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A-Kinase-Anchoring Protein (AKAP150) is expressed in Astrocytes and Upregulated in Response to Ischemia

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Abstract—A-kinase-anchoring proteins, AKAPs, are scaffolding proteins that associate with kinases and phosphatases, and direct them to a specific submembrane site to coordinate signaling events. AKAP150, a rodent ortholog of human AKAP79, has been extensively studied in neurons, but very little is known about the localization and function of AKAP150 in astrocytes, the major cell type in brain. Thus, in this study, we assessed the localization of AKAP150 in astrocytes and elucidated its role during physiological and ischemic conditions. Herein, we demonstrate that AKAP150 is localized in astrocytes and is up-regulated during ischemia both *in vitro* and *in vivo*. Knock-down of AKAP150 by RNAi depolarizes the astrocytic membrane potential and substantially reduces by 80% the ability of astrocytes to take up extracellular potassium during ischemic conditions. Therefore, upregulation of AKAP150 during ischemia preserves potassium conductance and the associated hyperpolarized membrane potential of astrocytes; properties of astrocytes needed to maintain extracellular brain homeostasis. Taken together, these data suggest that AKAP150 may play a pivotal role in the neuroprotective mechanism of astrocytes during pathological conditions.

Key words: potassium uptake, membrane potential, middle cerebral artery occlusion, siRNA, cortical astrocytes.

INTRODUCTION

A-kinase-anchoring proteins (AKAPs) are scaffolding proteins that recruit and direct protein kinases and phosphatases to a specific submembrane site and contribute to the regulation of phosphorylation events (Michel and Scott, 2002; Carnegie et al., 2009). Many studies have focused on the localization and role of AKAPs in brain, specifically the role of AKAP150 in neurons (Rosenmund et al., 1994; Carnegie and Scott, 2003; Snyder et al., 2005; Chen and Kass, 2005; Dell'Acqua et al., 2006; Zhang et al., 2011). In neurons, AKAP150 has been extensively described in *in vivo* and *in vitro* model systems (Rosenmund et al., 1994; Carnegie and Scott, 2003; Snyder et al., 2005; Chen and Kass, 2005; Dell'Acqua et al., 2006; Schnizler et al., 2008; Zhang et al., 2011; Jeske et al., 2011). Histologically AKAP150 along with the human homolog AKAP79 have been described in neuronal density fractions, cell bodies and dendrites (Sandoz et al., 2006; Lilly et al., 2005; Glantz et al., 1992; Carr et al., 1992; Klauck et al., 1996; Dell'Acqua et al., 1998; Sík et al., 2000; Gomez et al., 2002). Accumulating evidence demonstrates that these AKAPs associate with kinases and phosphatases and direct them to a specific subcellular compartment and modulate NMDAR, AMPAR, Kir2.1, TREK, TASK, TRP and other ion channel activity in neurons (Rosenmund et al., 1994; Dart and Leyland, 2001; Gomez et al., 2002; Sandoz et al., 2006; Schnizler et al., 2008; Zhang et al., 2011; Jeske et al., 2011; Efendiev et al., 2013).

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Abbreviations: AKAP, A-Kinase-Anchoring Protein; BBSS, bicarbonate-buffered balanced salt solution; tMCAO, transient middle cerebral artery occlusion.

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Despite the many advances in understanding the role of AKAP150 in neurons, there is a lack of knowledge about AKAP150 function in astrocytes. While given the anatomical location and abundance of astrocytes in cortex, in the literature, a single microscopy study was found showing AKAP150 immunoreactivity within glia (Lilly et al., 2005). However, it was a descriptive observation and the significance of their finding has yet to be determined. Therefore, the presence of AKAP150 in astrocytes and its importance during physiological and pathological conditions remains unknown. The goal of the present study was to address these questions both *in vitro* and *in vivo* by examining the localization of AKAP150 in astrocytes and assessing the role of AKAP150 on one of the key astrocytic functions, potassium uptake, during normal and pathophysiological ischemic conditions. Here we show that astrocytes express AKAP150 which is upregulated during ischemia and maintains potassium conductance and the associated hyperpolarized membrane potential of astrocytes.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out in accordance with a protocol approved by the Universidad Central del Caribe Institutional Animal Care and Use Committee. Adequate measures were taken to minimize pain or discomfort of experimental animals. Postnatal 1–2 day old Sprague–Dawley rats were used for preparing primary astrocytes cultures and adult Sprague–Dawley rats (250–300 g) were used for transient middle cerebral artery occlusion (tMCAO) experiments.

