Effect of Isosmotic Medium with Low Sodium Content on Mitochondria of Cultured Cerebellar Granular Cells

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Experiments with rhodamine-123 showed that incubation of cultured cerebellar granular cells in a medium with NaCl isosmotically replaced with sucrose reduced mitochondrial membrane potential. Elimination of Ca^{2+} or addition of $CoCl_2$, noncompetitive (MK-801) and competitive (APH) N-methyl-D-aspartate receptor antagonists abolished deenergizing effects of low-sodium solutions.

Key Words: *cerebellar granular cells; sodium; calcium; mitochondria*

Studies of ionic mechanisms of glutamate (Glu) toxicity showed that intracellular Na^+ and Ca^{2+} play an important role in neuronal damages [3,4]. Inhibition of Na^+ outward current potentiates toxic effects of Glu [1]. Extracellular Na^+ ions are involved in the Na^+/Ca^{2+} exchange, and their replacement with choline induced by Glu causes cell death [2] while replacement of extracellular Na^+ or with N-methyl-D-glucamine changes the direction of Na^+/Ca^{2+} exchange [8]. Moreover, transmembrane transport of Glu is Na^+-dependent [9], and the decrease in extracellular Na^+ concentration changes the direction of Glu transport and induces the release of endogenous Glu [10].

Thus, the role of Na^+ in neuronal degeneration remains unclear. Here we studied morphofunctional state of mitochondria in cultured granular cells from rat cerebellum during short-term isosmotic replacement of NaCl with sucrose.

MATERIALS AND METHODS

Dissociated cerebellar cells from 6-8-day-old Wistar rats were cultured as described elsewhere [2]. The medium contained 10% fetal bovine serum, 90% Eagle's minimum essential medium, 0.8% glucose, 0.1 U/ml insulin, 2 mM glutamine, and 10 mM HEPES. On day 2 of *in vivo* culturing, K^+ concentration in the medium was increased from 5.6 to 25 mM. The effects of extracellular Na^+ on mitochondria of granular cells were studied in 7-8-day cultures. NaCl was replaced with sucrose [4]. Cultures were incubated in a medium containing (in mM) 137 NaCl (or 274 sucrose), 5.6 KCl, 0.35 Na_2HPO_4, 12 $NaHCO_3$, 2.3 $CaCl_2$, and 11 glucose for 20 min.

$CoCl_2$ (1 mM) and noncompetitive selective antagonist of N-methyl-D-aspartate (NMDA) receptors (+)-5-methyl-10,11-dihydroxy-5H-dibenzo-(α,δ) cycloheptene-5,10-iminohydromaleate (MK-801, 10 μM) or competitive selective antagonist of NMDA receptors D,L-2-amino-7-phosphonoheptanoate (APH, 250 μM) were added to the incubation medium with $CaCl_2$ to block Ca^{2+} channels. The cells were intravitally stained with rhodamine-123 dissolved in the incubation medium (5 μg/ml, 10 min) to determine the functional state of mitochondria by visualizing their membrane potential [7]. Fluorescence of stained cells was examined under a Reichter microscope.

RESULTS

After a 20-min incubation of cultured cerebellar granular cells in a medium with normal Na^+ content, mito-

