Abstract

Gliotransmitter-mediated Ca^{2+} response and its modulation by

neurotrophic factors in astrocytes

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【Introduction】

Astrocytes, one of the glial cells, are known to release various bioactive substances called gliotransmitters, and are known to be involved in a wide range of physiological functions such as synaptic transmission and cerebral blood flow. Many gliotransmitters activate G-protein-coupled receptors on the plasma membrane and increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular sources. It has been pointed out that ATP, one of the gliotransmitters, is involved in synaptic transmission and blood flow regulation by acting on adjacent cells, including astrocytes, neurons, and blood vessels.

Other biologically active substances that act on astrocyte Ca^{2+} dynamics include bradykinin and neurotrophic factor. Bradykinin, produced during inflammation and cerebral ischemia, elevates $[Ca^{2+}]_i$ in astrocytes via activation of B2 receptors. Brain-derived neurotrophic factor (BDNF) is a protein released from synaptic junctions that binds to high-affinity nerve growth factor receptors (TrkB) on neurons and astrocytes, and has been reported to increase $[Ca^{2+}]_i$, though the details are not clear.

In order to clarify the signaling mechanism in astrocytes, the regulation mechanism of Ca^{2+} response and the interaction of BDNF with it were analyzed using a rat glial cell-derived cell line (C6 cells) and glial primary cells.

【Materials & Methods】

Astrocyte primary culture cells were prepared by treating the minced-cerebral cortex of 11-week-old male Wister rats with trypsin for 10 min, allowing fibroblasts to attach on fibronectin-coated dishes, and then seeding the detached cells into polyethyleneimine-coated measurement chambers. These adult-rat astrocyte were cultured in Dulbecco's Modified Eagle Medium including 10% Fetal bovine serum. C6 cells were cultured in RPMI-1640 including 10% Fetal bovine serum. Cells in the recording chambers were cultured in the presence of adenovirus vector for expressing GCaMP6s for 2 days. GCaMP6s-expressed cells were washed with HBSS-H and allowed to rest for at least 30 min prior to the Ca^{2+} measurement. To monitor GCaMP6s fluorescence, cells were excited at 480 nm, and emitted fluorescence at 530 nm were recorded using an inverted fluorescence microscope TE-2000 (Nikon) coupled with EM-CCD camera and controlled with AQUACOSMOS imaging software (Hamamatsu-photonics). These experiments were performed at room temperature.

【Results & Discussion】

Stimulations of C6 cells with ATP at concentrations above 3μM induced rapid increases in $[Ca^{2+}]_i$ in the entire cell body, and subsequent sustained elevation of $[Ca^{2+}]_i$. In the absence of

extracellular Ca^{2+}, ATP caused a transient increase in $[Ca^{2+}]_i$ without the sustained Ca^{2+} response. These ATP-induced Ca^{2+} responses in C6 cells were inhibited by the P2Y receptor antagonist Suramin. Adult rat astrocytes exhibited Ca^{2+} responses upon the stimulation of ATP and bradykinin.

ATP stimulation elicited a Ca^{2+} wave from the cell processes toward the cell body, whereas bradykinin stimulation elicited a Ca^{2+} wave from the cell body toward the cell processes. ATP-induced Ca^{2+} responses were inhibited by suramin, ATP caused Ca^{2+} response in C6 cells and rat astrocytes via P2Y receptors.

It was found that low concentrations of ATP (< 1 μM) induced localized elevations of $[Ca^{2+}]_i$ at cell processes. Localized Ca^{2+} oscillation was also observed in the cell processes with the local ATP stimulation at the cell body or the cell processes using caged ATP, regardless of the site of light exposure. In addition, Ca^{2+} oscillations at cell processes were inhibited by FCCP, which decreases the mitochondrial membrane potential, and increased by CAtr, which activates mPTP. These results indicate a high sensitivity of cell protrusions to ATP and the involvement of mitochondria in their Ca^{2+} response.

It was confirmed that adult rat astrocytes express TrkB-T1 as a BDNF receptor and that direct stimulation with BNDF does not induce a Ca^{2+} response. However, it was found that BDNF stimulation increased the sensitivity of astrocytes to ATP and bradykinin. When BDNF-unstimulated astrocytes were stimulated with bradykinin, 7.8% and 27% of the cells exhibited Ca^{2+} responses at 0.3 nM and 1 nM, respectively. In the astrocyte with 1-hr stimulation of BDNF, bradykinin-induced Ca^{2+} responses were observed in 76 % and 94 % of the cells at 0.3 nM and 1 nM, respectively. Similarly, ATP stimulation of BDNF-unstimulated astrocytes elicited Ca^{2+} responses in 3.9 % of the cells at 0.3 μM and 43 % at 3 μM, whereas in BDNF-stimulated cells, 43 % of the cells responded at 0.3 μM and 89 % at 3uM. The enhancement of bradykinin-induced Ca^{2+} response was further increased by 12-hrs stimulation with BDNF.

【Conclusion】

It was shown that astrocytes have higher ATP sensitivity in the cell processes than in the cell body. This ATP sensitivity in cell processes is thought to contribute to intercellular communication in astrocytes. The involvement of mitochondria in the Ca^{2+} response at the cell processes suggests that the signal transduction between astrocytes may be affected by oxidative stress. In addition, it was found that the sensitivity of astrocytes to ATP and bradykinin was strongly enhanced by BDNF. This finding represents a novel mechanism for the functional modulations of astrocyte sensitivity by factors released from neurons.