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Short Communication

A new approach to inhibiting astrocytic IP_3-induced intracellular calcium increase in an astrocyte–neuron co-culture system

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Abstract

Astrocytes exhibit dynamic Ca^{2+} mobilization, such as Ca^{2+} wave and Ca^{2+} oscillation, via an inositol 1,4,5-triphosphate-induced Ca^{2+} release (IICR)-dependent mechanism. The physiological functions of astrocytic Ca^{2+} mobilization, however, are poorly understood. To investigate this issue, we created a plasmid encoding an enhanced green fluorescent protein-tagged inositol 1,4,5-triphosphate absorbent protein and expressed it in cultured astrocytes. Expression of this protein inhibited both IICR and the Ca^{2+} wave in cultured astrocytes. By combining this method to the single cell electroporation technique, we were able to inhibit IICR specifically in astrocytes in an astrocyte–neuron co-culture system. Our approach provides a useful tool for direct examination of the physiological role of astrocytic Ca^{2+} signaling on neuronal function.

Theme: Cellular and molecular biology
Topic: Neuroglia and myelin

Keywords: IP3; IP3 scavenger; Astrocyte; Calcium wave; Single cell electroporation

Accumulating evidence indicates that astrocytes participate in the regulation of neuronal synaptic transmission [2,3,6,11,18,19,23,26]. It is now clear that astrocytes express a heterogeneous pattern of functional receptors to a variety of neurotransmitters, neuromodulators, and neurohormones. Studies involving initially cell cultures, and subsequently brain slices, have revealed that astrocytes respond to synaptically released transmitters, including glutamate, noradrenaline, histamine, acetylcholine, ATP, and γ-aminobutyric acid, accompanied by elevation of internal Ca^{2+} levels [7,11,24,28] through intracellular 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release (IICR) [27,28]. On the other hand, it has been also reported that hippocampal astrocytes in situ exhibit IICR-dependent calcium oscillations that occur independently of neuronal activity [17]. Intracellular Ca^{2+} elevation triggers the release of glutamate and aspartate from the astrocytes themselves [4,13,14,20–22]. However, the physiological consequences of intracellular Ca^{2+} elevation and Ca^{2+}-triggered transmitter release in astrocytes are largely unknown. To address this issue, we developed here a methodology for specific inhibition of astrocytic Ca^{2+} signaling in an astrocyte–neuron co-culture system.

We first investigated the IP_3 dynamics in cultured astrocyte. Astrocytes responded to either mechanical stimulation or bath application of ATP, with elevation of intracellular IP_3 and IICR (see Supplemental Fig. 1). To inhibit IICR, we used a single cell electroporation technique

Abbreviations: IPS, IP_3 scavenger; MPS, a point mutation of IPS

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[10] to express IP_3 scavenger protein (IPS; Fig. 1a), the amino acid residues 224–604 of mouse type 1 IP_3 receptor. It has been previously demonstrated that IPS has 500- to 1000-fold higher (K_d = 0.092 nM) binding affinity to IP_3 than IP_3 receptor (K_d = 83 nM) [25]. Briefly, mRNA of mouse type 1 IP_3 receptor was purified from mouse brain,

