Uptake of aluminum amino acid complexes by cultured astrocytes

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Abstract

The form by which Al enters brain cells as well as the intracellular consequences of Al in relation to neurodegenerative diseases remains unresolved. In this report, Al was differentially taken up from Al amino acid complexes by primary culture of cortical astrocytes. Aluminum uptake from different amino acid complexes in the presence and absence of the respective amino acid transporter blockers were compared. The results indicate that none of the amino acid transporter blockers, as well as ouabain, employed in the present study apparently inhibited the uptake of Al. There is a possibility that passive diffusion, influenced by concentration gradient and exposure time, is a major mechanism involved in the Al transport in the forms employed here. The apoptotic effect of Al amino acid complex on astrocytes was also confirmed in the present study with evidence of nuclear shrinkage and chromatin condensation that occurred in more than 20% of the cells, as early as 3 days and also at concentrations as low as 0.0125 mM.

Key words: aluminum uptake, apoptosis, astrocyte, Hoechist33258 dye, glutamate and glycine transporters

Introduction

Aluminum is a ubiquitous element used extensively in contemporary life, nevertheless, evolution has not conferred essentiality or utility on it as far as is known in biological system. Although newer evidences continue to emerge in support of aluminum's participation in neurodegenerative diseases [1], the subject remains controversial. Unless the controversial role of Al as an environmental factor in the pathogenesis of neurodegenerative diseases is well resolved therefore, it will continue to be a subject of man's curiosity. Thus, it is important to clarify the metabolism of Al in brain. The forms of Al being taken up by brain cells have not been specified so far. Astrocytes have been recognized as the primary or potential target for Al toxic action [2], therefore, Al amino acid complex as possible form of Al internalization by astrocytes is the subject of the present report. The ability of cultured astrocytes to differentially internalize Al in complex with some amino acids is reported here. We also report here the possible mechanism of uptake of Al amino acid complex and the apoptotic effect of internalized Al.

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Methods

Primary culture of cortical astrocytes was prepared from 4 - 5 day old mice. The cells were grown in DMEM/F12 (or DMEM during experimentation) containing 15% fetal calf serum and 0.05 mg/ml gentamicin, incubated at 37 °C in humidified atmosphere of 5% CO_2 and were used for experiments after second passages by trypsinization. In all experiments, the cells were stressed with 0.1 mM Al amino acid complex for times ranging between 0.5 - 8h, except otherwise stated. In order to test the effects of transporter blockers, the cells were exposed to 0.1 mM each of dihydrokainic acid or sarcosine, a selective blocker of EAAT2 or GlyT1 respectively, as well as trans-pyrollidine-2,4-dicarboxylic acid or doxepin, a non-selective blocker of glutamate or glycine transporter respectively for 30 min (induction time). EAAT2 (also known as GLT-1) and GlyT1 are the dominant CNS astrocytic glutamate and glycine transporters respectively. The cells were then stressed with the respective Al amino acid complex (0.1 or 1 mM). In another experiment, the cells were exposed to 0.1 mM ouabain. Uptake of the respective Al amino acid complex in the presence and absence of transporter blockers were compared. Al content was measured by electrothermal atomic absorption spectrophotometer with Zeeman background correction, following wet digestion with ultra pure HNO_3 (Kanto Kagaku) and results expressed in ngAl/μg prot. For apoptotic study, the cells were exposed to graded doses (0.0125 – 0.1 mM) of Al(Gly) complex for 6h after which

the medium was exchanged and the cells cultured for the next 1 – 10 days in normal medium. The cover slips on which the cells were cultured were carefully removed, washed with PBS, fixed with methanol (-20 °C), stained with Hoechist33258 dye, and then observed under fluorescence microscope.

Results and Discussion

Aluminum from different complexes of amino acid was differentially internalized by cultured astrocytes (Fig. 1). Thus the amino acids do not only solubilize Al but also influence its uptake. It is expected that the amino acid transporter blockers may influence the uptake of Al in complex with the respective amino acids, however none of the transporter blockers employed in the present study apparently inhibited the uptake of Al. Unexpectedly, some of the blockers rather enhanced the uptake (Fig. 2 and 3).

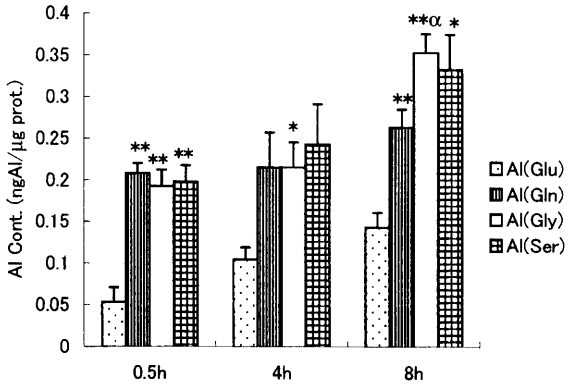

Figure 1. Cellular accumulation of Al following 0.5 - 8h exposure of primary culture of cortical astrocytes to 0.1 mM of Al(Glu), Al(Gln), Al(Gly) and Al(Ser). Plotted values represent the mean ± SE (n=4). ** and * indicate statistical difference from Al(Glu) at $p<0.01$ and 0.05 respectively, while α indicates statistical difference from Al(Gln) at $p<0.05$ using Welch's t test.

