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Research Paper

Dissipation of transmembrane potassium gradient is the main cause of cerebral ischemia-induced depolarization in astrocytes and neurons

Yixing Du[a], Wei Wang[a,b], Anthony D. Lutton[c], Conrad M. Kiyoshi[a], Baofeng Ma[a], Anne T. Taylor[a], John W. Olesik[c], Dana M. McTigue[a], Candice C. Askwith[a], Min Zhou[a,*]

[a] Department of Neuroscience, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA
[b] Department of Physiology, School of Basic Medicine, Huazhong University of Science and Technology, Wuhan 430030, China
[c] Trace Element Research Laboratory, The Ohio State University, Columbus, OH 43210, USA

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ABSTRACT

Membrane potential (V_M) depolarization occurs immediately following cerebral ischemia and is devastating for the astrocyte homeostasis and neuronal signaling. Previously, an excessive release of extracellular K^+ and glutamate has been shown to underlie an ischemia-induced V_M depolarization. Ischemic insults should impair membrane ion channels and disrupt the physiological ion gradients. However, their respective contribution to ischemia-induced neuronal and glial depolarization and loss of neuronal excitability are unanswered questions. A short-term oxygen-glucose deprivation (OGD) was used for the purpose of examining the acute effect of ischemic conditions on ion channel activity and physiological K^+ gradient in neurons and glial cells. We show that a 30 min OGD treatment exerted no measurable damage to the function of membrane ion channels in neurons, astrocytes, and NG2 glia. As a result of the resilience of membrane ion channels, neuronal spikes last twice as long as our previously reported 15 min time window. In the electrophysiological analysis, a 30 min OGD-induced dissipation of transmembrane K^+ gradient contributed differently in brain cell depolarization: severe in astrocytes and neurons, and undetectable in NG2 glia. The discrete cellular responses to OGD corresponded to a total loss of 69% of the intracellular K^+ contents in hippocampal slices as measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). A major brain cell depolarization mechanism identified here is important for our understanding of cerebral ischemia pathology. Additionally, further understanding of the resilient response of NG2 glia to ischemia-induced intracellular K^+ loss and depolarization should facilitate the development of future stroke therapy.

1. Introduction

In brain ischemia, the neuronal and glial cell depolarization appears as one of the earliest pathological events and is devastating for neuronal signaling and astrocyte homeostasis. A hypoxia-induced depolarization was initially observed in neurons and glia from *in vivo* sharp electrode recording, and the extracellular K^+ increase was indicated as the underlying mechanism (Muller and Somjen, 2000; Leblond and Krnjevic, 1989). Later on, excessive glutamate release from various sources has been identified as an additional mechanism contributing to neuronal depolarization (Kimelberg et al., 1990; Rossi et al., 2000; Fleidervish et al., 2001).

In hippocampal slices, the first sign of neuronal damage, anoxic depolarization, occurs only 7 min after oxygen-glucose deprivation (OGD) initiation with the subsequent loss of spikes in 15 min (Zhang et al., 2009; Zhang et al., 2008; Allen et al., 2005). In contrast, astrocytes can withstand OGD for up to 1 h with a fully recoverable V_M[9]. Neurons and glial cells differ strikingly in their ion channel expression (Cahoy et al., 2008) and membrane ion channels are among the first line of defense in the face of ischemic injury. It is possible that astrocytic leak-type K^+ channels and neuronal voltage-gated Na^+, Ca^{2+} and K^+ channels may exhibit different susceptibilities to ischemic insults (Rossi et al., 2007; Martin et al., 1994). This hypothesis is plausible because the functional state of voltage-gated and leak-type K^+ channels can be either up- or down-regulated by ATP/GTP-dependent phosphorylation through various kinases in neurons and glia. Thus, these channels may respond to ischemia-induced energy failure differently (Scheuer, 2011; Maier, 2011; Shah and Aizenman, 2014; Vacher and Trimmer, 2011; Rojas et al., 2007). What remains unknown is whether leak-type K^+ channels are less susceptible to ischemic insults, therefore, offering astrocytes a channel-based protective mechanism in stroke brain.

* Corresponding author at: 4066C Graves Hall, 333 West 10th Avenue, Columbus, OH 43210, USA.
E-mail address: zhou.787@osu.edu (M. Zhou).

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The resting V_M of a cell is established upon a physiological transmembrane K^+ gradient, 140 mM inside and 3.5 mM outside the cell, which is maintained by the Na^+-K^+-ATPase (Na^+-pump). A failure in ATP production resulting from cerebral ischemia should disable Na^+-pump activity. This, in turn, dissipates the intracellular K^+ content in a process termed K^+ gradient dissipation that results in depolarization of brain cells (Hansen, 1985; Rothman and Olney, 1986). However, the extent to which this K^+ gradient dissipation contributes to OGD-induced depolarization is unknown.

To examine how ischemic conditions acutely affect the function of ion channels and physiological ion gradients in neurons, astrocytes, and NG2 glia, we purposely used a short-term OGD paradigm to avoid long-term ischemic conditions induced secondary injury and cell death *in situ* (Rossi et al., 2007).

