BRAIN REGIONAL DIFFERENCES OF CELLULAR RESPONSES TO NEUROTOXIC INSULTS IN CULTURED ASTROCYTES

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Introduction

L-Glutamate is the major excitatory neurotransmitter as well as an important neurotoxin. Extracellular glutamate levels are elevated following ischemia, hypoglycemia, and trauma. Glutamate receptor-mediated excitotoxicity have been implicated in a number of disease processes such as stroke, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. One consequence of elevated glutamate levels is cell swelling. Such swelling occurs primarily in astroglial cells. Astrocytes are important in the control of extracellular water contents and ion concentrations in mammalian brain[1]. Dysfunction of astrocytes would lead to a sequence of pathological events such as loss of cellular volume control, an increase of brain tissue volume, both cellular and vasogenic edema, a rise in intracranial pressure, cerebral herniation and finally, arrest of cerebral circulation. It is now clear that astrocytes do not represent a uniform population of cells and that properties of astrocytes from one brain region ma be strikingly different from those of other regions. Regional defferences in their capacity to take up glutamate, serotonin and catecholamines and in the second messenger response have been described. Astrocytes from different regions exert different trophic or morphogenetic influences on neurons. An Astorcytic response to various stimuli has been reported to be different with different brain areas. In the present study, the regional differences in glutamate-induced swelling of cultured astrocytes from rat cerebral cortex, hippocampus and cerebellum were examined. And glutamate transporters GLAST and GLT and water transporter, aquaporin expressions in various astrocytic cultures were measured, in order to clarify the mechanism of the regional differences of glutamate-induced toxic response.

Methods and Materials

Astrocyte cell cultures

Cultured rat astrocytes were prepared from cerebral cortex, hippocampus, and cerebellum of 1-2 day old Sprague-Dawley rats[2]. Briefly, the dissociated cells were suspended in the culture medium and plated into 75cm^2 culture flask. After seeding, the cells were incubated in a humidified 5% CO_2 and

95% air atmosphere at 37°C and medium was changed twice a week. After the cells became confluent, microglia and oligodendrocytes were removed by shaking at 180rpm for 18h at 37°C and then the astrocytes were subcultured onto multi-well culture plate. Cells were differentiated by the addition of 0.5mM dB cAMP.

Measurement of intracellular water space and [^{3}H] glutamate uptake and transporters

Intracellular water space and intracellular glutamate uptake of cultured astrocytes were determined by equilibrium accumulation of [^{3}H] OMG and [^{3}H] glutamate[3]. The expression of GFAP and transporter was measured by immunohistochemistry.

Results and Discussion

Fig.1 shows that cultured astrocytic swelling (measured by [^{3}H] OMG space) of cerebral cortex and hippocampus by glutamate responded to an increase in its concentration. Glutamate produced astrocytic swelling in both cerebral cortex and hippocampus, in the same manner, showing maximal effect in 0.5mM concentration (cortex; 6.9→ 16.1ul/mg protein, hippocampus; 5.9→14.1 ul/mg protein). However, in cerebellum, glutamate did not produce astrocytic swelling.

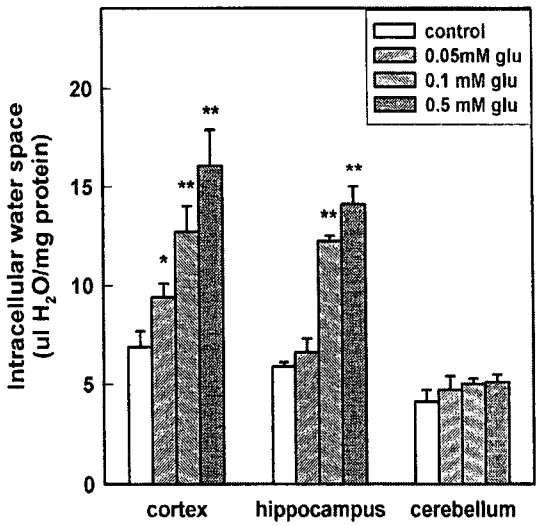

Fig. 1. Differential response of glutamate-induced astrocytic swelling in dibutyryl-cAMP treated cultures from different regions of the rat brain. Astrocytes cultured 3-4 weeks in vitro were treated with 0.5mM dibytyryl-cAMP for the differentiation of precesses. Cells were washed and preincubated in HEPES-buffered medium for 30min, and then treated with 0.5mM gutamate for 1hr at 37°C. Intracellular water space was measured by [^{3}H] OMG uptake for 20min at the end of the incubation. *$p<0.05$, **$p<0.01$ compared to control

The regional differences in glutamate uptake were also examined in astocytes from the different brain areas of rats. Fig. 2 and 3 show that the ability of astrocytes to take up glutamate varied between cortical and hippocampal astorcytes, where they were high, and astrocytes derived from the cerebellum, where glutamate uptake was relatively low. This difference is correlated with the difference of glutamate-induced swelling. The Vmax for glutamate uptake into cerebellum astrocytes was signigicantly lower (6.7 nmol/mg protein/min) than that for cerebral cortex and hippocampus astrocytes (13.0 and 12.0 nmol/mg protein/min, respectively). It has been suggested that Na+-dependent glutamate uptake is a possible mechanism of glutamate-induced swelling.