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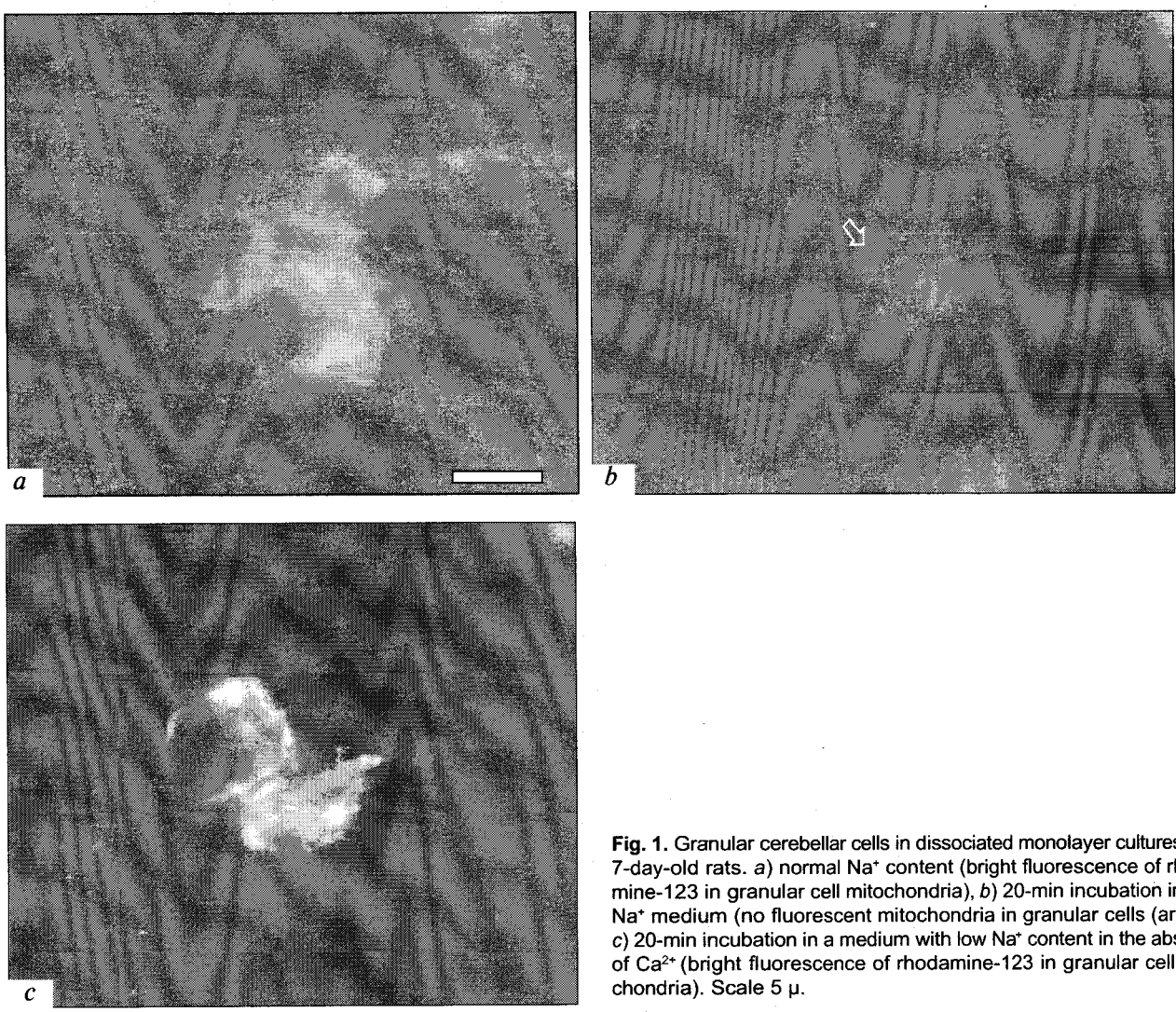

Fig. 1. Granular cerebellar cells in dissociated monolayer cultures from 7-day-old rats. *a*) normal Na^+ content (bright fluorescence of rhodamine-123 in granular cell mitochondria), *b*) 20-min incubation in low-Na^+ medium (no fluorescent mitochondria in granular cells (arrow)), *c*) 20-min incubation in a medium with low Na^+ content in the absence of Ca^{2+} (bright fluorescence of rhodamine-123 in granular cell mitochondria). Scale 5 μ.

chondria actively accumulated rhodamine-123 and fluoresced in blue light (450-490 nm, Fig. 1, *a*). Mitochondria of granular cells incubated in a low-sodium medium did not accumulate rhodamine-123, *i.e.*, were deenergized (Fig. 1, *b*).

Our previous experiments showed that excessive Ca^{2+} influx into granular cells induced by Glu rapidly decreases mitochondrial membrane potential in these neurons [5,6]. To estimate whether or not the decrease in mitochondrial membrane potential depends on Ca^{2+} influx into granular cells, Ca^{2+} ions were removed or $CoCl_2$ (Ca^{2+} channel blocker) was added. This procedure completely prevented deenergization of mitochondria (Figs. 1, *c* and 2, *a*).

At low extracellular Na^+ concentrations, Ca^{2+} ions enter the neuron through NMDA channels or reversed Na^+/Ca^{2+} exchanger. The inhibition of Na^+/Ca^{2+} exchanger by Glu potentiates its destructive effect [2]. The data suggest that in our experiments, Ca^{2+} entered the neuron through NMDA channels, but not via Na^+/Ca^{2+} exchanger. To verify this hypothesis NMDA channels were blocked with noncompetitive selective antagonist MK-801. Mitochondria of granular cells retained the membrane potential and accumulated rhodamine-123 (Fig. 2, *b*). Transmembrane transport of Glu dependens of Na^+ concetration [9]. Experiments on hippocampal slices showed that the decrease in extracellular Na^+ impairs inward transport of endogenous Glu and its accumulation in cells [10]. To determine whether or not opening of NMDA channels was induced by endogenous Glu, the cells were incubated in a low-sodium medium containing APH, a competitive selective antagonist of NMDA receptors. This blocker abolished deenergization of mitochondria in granular cells (Fig. 2, *c*).

