultured under a humidified atmosphere of 5% CO_2 at 37 °C in DMEM (Life Technologies, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (Biowest, Nuaillé, France), 100 IU/ml benzylpenicillin potassium (Wako Pure Chemical Industries, Ltd., Osaka), 100 μg/ml streptomycin sulphate (Wako Pure Chemical Industries, Ltd.), and 2 mM GlutaMax-I (Life Technologies).

2.2. PCR

Total RNA isolation and reverse transcription of 1321N1 RNA were performed as described previously.[22] Briefly, 1321N1, normal human astrocyte (Lonza, Basel, Switzerland), and whole human brain (Takara Bio Inc., Shiga) cDNAs were amplified using the KOD SYBR qPCR) real time-PCR master mix (Toyobo, Osaka), and run on 3-step-PCR for 40 cycles (98 °C for 10 s, 60 °C for 10 s, then 68 °C for 30 s). Specific primers for the genes of H_1–H_4 receptors (*HRH1–4*) and β-actin (*ACTB*) were used for amplification (Table 1).

2.3. Intracellular Ca^{2+} measurement

1321N1 cells, cultured on glass-bottom dishes, were incubated with 5 μM Fluo-4-AM dye (Dojindo, Kumamoto) for 30 min, followed by incubation with Hank's balances salt solution (HBSS) for 10 min. The buffer was replaced with fresh HBSS, and fluorescence intensity was detected using an inverted microscope (Olympus, Tokyo). Histamine (Sigma–Aldrich, St. Louis, MO, USA) or a histamine receptor agonist (H_1 receptor: 2-pyridylethylamine [2-PEA], H_2 receptor: dimaprit, H_3 receptor: immethridine [all purchased from Tocris, Bristol, UK], or H_4 receptor: 4-methylhistamine [Santa Cruz Biotechnology, Dallas, TX, USA]) was added after 30 s. Images were analysed using MetaMorph software (Molecular Devices, Sunnyvale, CA, USA).

For fluorescent assay, cells were seeded into 96-well clear-bottom microplates and prepared as described above. Baseline fluorescence was recorded for 20 s before the addition of histamine or a histamine receptor agonist. Fluorescence intensity was measured using a Flexstation® 3 microplate reader and analysed using Softmax Pro 5 software (Molecular Devices).

Independent of the recording method, antagonist studies were performed as follows: after incubation with Fluo-4-AM, cells were incubated with a histamine receptor antagonist (H_1 receptor: levocetirizine [Tokyo Chemical Industry, Tokyo], H_2 receptor: ranitidine [Sigma–Aldrich], H_3 receptor: JNJ10181457, or H_4 receptor: JNJ7777120 [both from Tocris]), an $InsP_3$ receptor antagonist (aminoethoxydiphenyl borate [2-APB]), or a PLC inhibitor (U73122) (both from Cayman Chemicals, Ann Arbor, MI, USA) for 10 min, and subsequently, fluorescence was measured. The inhibitory drugs were present in pre-treatment solutions as well as in the experimental solutions. In some experiments, the sarcoendoplasmic reticulum Ca^{2+} transport ATPase (SERCA) inhibitor thapsigargin (Wako Pure Chemical Industries, Ltd.) was added for 10 min after the first histamine stimulation but prior to the second histamine stimulation.

2.4. Glutamate release assay

1321N1 cells cultured in 24-well plates were washed and incubated with HBSS for 30 min at 37 °C 5% CO_2. Subsequently, cells were washed again and incubated with HBSS vehicle or HBSS containing a histamine receptor antagonist (H_1 receptor: levocetirizine and fexofenadine [Tokyo Chemical Industry] or H_2 receptor: ranitidine and famotidine [Tokyo Chemical Industry]) and PLC-inhibitor U73122 or its inactive analogue U73343 (Abcam, Cambridge, UK) for 10 min, before the medium was exchanged with medium containing histamine or histamine receptor agonists (H_1 receptor: 2-PEA; H_2 receptor: dimaprit), or containing histamine together with inhibitory drugs. The supernatant was collected after 5 min and subjected to high-pressure liquid chromatography (HPLC).

2.5. HPLC measurement

Glutamate was measured using an HPLC system, as described previously.[23] In this study, samples were analysed using an FA-3ODS separation column, HTEC-500 electrochemical detection system, and M-504G auto-sampling injector (Eicom, Kyoto).

2.6. cAMP response-element binding protein phosphorylation assay

1321N1 cells cultured in 6-well plates were washed and treated with 0.1 mM histamine, with or without 0.2 μM ranitidine, for 5 min. The reaction was terminated, and the cells were lysed in radioimmunoprecipitation assay buffer containing 1 mM sodium orthovanadate (New England Biolabs, Ipswich, MA, USA) and cOmplete™ Protease Inhibitor Cocktail® (Roche, Basel, Switzerland). Total protein concentrations were measured using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Western blot

Ten μg of extracted protein was separated on a 12% sodium dodecyl sulphate-polyacrylamide gel, and transferred onto a polyvinylidene fluoride membrane. The membrane was blocked in 5% non-fat dry milk solution for 1 h, and incubated with primary IgG rabbit anti-cAMP response-element binding protein (CREB) (1:5000), IgG rabbit anti-phospho-CREB (1:5000), or IgG rabbit anti-β-actin (1:1000) antibody (all purchased from Cell Signalling Technology, Danvers, MA, USA) overnight at 4 °C. After washing, the membrane was incubated with peroxidase-linked anti-rabbit IgG antibody (Jackson ImmunoResearch Inc., West Grove, PA, USA; 1:2000), and

Table 1
Primer sequences for human histamine receptor genes.

Gene	Primer	Sequence	Product size (bp)
HRH1	Sense	5′ -TACTGAAACCAGCCAGGGAGT -3′	402
	Antisense	5′ -ACTGAAAATGGACGCTGTGC -3′	
HRH2	Sense	5′ -TGGTTTCCCTACTTCACCGC -3′	348
	Antisense	5′ -AAGCCATGGTCTGTCTGTGG -3′	
HRH3	Sense	5′ -TCACCCGAGCGGTCTCATAC -3′	430
	Antisense	5′ -TCACCCACCCCATACCTGTG -3′	
HRH4	Sense	5′ -TTTGTGGGTGTGATCTCCATTCC -3′	489
	Antisense	5′ -TCCACAGATGTTGGAAGAGACAG -3′	
ACTB	Sense	5′ -CGCCCTATAAAACCCAGCGG -3′	489
	Antisense	5′ -AACATGATCTGGGTCATCTTCTCG -3′	

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