Astrocyte primary cultures

Primary cortical astrocyte cultures were prepared from neocortex of 1- to 2-day-old rats as previously described (Kucheryavykh et al., 2007).

Simulated ischemia

Astrocytes were exposed to hypoxia/hypoglycemia conditions for 24 h to simulate ischemia *in vitro* as previously described in Rivera-Pagán et al. (2015). Briefly, astrocytes were plated in petri dishes and to achieve hypoxia/hypoglycemic conditions the medium from cultures was removed, cells were gently rinsed and a bicarbonate-buffered balanced salt solution (BBSS) containing: 127 mM NaCl, 3 mM KCl, 19.5 mM $NaHCO_3$, 1.5 mM NaH_2PO_4, 1.4 mM $MgSO_4$, 1 mM $CaCl_2$ and 2.5 mM D-glucose and previously gassed with 5 min of 95%N_2 and 5%CO_2 was applied to the cells. Afterward, cells were placed in a chamber flooded with 95% N_2 and 5%CO_2 and incubated at 37 °C for 24 h. Control cells were incubated in a BBSS solution containing 25 mM D-glucose and incubated at 37 °C for 24 h under normoxic conditions (95%air and 5%CO_2). We have previously shown using electrophysiological techniques that astrocytes exposed to these conditions are viable (Kucheryavykh et al., 2009).

RNA interference by small double-stranded RNAs

As we previously described (Kucheryavykh et al., 2007), the cells were transfected using HiPerfect transfection reagent (Qiagen, Germantown, MD, USA) and the Fast-Forward Protocol for Transfection of Adherent Cells with siRNA recommended by the manufacturer (Qiagen, S102000271). Briefly, 20 nM of siRNA-targeting AKAP150 and 12 μL of HiPerfect were diluted in 100 μL of culture medium without serum. The mix was incubated for 10 min at room temperature to allow the formation of transfection complexes. The complexes were then added to the cells in a drop-wise fashion giving a final volume of 2 mL. The plate was gently swirled to evenly distribute the transfection complexes and cells were incubated at 37 °C for four days. Cell were either used for electrophysiological studies or harvested and AKAP 150 protein expression was measured and compared in control (no transfection reagent), mock-transfected (transfection reagent (HiPerfect) without siRNA) and AKAP150 siRNA transfected astrocytes (transfection reagent (HiPerfect) with siRNA).

SDS–PAGE and western blotting analysis

Astrocytes were pelleted and resuspended in lysis buffer (pH 7.5) containing: (in mM) Tris–HCl 20, NaCl 150, EDTA 1.0, EGTA 1.0, PMSF 1.0, 1% Triton X-100, and an additional mixture of peptide inhibitors (leupeptin, bestatin, pepstatin, and aprotinin). Total protein of homogenates were determined with the Bradford protein assay (Bio-Rad), followed by addition of an appropriate volume of Urea sample buffer (62 mM Tris/HCl pH 6.8, 4% SDS, 8 M Urea, 20 mM EDTA, 5% β-Mercaptoethanol, 0.015% Bromophenol Blue) to load 15 μg of protein per lane. Next, samples were boiled in a water bath at 95 °C for 10 min, spun to pellet debris, and immediately run on 7% SDS–polyacrylamide gels. Western blotting was performed as previously described (Kucheryavykh et al., 2009) using rabbit polyclonal antibodies against AKAP150 (1:200; Santa Cruz Biotechnology, Dallas, TX, USA; Cat# sc-10765, RRID: AB_2289482). Final detection was performed with enhanced chemiluminescence methodology (SuperSignal® West Dura Extended Duration Substrate; Pierce, Rockford, IL, USA) as described by the manufacturer, and the intensity of the signal was measured in a gel documentation system (Versa Doc Model 1000, Bio Rad, Hercules, CA, USA). In all cases, intensity of the chemiluminescence signal was corrected for minor differences in protein content after densitometry analysis of the India ink-stained membrane.

Patch-clamp electrophysiology: voltage and K+ steps

Membrane potentials and currents were measured with the single-electrode whole-cell patch-clamp technique. Two Narishige hydraulic micromanipulators (Narishige, MMW-203, Amityville, NY, USA) were used for (1) voltage-clamp recording, and (2) positioning a micropipette with 30- to 50-μm tip diameter for the

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