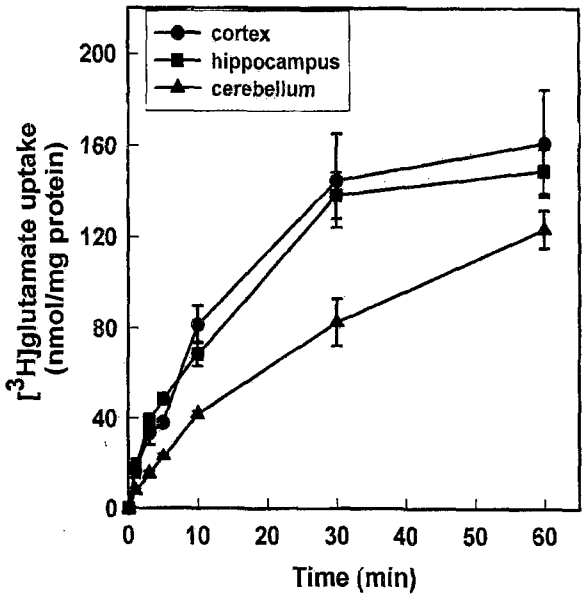

Fig. 2. Differential time response of glutamate uptake to cultured astrocytes from different regions of the rat brain. Astrocytes cultured 3-4 weeks in vitro were treated with 0.5mM dibutyryl-cAMP for differentiation of processes. Cells were washed and preincubated in HEPES-buffered medium for 30min, and then treated with 0.05mM [^{3}H] glutamate for various time at 37°C. A washing with ice-cold media terminated incubations.

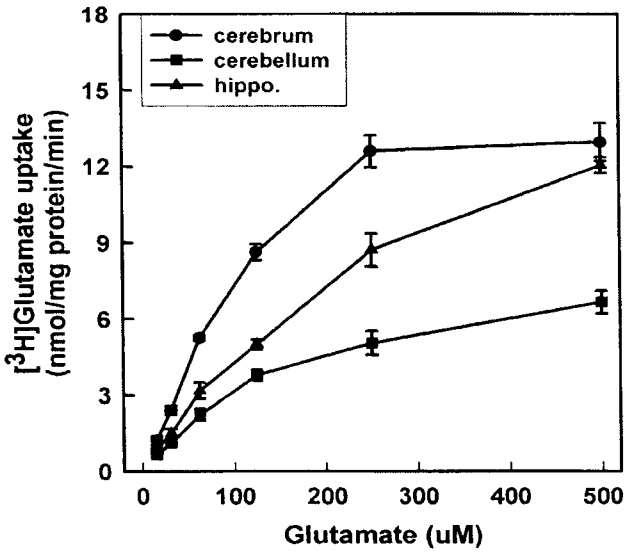

Fig. 3. Differential velocity of glutamate uptake into to cultured astrocytes form different regions of the rat brain. Cells were washed and preincubated in HEPES-buffered medium for 20min, and then the media were exchanged with corresponding media containing various concentrations of [^{3}H] glutamate and the cells incubated for further 5min. incubations were terminated by a washing with ice-cold media. The following Vmax were calculated: Cerebrum, 13.0; Hippocampus, 12.0; Cerebellum, 6.7nmol/mg protein/min

In the three regions, more than 95% of the cultured cells showed glial fibrillary acidic protein (GFAP) immunoreactivity. To further clarify the regional differences of astrocytic responses, we performed immunostaining of the cells against S100, apuaorin, and glutamate transporters (GLT and GLAST). Immunoreactivity of GLT, one of markers of glutamate transporters, which is expressed at low levels in cultured astrocytes, did not show any differences in three regions. However, immunoreactivities of GLAST, the other astroglial glutamate transporter, and aquaporin, water transporter, were significantly lower in cerebellum than in cerebral cortex and hippocampus. This evidence may explain the regional differences of glutamate-induced astrocytic swelling and glutamate uptake.

References

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