Toxic Effect of Glutamate Causes Mitochondria Damage in Granule Cells of Dissociated Cultures of Rat Cerebellum

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It is shown that treating rat cerebellum with glutamate in a neurocytotoxic concentration causes a drop of the mitochondrial membrane potential in granule cells and leads to ultrastructural alterations of mitochondria in these neurons.

Key words: *glutamate, granule cells of cerebellum, mitochondria*

Intensive research into the mechanisms of neurocytotoxic action of excitatory amino acids (EAA), which interact with the glutamate receptors, is now underway. It has been shown that hyperactivation of glutamate receptors leads to calcium overloading of neurons [5,10], a drop of the intracellular pH [3], activation of Ca^{2+}-dependent proteolytic and lipolytic enzymes [6,12], and, finally, to irreversible neuron damage and death. It is known that many cell-damaging compounds adversely affect primarily the mitochondria [4,7]. However, the influence of toxic doses of glutamate (GLU) on the functional status of mitochondria in the cells remains unclear [8]. The present study examines this problem.

MATERIALS AND METHODS

A suspension of granule cells was isolated from the cerebellums of 7-8-week-old Wistar rats using the modified enzyme dissociation method. Cells were cultured 7-8 days on poly-L-lysine-coated slides in plastic dishes using the method developed in our laboratory [2]. The culture medium consisted of minimal Eagle medium supplemented with 10% fetal calf serum, 5% glucose, 0.1 U/ml insulin, 2 mM glutamine, and 10 mM HEPES. On the second day of *in vitro* incubation the potassium concentration was raised from 5.6 to 25 mM. Glutamate treatment (100 μM) was conducted for 15 min at 20°C in a balanced salt solution (BSS) containing 154 mM NaCl, 5.6 mM KCl, 0.35 mM Na_2HPO_4, 3.6 mM $NaHCO_3$, 2.3 mM $CaCl_2$, 5.6 mM glucose, and 10 mM HEPES, pH 7.5-7.6. Control cultures were incubated for 15 min in the same media without glutamine. For assessment of mitochondria functional status the cells were stained with rhodamine 123 dissolved in BSS (10 μg/ml, 10 min). This method makes it possible to determine the presence of a membrane potential on mitochondria [9]. Fluorescence of stained cells was observed in an Opton-3 photomicroscope at exciting light wavelength 450-490 nm. Living cells were photographed using RF-3 photofilm.

For electron microscopy, 10 min after GLU treatment the cell cultures were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) and then additionally fixed in 1% OsO_4 solution. Samples were contrasted with uranyl acetate, dehydrated in alcohol solutions, and embedded in Epon-812 using the standard technique. Ultrathin sections were obtained on an LKB-3 ultramicrotome, mounted on lenses coated with polyvinyl film, and stained with lead acetate after Reynolds. Sections were examined and photographed in an HU-11 electron microscope with accelerating voltage 75 kV.