There is a possibility that other mechanisms influenced by concentration gradient are involved in the Al transport in the forms employed here, more so, when more Al was taken up at higher concentration (Fig. 2) and longer exposure time (Fig. 1 and 3). It has been recently suggested that the fact that a substance solubilizes Al does not necessarily imply that its transfer into cytoplasm follows the receptor-mediated pathway of the solubilizing agent [3]. Ouabain has also failed to block the uptake of Al in complex with glycine in the present study (Fig. 3). Thus, uptake of Al in the present forms can be said to be mainly by passive transport and this agrees with previous findings [4].

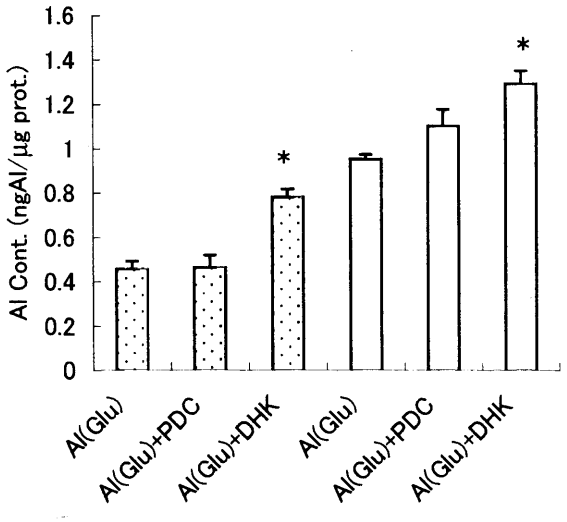

Figure 2. Cellular accumulation of Al following 6h exposure of primary culture of cortical astrocytes to 0.1 mM (dotted bar) or 1 mM (plain bar) of Al(Glu) in the presence and absence of PDC, trans-pyrollidine-2,4-dicarboxylic acid; and DHK, dihydrokainic acid. Plotted values represent the mean ± SE (n=4). * indicates statistical difference from control condition (i.e., in the absence of blockers) at $p<0.05$ using Welch's t test.

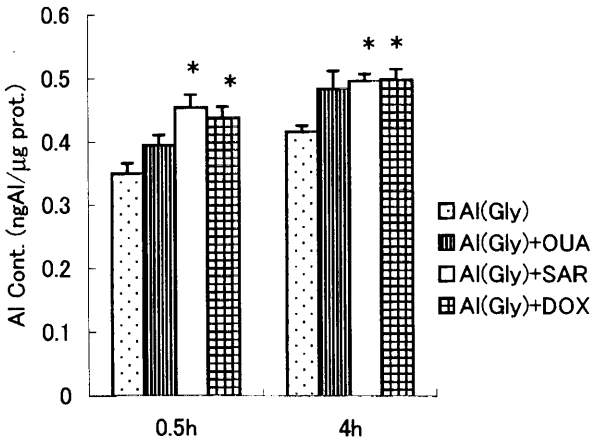

Figure 3. Cellular accumulation of Al following 0.5 or 4h exposure of primary culture of cortical astrocytes to 0.1 mM Al(Glu) in the presence and absence of OUA, ouabain; SAR, sarcosine; and DOX, doxepine. Plotted values represent the mean ± SE (n=4). * indicates statistical difference from control condition (i.e., in the absence of blockers) at $p<0.05$ using Welch's t test.

Apoptosis is a major form of cell death, characterized by a series of distinct morphological and

biochemical alterations. It is an important process in a wide variety of different biological systems, including normal cell turnover, the immune system, embryonic development, metamorphosis and hormone-dependent atrophy as well as in chemical-induced cell death. There are emerging evidences that Al is capable of inducing apoptosis in astrocytes [5]. The apoptotic effect of Al amino acid complex on cultured astrocytes is further confirmed in the present study with evidence of nuclear shrinkage and chromatin condensation that occurred in more than 20% of the cells. The significance of the present finding is that nuclear shrinkage and chromatin condensation occurred as early as three days following 6h exposure to test compounds and also at concentrations as low as 0.0125 mM. This is much lower concentration and earlier effect than those in previous reports [5]. Although the route of Al transport was yet to be clarified, Al amino acid complex is still candidate species relevant to its internalization.

References

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