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CELL-SPECIFIC MODULATION OF MONOCARBOXYLATE TRANSPORTER EXPRESSION CONTRIBUTES TO THE METABOLIC REPROGRAMING TAKING PLACE FOLLOWING CEREBRAL ISCHEMIA

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Abstract—Monocarboxylate transporters (MCTs) are involved in lactate trafficking and utilization by brain cells. As lactate is not only overproduced during ischemia but its utilization was shown to be essential upon recovery, we analyzed the expression of the main cerebral MCTs at 1 and 24 h after an ischemic insult induced by a transient occlusion of the left middle cerebral artery (MCAO) in CD1 mice (n = 5, 7 and 10 for control, 1 and 24 h groups, respectively). After 1 h of reperfusion, an upregulation of the three MCTs was observed in the striatum (MCT1 ipsilateral 2.73 ± 0.2 and contralateral 2.01 ± 0.4; MCT2 ipsilateral 2.1 ± 0.1; MCT4 ipsilateral 1.65 ± 0.1) and in the surrounding cortex of both the ipsilateral (MCT1 2.4 ± 0.4; MCT2 1.62 ± 0.2; MCT4 1.31 ± 0.1) and contralateral (MCT1 2.78 ± 0.4; MCT2 1.76 ± 0.2) hemispheres, compared to the corresponding sham hemispheres. An increase of MCT1 (ipsilateral 2.1 ± 0.2) and MCT2 (contralateral 1.9 ± 0.1) expression was also observed in the hippocampus, while no effect was observed for MCT4. At 24 h of reperfusion, total MCT2 and MCT4 expressions were decreased in the striatum (MCT2 ipsilateral 0.32 ± 0.1 and contralateral 0.63 ± 0.1; MCT4 ipsilateral 0.59 ± 0.1) and the surrounding cortex (MCT4 ipsilateral 0.67 ± 0.1), compared to the sham. At the cellular level, neurons which usually express only MCT2 strongly expressed MCT1 at both time points. Surprisingly, staining for MCT4 appeared on neurons and was strong at 24 h post-insult, in the striatum and the cortex of both hemispheres. A similar expression pattern was observed also in the ipsilateral hemisphere of the sham operated animals at 24 h. Overall, our study indicates that cell-specific changes in MCT expression induced by an ischemic insult may participate to the metabolic adaptations taking place in the brain after a transient ischemic episode.

Key words: astrocyte, neuron, microvessel, monocarboxylate transporter, ischemia.

INTRODUCTION

Stroke is the second leading cause of death worldwide (World Health Organization 2014; http://www.who.int/mediacentre/factsheets/fs310/en) and the commonest cause of neurological disability in the developed world (Murray and Lopez, 1996; MacDonald et al., 2000). Ischemic stroke, resulting from an artery occlusion, is the most common subtype of stroke. In this case, oxygen and glucose supply from the blood is disrupted, leading to energy failure in the concerned area and causing damage to the brain tissue. Brain metabolism therefore plays a critical role in the pathophysiological mechanisms underlying neuronal damage in stroke and recovery. Glucose is the primary energy substrate of the brain under physiological conditions, but the brain is also able to oxidize other intermediate metabolites, notably lactate (Boumezbeur et al., 2010; Wyss et al., 2011). Lactate seems to occupy a central position under ischemic conditions. Indeed, during cerebral ischemia, lactate accumulates in the extracellular space due to the anaerobic metabolism of glucose. Levels can rise from 1.5 to 3.5 mM in the normal brain to 15–25 mM in the ischemic brain (Rehncrona et al., 1981; Folbergrova et al., 1992; Wagner et al., 1992). Despite a long history of negatively perceived role in ischemia, lactate has been shown to have neuroprotective properties in hypoxic conditions *in vitro* (Schurr et al., 1997a,b; Berthet et al., 2009), but also in models of ischemia *in vivo* (Schurr et al., 2001; Berthet et al., 2009, 2012). Indeed, Berthet et al. (2009, 2012) demonstrated that administration of lactate after reperfusion, either intracerebroventricularly or intravenously, decreased significantly the lesion size in mouse brain and improved the neurological outcome upon recovery from a transient middle cerebral artery occlusion (tMCAO).

Lactate trafficking between cells is facilitated by a family of proton-dependent carriers named monocarboxylate transporters (MCTs). Three of them have been

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; MCAO, middle cerebral artery occlusion; MCTs, monocarboxylate transporters; PBS, phosphate-buffered saline; rCBF, regional cerebral blood flow; TBS, Tris-Buffered-Saline.

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clearly characterized as lactate transporters in the central nervous system (Pierre and Pellerin, 2005). MCT1 is predominantly found on endothelial cells forming cerebral blood vessels as well as on glial cells including astrocytes (Gerhart et al., 1997; Hanu et al., 2000; Pierre et al., 2000), oligodendrocytes (Rinholm et al., 2011; Lee et al., 2012) and tanycytes (Cortés-Campos et al., 2011). MCT2 is considered the main neuronal transporter (Pierre et al., 2002; Debernardi et al., 2003). Finally, MCT4 is exclusively expressed by astrocytes but its precise role is not well understood up to now (Rafiki et al., 2003; Pellerin et al., 2005; Rosafio and Pellerin, 2014). Interestingly, Schurr et al. (2001) showed that inhibition of lactate transport through the MCTs increases neuronal damage after cardiac arrest-induced transient global cerebral ischemia in a rat model, thus suggesting a critical role for these transporters in the recovery from ischemia. In this study, we investigated the changes in cerebral MCT expression pattern occurring in a MCAO ischemic mouse model.