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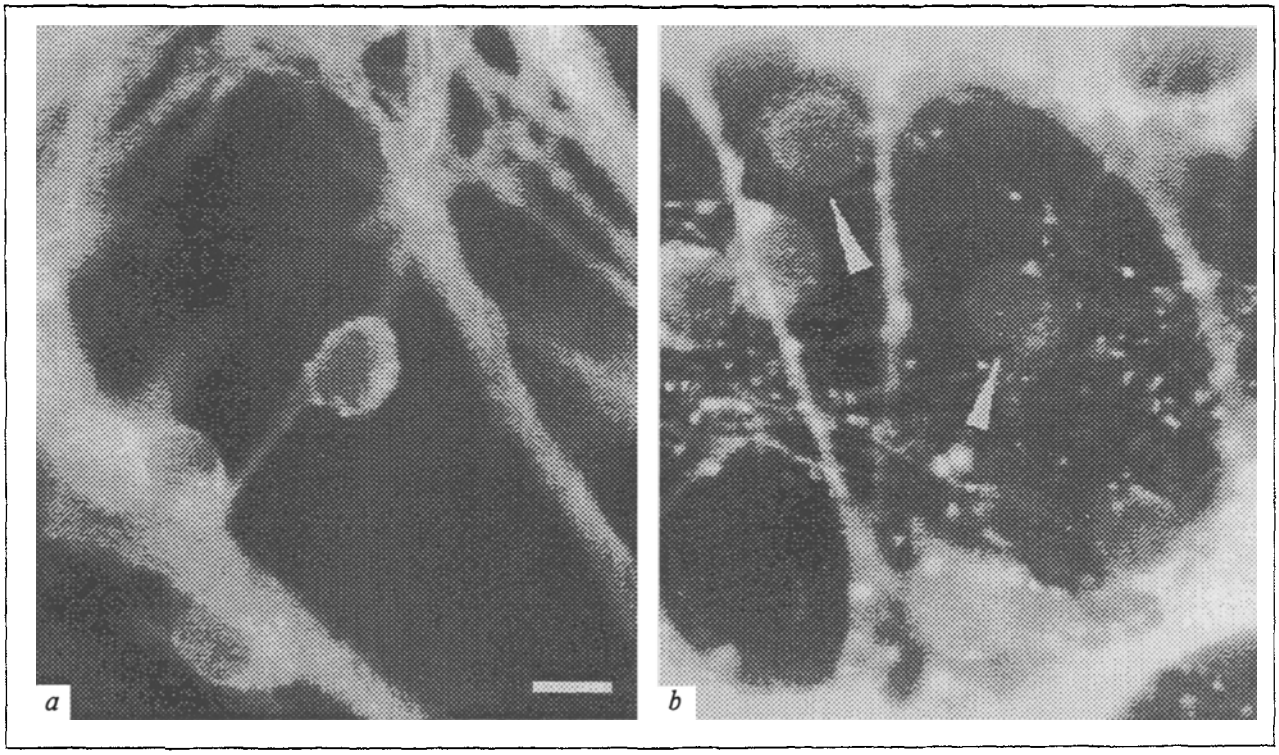

Fig. 1. Granule cells from 7-day-old rat cerebellum in monolayer dissociated culture. Vital staining by rhodamine 123. Scale 5 μ. *a*) granule cells from control culture with intensively fluorescing rhodamine 123 accumulated in mitochondria. *b*) glutamate-treated (100 μM, 15 min) cell culture. No fluorescing mitochondria in granule cells (marked with arrows).

RESULTS

In the experiments mitochondria of granule cells in control cultures, incubated 15 min in BSS, actively accumulated rhodamine 123, as is seen by the intense fluorescence under exciting blue light (λ=450-490 nm) (Fig. 1, *a*). Since cell size is approximately 7-10 μ (and the nucleus takes up most of the room, individual mitochondria are not clearly distinguished upon light microscope observation. Mitochondria fluorescence of almost all granule cells in GLU-treated cultures (15 min, 100 μM in BSS) was quenched and only weak, diffuse staining of the cytoplasm remained (Fig. 1, *b*). It is known that the capacity of mitochondria for rhodamine 123 accumulation reflects their functional status and determines the presence of a membrane potential [9]. Thus, toxic GLU action causes a membrane potential drop (deenergization) in mitochondria of cerebellum granule cells.

For assessment of mitochondrial morphophysiological transformations and for verification of the data obtained in experiments with rhodamine 123, electron microscopy was carried out. Mitochondria in granule cells from control cultures have the normal ultrastructure: there are clearly observed, uniformly thick cristae and the electron density of the matrix was greater than that of the cell cytoplasm (Fig. 2, *a*).

Mitochondria in experimental GLU-treated cultures were larger than in the control group, the electron density of their matrix was lower, most cristae were destroyed and the few intact ones were deformed (Fig. 2, *b*). Such ultrastructural alterations of mitochondria indicate a functional disturbance of these organelles. Earlier the same mitochondria ultrastructural alterations in rat brain neurons were shown after 5-hour hypoxia or after 2.5-hour oxygen-glucose deprivation [13]. It may be that mitochondria destruction under conditions of hypoxia is the result of GLU cytotoxic action on neurons. This assumption is supported by the fact that ultrastructural damage of mitochondria under hypoxia or oxygen-glucose deprivation conditions is prevented by glutamate antagonists [13].

