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Role of mitochondria in kainate-induced fast Ca^{2+} transients in cultured spinal motor neurons

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Abstract

Motor neuron death in amyotrophic lateral sclerosis (ALS) has been linked to selective vulnerability towards AMPA receptor-mediated excitotoxicity. We investigated intracellular mechanisms leading to impairment of motor neuron Ca^{2+} homeostasis with near physiological AMPA receptor activation. Using fast solution exchange on patch-clamped cultured neurons, kainate (KA) was applied for 2 s. This induced a transient increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) for seconds. Inhibition of the mitochondrial uniporter by RU-360 abolished the decay of the Ca^{2+} transient and caused immediate $[Ca^{2+}]_c$ overload. Repetitive short KA stimulation caused a slowing of the decay of the Ca^{2+} transient and a gradual increase in peak and baseline $[Ca^{2+}]_c$ in motor neurons, but not in other neurons, indicating saturation of the mitochondrial buffer. Furthermore, mitochondrial density was lower in motor neurons and, in a network of neurons with physiological synaptic AMPA receptor input, RU-360 acutely induced an increase in Ca^{2+} transients. We conclude that motor neurons have an insufficient mitochondrial capacity to buffer large Ca^{2+} elevations which is partly due to a reduced mitochondrial density per volume compared to non-motor neurons. This may exert deleterious effects in motor neuron disease where mitochondrial function is thought to be compromised.

Keywords: Amyotrophic lateral sclerosis; AMPA receptors; Excitotoxicity; Calcium-induced calcium release; Mitochondrial uniporter; Calcium imaging; Patch-clamp

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the death of motor neurons in the motor cortex, brain stem and spinal cord. This selective motor neuron degeneration results in death of the patient after a mean disease duration of 3 years. The only drug proven to slow ALS in patients is riluzole which mediates at least part of its neuroprotective effect via a presynaptic reduction of glutamatergic stimulation of motor neurons [1].

About 10% of ALS cases are familial and 20 % of these are caused by mutations in the superoxide dismutase 1 (SOD1) gene. Despite intensive research, the exact nature of the gain of function for which mutations in SOD1 are responsible is not yet clarified (for a review see Ref. [2]) but AMPA receptor-mediated excitotoxicity seems to be involved. First, mutant SOD1 can damage the glial glutamate transporter responsible for the removal of glutamate from the synaptic cleft [3]. Second, AMPA receptor antagonists prolonged

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survival in the mutant SOD1-mouse model [4–6]. Last but not least, physiological AMPA receptor activation increased both the formation of toxic aggregates and death of mutant SOD1 expressing motor neurons which was prevented by selective blockers of Ca^{2+}-permeable AMPA receptors and by increased expression of calbindin D-28k [7].

In 90% of ALS cases, the cause is unknown. An intrinsic selective vulnerability of motor neurons to AMPA receptor-mediated excitotoxicity was postulated, but the mechanisms involved are only partially understood. Motor neurons appear to have AMPA receptors with a higher relative Ca^{2+}-permeability than other neuronal populations. The Ca^{2+} permeability of the rapidly inactivating AMPA receptors [8] is determined by the absence or presence of the GluR2 subunit in the receptor complex; receptors containing GluR2 have a very low relative Ca^{2+} permeability compared to GluR2-lacking receptor channels [9]. The change of a neutral glutamine (Q) into a positively charged arginine (R) at the Q/R site of the GluR2 pre-mRNA via posttranscriptional editing is responsible for the low Ca^{2+} permeability of GluR2-containing AMPA receptors [10]. This change seems to act as a developmental switch to shift the function of AMPA receptor activation from mediator of cell development to transmitting excitatory input to neurons [11]. Low expression levels of the GluR2 subunit or a reduced editing efficiency of GluR2 can explain the high Ca^{2+} permeability and both mechanisms were found to contribute to motor neuron degeneration in ALS [12–17].