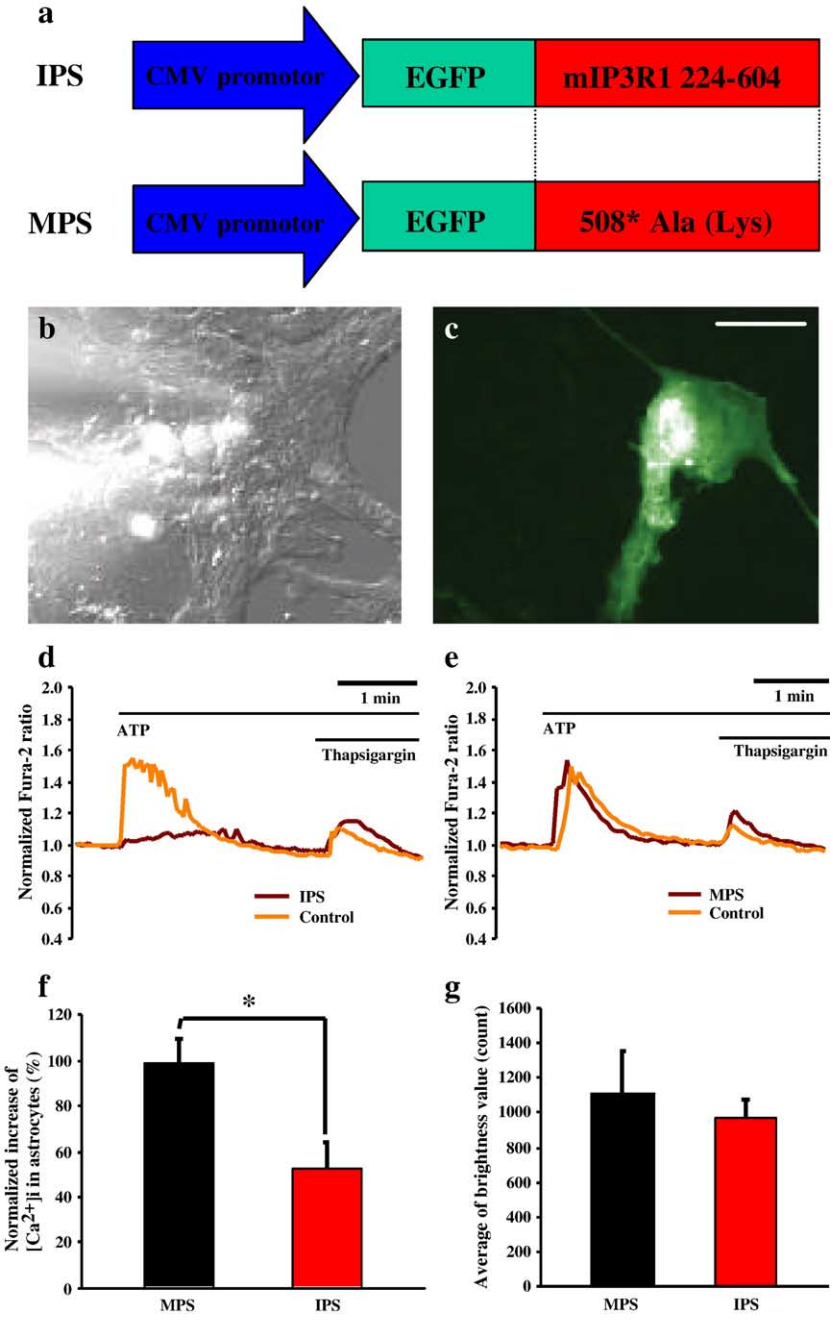

Fig. 1. Inhibition of IICR in IPS-expressing astrocytes. (a) Structures of IPS and MPS. (b) Electroporation of IPS into a single astrocyte. DIC image depicts single cell electroporation. The tip of a micropipette was positioned on the membrane surface of the target astrocyte. (c) Detection of EGFP fluorescent signal in the same cell 4 h later. (d–f) Intracellular Ca^{2+} imaging with fura-2. In these experiments, both ATP (10 μM) and thapsigargin (1 μM) were applied in a Ca^{2+}-free extracellular solution. (d) Representative data reveal that ATP induced a transient rise in intracellular Ca^{2+} concentration in naïve control astrocytes but not in IPS-expressing astrocytes. In contrast, subsequent application of thapsigargin evoked an intracellular Ca^{2+} increase in both control and IPS-expressing astrocytes. (e) Representative data show that both ATP and thapsigargin induced a transient rise in intracellular Ca^{2+} concentration in both naïve astrocytes and MPS-expressing astrocytes. (f) Summary of ATP-induced elevation in intracellular Ca^{2+} concentration. Scale bar = 50 μm. (g) Expression levels of MPS and IPS were examined by Aquacosmos particle analysis program. The average brightness value of EGFP was not significantly different between MPS- and IPS-expressing astrocytes. Intracellular Ca^{2+} imaging was conducted by employing Ca^{2+}-specific fluorescent probes, fura-2 AM. Cells were incubated for 60 min at 37 °C in Hank's balanced salt solution (HBSS) plus HEPES (25 mM) buffer (HEPES–HBSS) containing 5–10 μM fura-2 AM and 0.05% pluronic detergent; subsequently, cells were washed twice with HEPES–HBSS, then incubated at room temperature for 60 min. Cells were exposed to excitation wavelengths of 340 and 380 nm in order to measure fura-2 fluorescence; emission was recorded at 510 nm with a video-based imaging system (Aquacosmos, Hamamatsu Photonics, Shizuoka, Japan).