Thus, the data obtained show that GLU action on glutamateceptive neurons causes mitochondria deenergization and damage. Similar results, indicating that EAA action leads to a drop of the energy level of brain cells, were obtained by measurement of the ATP level in homogenate of cerebellum cells [1]. It is possible that mitochondria deenergization is the main cause of cell death not only for exogenous GLU action but also under the

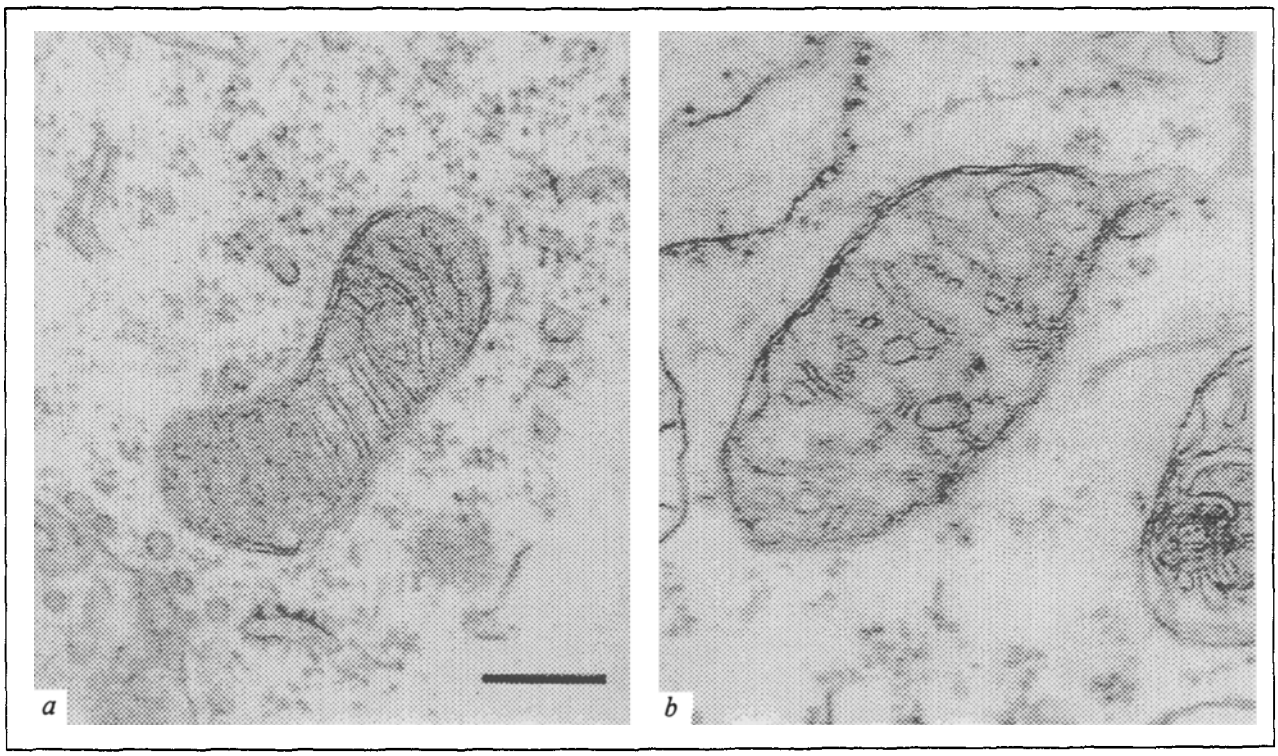

Fig. 2. Ultrastructure of granule cell mitochondria in culture of 7-days-old rat cerebellum. Scale 0.2 μ. *a*) granule cell mitochondrion with normal ultrastructure in control culture: uniformly thick cristae observed; mitochondria matrix is more electron-dense than cell cytoplasm. *b*) glutamate-treated granule cells (100 μM, 15 min). Mitochondria are strongly swollen, almost all cristae have disappeared, and remaining ones are severely deformed. Mitochondria matrix is less electron-dense than in normal granule cell mitochondria.

influence of endogenous EAA in ischemia and brain hypoxia.

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