We show that the ion channels in neurons and glial cells are more resilient to acute OGD insults than previously anticipated. Meanwhile, the contribution of K^+ gradient dissipation to ischemia-induced membrane potential (V_M) depolarization is cell type dependent, with astrocytes and neurons severely affected and almost absent in NG2 glia. NG2 glia are progenitor cells by nature and distributed throughout the brain. Understanding of why the NG2 glia selectively resist to ischemia-induced intracellular K^+ loss and depolarization may shed lights on the novel strategy for stroke treatment in the future.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University. All experimental procedures were conducted in compliance with The Ohio State University IACUC guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, 8th edition. Experiments have been reported in accordance with ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. C57BL/6 N mice were used unless otherwise noted. Mice from postnatal day (P) 21–35 of both sexes weighing 9 to 16 g were used for brain slices and freshly dissociated astrocyte preparation; P0-P1 mice were used for hippocampal neuron culture preparation. mT/mG;PDGFRα-CreER^{T2} reporter mice from P21–35 of both sexes were used for identification of NG2 glia (Hesp et al., 2015).

2.2. Preparation of acute hippocampal slices

Hippocampal slices were prepared as described previously (Du et al., 2015; Ma et al., 2016). Briefly, after anesthesia with 8% chloral hydrate in 0.9% NaCl, mouse brains were rapidly removed from skulls and placed into ice-cold oxygenated (95% O_2 and 5% CO_2) slice cutting artificial Cerebrospinal Fluid (aCSF) solution with reduced Ca^{2+} and increased Mg^{2+} (in mmol/L): 125 NaCl, 3.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.1 $CaCl_2$, 3 $MgCl_2$ and 10 Glucose. Coronal hippocampal slices (250 μm thickness) were cut at 4 °C with a Vibratome (Pelco 1500), and then transferred to a nylon-net basket in an incubator filled with oxygenated standard aCSF (in mmol/L): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 3.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 Glucose (295 ± 5 mOsm; pH 7.3–7.4) and allowed to recover at room temperature for at least 1 h before recording.

2.3. Sulforhodamine (SR101) staining

For SR101 (Invitrogen) staining (Nimmerjahn et al., 2004), the slices were transferred and incubated in another aCSF-filled incubator for 30 min containing 0.6 μmol/L SR101 at 34 °C. Afterwards, the slices were transferred back to standard aCSF at room temperature before the experiments.

2.4. Fresh dissociation of hippocampal astrocytes

As previously described (Du et al., 2015; Ma et al., 2016), hippocampal slices at 250 μm thickness were sectioned from P21–35 mice. After incubation with SR101 for 30 min, the CA1 regions were dissected out, cut into small pieces (1 mm^2), and then transferred into oxygenated aCSF in a 1.5 mL microcentrifuge tube supplemented with 24 U/mL papain and 0.8 mg/mL L-cysteine. This enzymatic incubation lasted for 7 min at 25 °C. The loosened CA1 tissues were gently triturated 5–7 times with a fire-polished glass pipette and then transferred into the recording chamber, where the dispersed small tissue blocks contained single to multiple gap junction-coupled astrocytes with their domains well-preserved in SR101 staining. The number of coupled astrocytes varies among these tissue blocks, which therefore offers a new model, termed the “miniature syncytium”, for the study of astrocyte membrane ion channel and gap junction coupling (Du et al., 2015; Ma et al., 2016; Zhong et al., 2016).

2.5. Primary hippocampal neuron cultures

Primary hippocampal neuron cultures were prepared as previously described (Sherwood et al., 2011). Briefly, the hippocampus was dissected from P0-P1 mice, cut into small pieces, then transferred into Leibovitz's L-15 medium containing 0.38 mg/mL papain and 0.25 mg/mL bovine serum albumin, and then incubated at 37 °C for 13 min with 95% O_2 and 5% CO_2 gas currents over the medium's surface. After incubation, the dissected tissue was washed three times with M5–5 medium (Earle's minimal essential medium with 5% fetal bovine serum, 5% horse serum, 0.4 mmol/L L-glutamine, 16.7 mmol/L glucose, 5000 U/L penicillin, 50 mg/L streptomycin, 2.5 mg/L insulin, 16 nmol/L selenite, and 1.4 mg/L transferrin) and triturated. Dissociated cells were then centrifuged at the relative centrifugal force (RCF) of 0.048 × *g* for 4.5 min, and M5-5 media was aspirated. Supplemented Neurobasal-A media was then added and the cells were resuspended (1% B27 supplement containing antioxidants, 1% B27supplement without antioxidants, 0.5 mmol/L L-glutamine, 0.5 mg/mL gentamycin, 2.5 mg/L insulin, 16 nmol/L selenite, and 1.4 mg/L transferrin). Cells were plated in 24-well cell culture plates containing 10-mm poly-D-lysine-coated glass coverslips at a density of 5×10^4 cells per well. 10 μmol/L cytosine β-D-arabinofuranoside was added 48–72 h after plating. Neurons were maintained at 37 °C with 5% CO_2 for 10–14 days before experiments were performed.

2.6. Oxygen-glucose deprivation (OGD)

To simulate ischemic conditions *in situ* and *in vitro*, glucose was replaced with equimolar sucrose in aCSF, and oxygen was depleted by bubbling the solution with 95% N_2 and 5% CO_2 (pH 7.3–7.4) for 30 min prior to and throughout the 30 min OGD treatment in experiments (Xie et al., 2008).

2.7. Imaging acquisition

A fluorescent imaging system, Polychrome V system (Till Photonics, Germany) was used for high-resolution identification of SR101-positive astrocytes and eGFP-positive NG2 glia.

2.8. Measurement of intracellular K^+ contents from hippocampal slices

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to quantitatively measure the intracellular K^+ contents from hippocampal tissue (Wald et al., 2014; VJT et al., 2017). To ensure mass-equal in slices randomly distributed in the control and OGD groups, two acute hippocampal slices from the left and right hemisphere in each cut were paired as control and OGD. Slices were exposed to 30 min in either OGD conditions or normal aCSF (control). To remove the extracellular