AMPA-mediated excitotoxicity was studied extensively by exposing neurons to non-desensitizing AMPA receptor agonists such as kainate (KA). Motor neurons were more sensitive to Ca^{2+}-dependent degeneration after exposure to KA than other neurons [18]. KA induced a selective rise in cytoplasmic Ca^{2+} concentration, reactive oxygen species and mitochondrial Ca^{2+} overload in motor neurons [19–23]. Therefore, a specific AMPA receptor induced perturbation of mitochondrial function [24,25] has been implied in AMPA receptor-mediated excitotoxic motor neuron degeneration. Prevention of excitotoxic cell death by overexpression of Ca^{2+}-binding proteins suggests that motor neuron specific intracellular Ca^{2+}-buffering mechanisms may cause selective vulnerability [7,26].

In previous studies, KA has been applied for long periods of time, with a minimum of 15 s which caused an excessive rise in intracellular Ca^{2+} concentration for several minutes [18,20]. In order to examine Ca^{2+} buffering processes which act in the range of seconds, we generated intracellular Ca^{2+} transients by eliciting a defined AMPA receptor inward current for 2 s. At the same time, we prevented membrane depolarization and Ca^{2+} influx through NMDA receptors. We found evidence for activation of mitochondrial Ca^{2+} uptake after the inward current through AMPA receptors which saturated on repetitive KA application and lead to a persistent increase in cytosolic Ca^{2+} in motor neurons. This was at least partially attributed to a lower density of mitochondria in motor neurons.

2. Methods

2.1. Cell culture

Rat spinal neurons from embryonic day 14 were enriched for motor neurons and cultured on a glial feeder layer as previously described [23]. Briefly, ventral spinal cords were dissected from 14-day-old Wistar rat embryos in Hanks' balanced salt solution (HBSS), digested for 15 min in 0.05% trypsin in HBSS at 37 °C and triturated after treatment with DNase. Motor neurons were purified by centrifugation on a 6.3% OptiPrep (Axis-shield Poc AS, Oslo, Norway) cushion and seeded on a glial feeder layer pre-established on poly-L-ornithine and laminin coated 18-mm round glass coverslips. The culture medium consisted of L15 supplemented with sodium bicarbonate (0.2%), glucose (3.6 mg/ml), progesterone (20 nM), insulin (5 μg/ml), putrescine (0.1 mM), conalbumin (0.1 mg/ml), sodium selenite (30 nM), penicillin (100 IU/ml), streptomycin (100 μg/ml), chick embryo extract (5%) and horse serum (2%). The cultures were kept in a 7% CO_2-humidified incubator at 37 °C and used for measurements between days 6 and 13 in vitro.

In a second co-culture system, spontaneous Ca^{2+} transients were observed. Motor neurons were enriched and cultured on a Schwann cell feeder layer as previously described [27]. In short, Sprague Dawley rat embryos with gestational age of 14–15 days were dissected in Ca^{2+}- and Mg^{2+}-free cold Hanks balanced salt-solution (HBSS, PAA, Coelbe, Germany). Ventral horn sections of the lumbar spinal cord were dissociated with trypsin (0.06% in PBS). Motor neuron-rich cell fractions were extracted by centrifugation in OptiPrep density gradient medium (1:4 in HBSS). After resuspension in culture medium, cells were seeded at 1.7×10^4 cells/cm^2 on a layer of neonatal Schwann cells which were previously prepared by immunopanning with Thy1 antibody-coupled magnetic beads (Dynabeads, Dynal, Hamburg, Germany). Neurobasal medium with 2% horse serum, 2% B27-Supplement (GIBCO Invitrogen, Eggenstein, Germany), 0.5 mM L-glutamine, 25 μM β-mercaptoethanol and 10 ng/ml rHu BDNF (PromoKine, Heidelberg, Germany) was changed twice per week.

2.2. Fast solution exchange

The recording chamber (3 ml) was continuously superfused (10 ml/min). To achieve fast application and removal of KA without causing perturbation of the fluorescence signal, a custom made solution applicator was attached to the objective (Achroplan 0.75W, Zeiss, Jena, Germany) of the upright microscope (Optiphot-2, Nikon, Surrey, UK). The applicator mount directed two canulas (0.2 mm inside diameter) separated by 30° to the optic center with an outlet-object distance of 1.5 mm and a 30° angle to the recording chamber's surface. The perfusion rate was adjusted using a custom water-column based air pressure system fitted on 50 ml reservoir syringes. The digital outputs of the patch-clamp system were converted