Astrocytes protect neurons in the hippocampal CA3 against ischemia by suppressing the intracellular Ca2+ overload

博士（医学）　孫　伝奇

論文題目

Astrocytes protect neurons in the hippocampal CA3 against ischemia by suppressing the intracellular Ca^{2+} overload

(アストロサイトは細胞内Ca^{2+}の過負荷を抑制して虚血から海馬CA3ニューロンを保護する)

論文の内容の要旨

[Introduction]

Astrocytes have extensive contacts with neuronal synapses, axons, blood vessels, and even other adjacent glial cells, and participate in the regulation of information processing and synaptic transmission by expressing various neurotransmitters and receptors. In past decades, astrocytes have generally been considered as a supportive glial cell in the central nervous system. However, in recent years, studies on astrocytes have shown that they play multiple roles in neuronal functions, including both physiological and pathological processes. While evidence indicates that astrocytes could exert neuroprotective effects following ischemia, the possible underlying mechanisms remain unclear. We hypothesized that: 1) the astrocytes can be neuroprotective against ischemia; and 2) if so, the neuroprotective mechanisms could be suppressing Ca^{2+} overload in the neurons. We aimed to investigate the roles of astrocytes in the process of delayed neuronal death following transient forebrain ischemia.

[Materials and Methods]

Male, adult Sprague-Dawley rats weighing 280–320 g were used. The experimental protocol was approved by the Committee on Ethics in Animal Experimentation at Hamamatsu University School of Medicine.

Gliotoxin Injection: L-α-aminoadipic acid (L-α-AAA), an astrocyte-selective gliotoxin, was injected into the hippocampal CA3 region of rats to selectively damage astrocytes. A glass micropipette filled with a solution L-α-AAA (0.1 mol/L) was inserted into the right hippocampal CA3 through a 3 × 3-mm cranial window according to the stereotoctic coordination. A pressure pulse of 0.1 MPa was applied to the pipette to eject 10 μL of solution.

Immunofluorescence Staining: Brain slices were sectioned at days 3 and 10, and immunofluorescence staining of glial fibrillary acidic protein (GFAP), NeuN and DAPI staining were performed.

4-vessel-occlusion, Transient Forebrain Ischemia Model: Transient forebrain ischemia was administered 3 days after L-α-AAA injection. Bilateral vertebral arteries were

completely occluded through the alar foramina of the first cervical vertebra by cauterization. Then the bilateral common carotid arteries were then exposed and a balloon vessel-occluder was placed loosely around each artery. By tightening and releasing the balloons, we induced an ischemic period 10 min in duration followed by reperfusion.

In-situ Ca^{2+} Fluorescence Imaging with a Fiber-coupled Confocal Microscope: The fluorescence Ca^{2+} indicator, fluo3/AM was applied by the pressurized-bolus injection method. A glass micropipette filled with fluo3/AM solution (2.5 μL, 440 μmol/L) was stereotactically inserted slowly into the CA1 or the CA3 regions. A pressure pulse of 0.1 MPa was applied to inject the dye solution. Rats were stabilized for 30 min after injection. An imaging fiber coupled to the laser confocal microscope was then inserted into the CA1 or CA3 region to observe the changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Rats were stabilized for a further 5 min, and then fluorescence images were recorded every 30 s beginning 5 min before transient forebrain ischemia and continuing until 20 min after reperfusion.

Regional cerebral blood flow (rCBF) measurement: rCBF was measured by laser-Doppler flowmetry during ischemia and reperfusion in CA1 and CA3 regions. A needle probe (diameter 0.5 mm) was stereotactically inserted to the CA1 or CA3 regions through the cranial window. Data were recorded every 2 min from 10 min before transient forebrain ischemia to 20 min after reperfusion (40 min in total).

[Results]

L-α-AAA injection significantly decreased the number of astrocytes in CA3, but did not affect the pattern of rCBF changes upon ischemia/reperfusion. Seven days after transient forebrain ischemia, in rats receiving L-α-AAA, delayed neuronal death comparable with that in CA1 was observed in the CA3 region. In addition, the pattern of increase in $[Ca^{2+}]_i$ due to transient forebrain ischemia was completely changed in the hippocampal CA3. The loss of astrocytes induced a persistent increase in $[Ca^{2+}]_i$ in the CA3 region following transient ischemia, similar to what is observed in the CA1 region.

[Conclusion]

Our study observed different $[Ca^{2+}]_i$ changes in hippocampal CA1 and CA3 regions after transient forebrain ischemia, and astrocytes were found to protect neurons against transient forebrain ischemia by suppressing the intracellular Ca^{2+} overload of neurons. The possible mechanisms of the astrocytic neuroprotection we have observed are: 1) astrocytes may uptake excitatory neurotransmitter, glutamate, to suppress neuronal intracellular Ca^{2+} overload after brain ischemia; and 2) neurons/astrocyte ratio of CA1

subfield may be more than that of CA3 subfield, and then the astrocytes in CA1 are not enough to protect neurons against ischemia. This novel finding reveals a new approach to explaining the selective vulnerability of hippocampal CA1 neurons and the neuroprotective role of astrocytes after transient forebrain ischemia.