Thus, incubation of cultured granular cells in a low-sodium medium induces the release of endogenous Glu due to its impaired reuptake [10]. Accumulated endogenous Glu activates glutamate receptors, opens NMDA channels, and Ca^{2+} ions enter the neuron and decrease mitochondrial membrane potential of granular cells. The inhibition of NMDA channels prevents Ca^{2+}-dependent de-

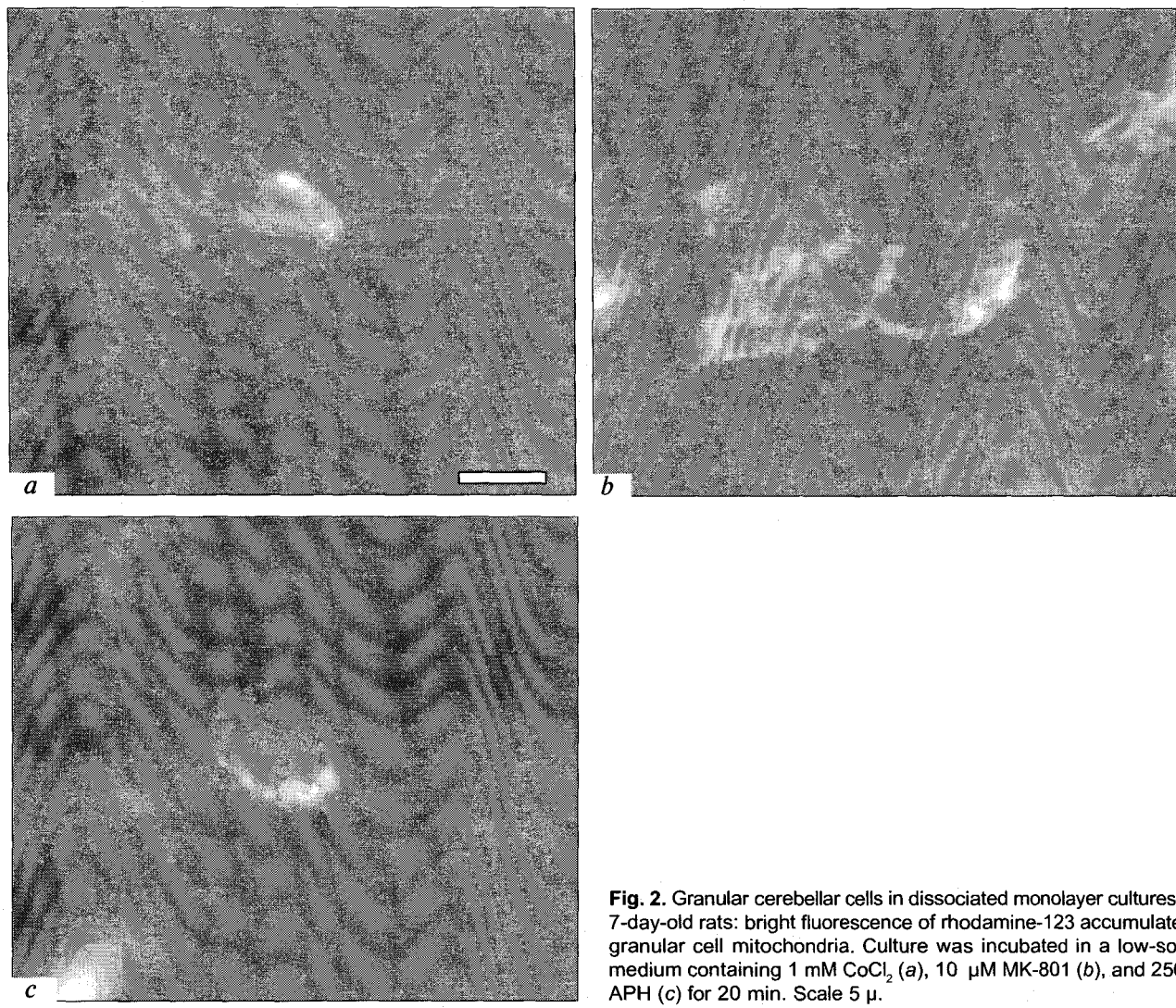

Fig. 2. Granular cerebellar cells in dissociated monolayer cultures from 7-day-old rats: bright fluorescence of rhodamine-123 accumulated by granular cell mitochondria. Culture was incubated in a low-sodium medium containing 1 mM $CoCl_2$ (*a*), 10 μM MK-801 (*b*), and 250 μM APH (*c*) for 20 min. Scale 5 μ.

energization of mitochondria in granular cells. Therefore, during the incubation of granular cells in a low-sodium medium, Ca^{2+} ions enter the neuron through NMDA channels, but not via reversed Na^+/Ca^{2+} exchanger.

Thus, Na^+ ions contribute to Glu toxicity [3,4] and control Glu transport and normal functioning of CNS neurons.

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