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Toxic Effect of Glutamate on Granular Cells of the Cerebellum Reduces Cell ATP Content. The Role of Calcium Ions

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The content of ATP in cultured cerebellar granular cells during and after glutamate intoxication is determined using the luciferin-luciferase method. A 15-min exposure to glutamate (100 μM in a Mg^{2+}-free medium) reduces the content of ATP to 63.8±3.01% of the initial value, followed by further drop of ATP during the postglutamate period. In a Ca^{2+}-free medium, the content of ATP during incubation with glutamate and in the postglutamate period drops to 89.2±4.8 and 74.7±3.6% of the initial level, respectively, while subsequent washout with a medium containing 1.8 mM Ca^{2+} results in a further decrease in the ATP content.

Key Words: *ATP; cerebellar granular cells; calcium; glutamate; toxicity*

It is known that hyperstimulation of glutamate receptors by a long-term (15-30 min) exposure of nerve cells to glutamate (GLU) results in a steady elevation of cell Ca^{2+} concentration ($[Ca^{2+}]_i$), leading to cell death [5,10,12]. However, despite extensive studies the mechanism of this phenomenon remains unclear. Until recently, the steady elevation of $[Ca^{2+}]_i$ in nerve cells after GLU exposure has been supposed to result from irreversible increase in Ca^{2+} permeability of the neuronal membrane [5,10]. Our studies demonstrated that Ca^{2+} overload of cells in the postglutamate period arises from inhibition of the processes responsible for the elimination of excessive Ca^{2+} from the cytoplasm, in particular, Na^+- and Ca^{2+}-exchange. The same conclusion has been recently made by L. Kiedrowski *et al.* [7].

Since Na^+- and Ca^{2+}-exchange and Ca^{2+}-pump largely depend on cell ATP concentration, the present study explored changes in cell ATP content during and after exposure of cerebellar granular cells to GLU.

MATERIALS AND METHODS

The study was performed on cerebellar granular cells from 7-8-day-old Wistar rats obtained as described previously [3]. The cell suspension was transferred to poly-L-lysine-coated coverslips and placed into 35-mm Petri plastic dishes. The cultures were placed

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into a CO_2-incubator (Juan) for 7-8 days at 5% CO_2, 95% air, and 98% humidity. On day 2, cytosine arabinoside (10 mM) was added to the culture medium to suppress the glial cell growth, and 25 mM KCl was added to promote the development of neurons [3].

Experiments were carried out on 7-8-day-old cultures. The cultures were washed with 20 mM HEPES in a control saline containing (in mM) 143 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 1 NaH_2PO_4, and 5 glucose, pH 7.4. All experiments were carried out at room temperature. GLU was dissolved in a Mg^{2+}-free medium. Ca^{2+}-free solutions were prepared by substituting Ca^{2+} in the control saline with 100 μM EGTA.

ATP was extracted from cerebellar granular cells with 2% trichloroacetic acid (TCA) containing 2 mM EGTA. The chosen TCA concentration yields the maximum extraction of ATP. The content of ATP in neurons was measured by luciferin-luciferase method [9] in a BKhL-06 bioluminometer and standardized to 1 mg protein measured by the method of Lowry.

The data were processed by ANOVA using the Student test.

Reagents were purchased from Sigma and Gibco-Biocult, luciferin-luciferase reagent was provided by Bioprocess Association, Russian Academy of Sciences.

RESULTS

In the present study, the neurotoxic effect of GLU on cultured nerve cells was reproduced in accordance with the protocol employed for the determination of $[Ca^{2+}]_i$, pH_i, and neuron survival [2,3,10]. In brief, cell culture was treated with 100 μM GLU in magnesium-free buffer at room temperature, and the medium was then replaced with the control saline. The cell content of ATP was measured before (basal), during, and after (postglutamate period) GLU exposure.

The basal ATP level was 4.2±0.3 μmol/mg protein (n=34). A 15-min exposure to GLU reduced the cell content of ATP to 63.8±3.01% of the initial value (n=25, p<0.001). A 30-min washout with the control saline led to a further drop of ATP to 43.1±3.5% of the initial level (n=12, p<0.001, Fig. 1, *a*).