EXPERIMENTAL PROCEDURES

All experiments were conducted in accordance with the Swiss guidelines for animal experimentation and approved by the veterinary authority.

tMCAO in the mouse

Male CD1 mice (body weight 26–35 g, 5–7 weeks of age; Charles River, L'arbresle, France) were anesthetized with isoflurane (1.5–2% in nitrous oxide/oxygen 70%/30%) using a face mask. Body temperature was maintained at 37.0 ± 0.5 °C throughout surgery using a heating pad (FHC Inc., Bowdoinham, ME, USA). Regional cerebral blood flow (rCBF) was measured and continuously recorded throughout the operation in all animals by laser-Doppler flowmetry (Perimed, Craponne, France) with a flexible probe fixed on the skull (1 mm posterior and 6 mm lateral from bregma). Transient (30 min) focal cerebral ischemia was induced by occlusion of the left MCA with an intra-arterial suture as described previously (Longa et al., 1989; Berthet et al., 2009). Briefly, the left common carotid artery and the left external carotid artery were exposed and ligated following a ventral midline neck incision. Ischemia was induced by inserting a silicon-coated nylon monofilament (0.17 mm diameter) through the left common carotid artery into the internal carotid artery until mild resistance was felt and a drop to less than 20% of initial rCBF was registered. rCBF was monitored and maintained below 20% of the baseline level during ischemia. Reperfusion was considered successful if the rCBF rose above 50% of baseline. Sham mice underwent exclusively the common carotid artery ligation during 30 min.

At the beginning of the operation, mice were administered 0.025 mg/kg of buprenorphine subcutaneously for post-surgery analgesia. Once the animals were awake, they were housed overnight in an incubator at 28 °C.

Western blotting

For protein expression experiments, young adult male mice were subjected to 30 min MCAO as described above and sacrificed either 1 h or 24 h after reperfusion. Striatum (caudate and putamen), primary motor and somatosensory cortex and hippocampus were collected using a rodent brain matrix (adult mouse, coronal. ASI Instruments, Warren, MI, USA). Total proteins were extracted from brain tissues by cellular lysis in ice-cold RIPA buffer (#9806, Cell Signaling, Beverly, MA, USA) supplemented with a mixture of protease inhibitors (Complete 11257000; Roche, Basel, Switzerland). Protein quantification was performed with the Pierce BCA Protein Assay kit (#23227, Thermo Fisher Scientific, Pierce, Lausanne, Switzerland) and about 20 μg of proteins were denatured (95 °C) during 5 min in SDS–PAGE sample buffer (60 mM Tris–HCl pH 6.8, 5% SDS, 6.6% glycerol, 5 mM EDTA, 5% β-mercaptoethanol and 0.1% bromophenol blue). Samples were separated on a 10% acrylamide running gel and a 4% acrylamide stacking gel using an Electrophoresis Unit (Bio-Rad, Cressier, Switzerland). Proteins were then electroblotted onto nitrocellulose membranes (0.45 μm; #162-0115, Bio-Rad, Cressier, Switzerland) using an Electrophoresis Unit. Non-specific binding sites were blocked for 1 h at room temperature with a solution of Tris-Buffered-Saline (TBS; 50 mM Tris-HCl pH 7.5, 150 mM NaCl) supplemented with 0.1% Tween-20 and containing 10% (wt/vol) of skimmed milk. Blots were then incubated overnight at 4 °C with specific primary antibodies in TBS-T 0.1% containing 1% skimmed milk: polyclonal rabbit anti-mouse MCT1 (1:1000 dilution; (Pierre et al., 2000)), polyclonal rabbit anti-mouse MCT2 (1:1000 dilution; (Pierre et al., 2000)) or polyclonal rabbit anti-mouse MCT4 (1:200 dilution; #sc-50329; SantaCruz, Heidelberg, Germany). Blots were washed three times in TBS-T 0.1% and subsequently incubated 2 h at room temperature with horseradish peroxidase-conjugated donkey anti-rabbit IgG (#NA9340V, 1:10.000 dilution; GE Healthcare, Glattbrugg, Switzerland). After being washed three times in TBS-T 0.1%, blots were processed using Immun-StarTMWesternCTM Chemiluminescent Kit (#170-5070, Bio-Rad, Cressier, Switzerland). Chemiluminescence detection was performed with the ChemiDoc™ XRS System (#170-8070, Bio-Rad, Cressier, Switzerland). Total protein content assay was performed with the Pierce Reversible Protein Stain kit (#24580, Thermo Fisher Scientific, Pierce, Lausanne, Switzerland) and revealed with the ChemiDoc™ XRS System (#170-8070, Bio-Rad, Cressier, Switzerland). Both types of labeling were quantified with the ImageLab 3.0 software (Bio-Rad, Cressier, Switzerland) and MCT protein expression was normalized to the total protein content. All data were normalized to the corresponding ipsilateral area of the sham.

Immunohistochemistry

Mice were injected intraperitoneally with a lethal dose of pentobarbital (10 mL/kg, Sigma–Aldrich, Buchs, Switzerland) and then perfused with 150 mL of 4%