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学位論文題目	Accumulation of aluminum by primary cultured astrocytes from aluminum amino acid complex and its apoptotic effect （アルミニウムアミノ酸錯体を用いた初代培養アストロサイトによるアルミニウムの蓄積とアポトーシス効果）
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学位論文の内容の要旨

Recent findings have implicated astrocytes as the principal target for aluminum toxic action. Thus primary culture of astrocytes would provide a good model for evaluating neurotoxic effect. Unfortunately, the majority of works on the toxic effects of aluminum has involved an examination of the direct effects of aluminum on neuronal cells while works on the toxic effects of aluminum on astrocytes are lagging behind. However, the astroglial environment of neurons provides metabolic and trophic support and contributes to local modulation of synaptic efficacy at excitatory inputs and represents an important regulator of glutamatergic communication between dependent synapses by setting the parameters of diffusion in the extra cellular space. Defects in these functions may be precedent to neurodegeneration. Moreover, the form by which aluminum enters brain cells as well as the intracellular consequences of aluminum remains unresolved. Furthermore, aluminum salts or doses that are unlikely in human system have been employed in toxicity studies so far. In order to address these issues, we have investigated the uptake and apoptotic effect of aluminum amino acid complex on primary cultured astrocytes because these are fundamental in understanding the mechanism of aluminum neurotoxicity.

方　法

Primary astrocyte cultures were prepared from cerebral cortices of newborn ICR mice (postnatal days 5-7) by the method previously described, with some modifications, and incubated in D-MEM/F12 (growth phase) or D-MEM (experimental phase) and standard incubation conditions. Following the second passage after about 80 % confluent monolayer astrocytes, the cells were stressed with aluminum amino acid complexes freshly prepared at various concentrations (usually 0.0125 – 0.1 mM) in culture medium for various time periods (usually 0.5 – 24 h) depending on the study. Aluminum uptake of the harvested intact and lysed cells (control for internalized Al) was quantified by atomic absorption spectrophotometry. Mechanism of uptake was studied by employing respective amino acid transporter blockers and ouabain. Impact of metabolic perturbation on uptake was also studied by employing methionine sulfoximine (MSO). Quick cell proliferation assay kit was used for the quantification of cell proliferation and viability according to the manufacturer's guide. Apoptosis was morphologically analyzed using Hoechist33258 dye and fluorescence microscopy. The nuclear shrinkage in astrocytes was determined as changes in the nuclear perimeter (arbitrary unit) by the NIH Image for Windows.

結　果

Aluminum solubilized by various amino acids was differentially internalized by astrocytes (glycine > serine >> glutamine >> glutamate), but aluminum was not internalized from citrate complex following 24 h exposure. Inhibition of glutamine synthetase, by methionine sulfoximine, enhanced the uptake of aluminum from various amino acid complexes within 8 h except from glutamine complex. Blockade of selective - GLT-1 (EAAT2) and GlyT1, as well as non-specific transporters did not inhibit, or had no effect on uptake of aluminum in complex with the corresponding amino acids. Ouabain also failed to inhibit uptake of aluminum complexed with glycine. Pulse exposure to aluminum glycinate in the absence or presence of methionine sulfoximine caused apoptosis in over 25% of primary cultured astrocytes and apoptotic features such as chromatin condensation and fragmentation became evident as early as 3 d culture in normal medium. Lower doses (as low as 0.0125 mM) also caused apoptosis.

考　察

The present investigation revealed that aluminum solubilized by various amino acids was differentially internalized by astrocytes as ascertained by comparing the uptake by living cells with that of lysed astrocytes. Surprisingly, there was no apparent uptake of aluminum from citrate complex at 24 h exposure time compared to control. The differential internalization of aluminum observed in the present study demonstrates diverse nature of the complexes formed. Citrate may not be a physiologically relevant form of aluminum for cellular uptake, but an aluminum chelator both in extra- and intra-cellular fluids in order to detoxify it. The perturbation of astrocytes in the present work with the attendant increase in aluminum uptake seems to provide an interesting model for the clarification of the cause of excessive accumulation of

aluminum in Alzheimer's disease (AD) brain. Impairment of the normal glutamate-glutamine-cycle is known to be characteristic of AD. The failure of the blockers employed to inhibit aluminum uptake eliminates the possibility of amino acid transporters or Na^+/K^+-ATPase as being responsible for its uptake but seems to suggest passive diffusion. However, passive diffusion alone may not sufficiently explain the differential uptake in the absence and presence of MSO, hence, another pathway of aluminum internalization may be implicated in addition to passive diffusion. The nuclear shrinkage and chromatin condensation that occurred as early as 3 d following 6h pulse exposure to 0.1 mM aluminum glycinate and also at concentrations as low as 0.0125 mM (7 d) is an earlier effect and much lower concentration than previously reported for primary astrocyte. MSO, alone or in combination with aluminum glycinate, also caused apoptosis in astrocyte. Thus aluminum can compromise astrocyte via apoptosis and this can spell doom for neurons.

結　論

Aluminum solubilized by amino acids, particularly glycine, could serve as better candidate for neurotoxicity studies. Citrate may be a chelator of aluminum rather than a candidate for its cellular uptake. Amino acid transporters may not participate in the uptake of aluminum solubilized by their substrates. Another pathway of aluminum internalization may be implicated in addition to passive diffusion but may not require energy in form of Na+/K+-ATPase. Impaired astrocyes metabolism can aggravate their accumulation of aluminum and aluminum can compromise astrocytes via apoptosis. Thus loss of astrocytic regulatory and supportive roles in CNS may be responsible for neurodegeneration observed in AD.

論文審査の結果の要旨

本研究は初代培養アストロサイトを用いて、アルミニウムの蓄積と毒性を検討したものである。アルミニウムのアミノ酸錯体はアストロサイトによく取り込まれることを原子吸光法を用いて示した。アミノ酸代謝を特異的に阻害するとアルミニウムの取り込みは有意に増加した。しかし、アミノ酸のトランスポーターはアルミニウムの取り込みには関与しなかった。また、ATP アーゼも関与していなかった。受動的拡散によって細胞内に入っていると考えられる。さらに、微量のアルミニウムアミノ酸錯体がアストロサイトにアポトーシスを起こすことを初めて示した。本論文の内容は、アルミニウムの中枢神経毒性の活性種はアルミニウムのアミノ酸錯体の可能性があることを初めて示したものであること、また、アルミニウムがアストロサイトに対する毒性をとおして神経変性疾患の環境要因となっている可能性があることを示したものであり、明らかに学術水準を高めたものと認める。