Washout with the control calcium-free solution (Ca^{2+} replaced with 100 μM EGTA) induced a less pronounced decrease in the ATP concentration during the postglutamate period: to 55.6±3.6% of the initial value *vs.* 43.1±3.5% in Ca^{2+}-containing postglutamate saline (p<0.05, n=16, Fig. 1, *a*).

The effects of GLU was much weaker when Ca^{2+} was absent both from control and GLU-containing solutions. The basal content of ATP was only slightly decreased in Ca^{2+}-free medium in comparison with that in the control Ca^{2+}-containing solution

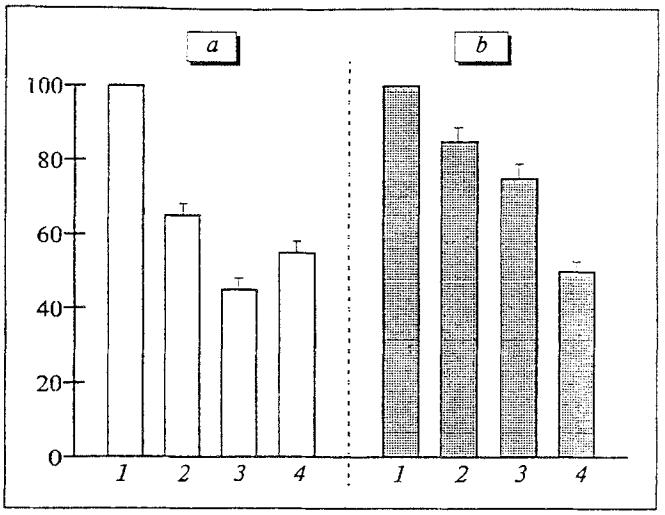

Fig. 1. Effect of glutamate (100 μM, without magnesium) on the ATP concentration in cultured cerebellar granular cells. The content of ATP before glutamate exposure is taken as 100%. Ordinate: ATP content, %. *a*) experiments carried out in the medium containing 1.8 mM Ca^{2+}: *1*) control; *2*) 15-min glutamate exposure; *3*) postglutamate period; *4*) postglutamate period in calcium-free medium (100 μM EGTA). *b*) experiments carried out in calcium-free medium (100 μM EGTA): *1*) control; *2*) 15-min glutamate exposure; *3*) postglutamate period; *4*) postglutamate period in medium containing 1.8 mM Ca^{2+}.

(3.58± 0.4 *vs.* 4.2±0.3 μmol/mg protein, n=29, Fig. 1, *b*). A 15-min GLU exposure in calcium-free medium and subsequent washout with calcium-free solution reduced the content of ATP to 89.2±4.8% (n=25) and 74.7±3.6% (n=29), respectively, from the initial level measured in calcium-free medium (Fig. 1, *b*). A more pronounced drop of ATP in the postglutamate period (to 51.1±1.9%) was observed when a 15-min GLU exposure was followed by washout with Ca^{2+}-containing control saline (n=15).

Thus, GLU induces a pronounced decrease in the ATP content of cultured nerve cells, which persists during the postglutamate period. What is the mechanism of these GLU-induced shifts in the neuronal content of ATP? It is likely that the observed changes in neuronal ATP concentration are related to disturbances in Ca^{2+} homeostasis during and after GLU exposure. This was confirmed by the fact that the effects of GLU depend on Ca^{2+}: the GLU-induced drop in ATP was less pronounced in calcium-free medium, while during the postglutamate period the replacement of calcium-free solution by the control one resulted in a more dramatic drop of ATP concentration (Fig. 1, *b*). These data suggest that a decrease in cell ATP concentration during and after GLU exposure results from excessive rise of the cytoplasmic Ca^{2+} concentration in neuron [5,8,10], leading to Ca^{2+} overload of the mitochondria [13] and inhibition of ATP synthesis. Since Na^+ and Ca^{2+}

exchange and Ca^{2+} and Na^{+} pumps are ATP-dependent, it can be hypothesized that the drop of ATP during and after neurotoxic GLU exposure results in their inhibition and, consequently, in accumulation of Na^{+} and Ca^{2+} in the cell [8,11]. It is not inconceivable that the decrease in the intracellular pH in neurons during and after GLU exposure [2,6] also results from the decrease in the ATP concentration [13].

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