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TISSUE-TYPE PLASMINOGEN ACTIVATOR MEDIATES NEUROGLIAL COUPLING IN THE CENTRAL NERVOUS SYSTEM

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Abstract—The interaction between neurons, astrocytes and endothelial cells plays a central role coupling energy supply with changes in neuronal activity. For a long time it was believed that glucose was the only source of energy for neurons. However, a growing body of experimental evidence indicates that lactic acid, generated by aerobic glycolysis in perivascular astrocytes, is also a source of energy for neuronal activity, particularly when the supply of glucose from the intravascular space is interrupted. Adenosine monophosphate-activated protein kinase (AMPK) is an evolutionary conserved kinase that couples cellular activity with energy consumption via induction of the uptake of glucose and activation of the glycolytic pathway. The uptake of glucose by the blood–brain barrier is mediated by glucose transporter-1 (GLUT1), which is abundantly expressed in endothelial cells and astrocytic end-feet processes. Tissue-type plasminogen activator (tPA) is a serine proteinase that is found in endothelial cells, astrocytes and neurons. Genetic overexpression of neuronal tPA or treatment with recombinant tPA protects neurons from the deleterious effects of metabolic stress or excitotoxicity, via a mechanism independent of tPA's ability to cleave plasminogen into plasmin. The work presented here shows that exposure to metabolic stress induces the rapid release of tPA from murine neurons but not from astrocytes. This tPA induces AMPK activation, membrane recruitment of GLUT1, and GLUT1-mediated glucose uptake in astrocytes and endothelial cells. Our data indicate that this is followed by the synthesis and release of lactic acid from astrocytes, and that the uptake of this lactic acid via the monocarboxylate transporter-2 promotes survival in neurons exposed to metabolic stress. Published by Elsevier Ltd. on behalf of IBRO.

Key words: tissue-type plasminogen activator (tPA), glucose metabolism, neuroprotection, adenosine monophosphate-activated protein kinase (AMPK), monocarboxylate transporter-2 (MCT-2).

INTRODUCTION

Astrocytes constitute a functional and anatomical link between neurons and endothelial cells. Indeed, almost 95% of the vascular surface of the blood–brain barrier (BBB) is ensheathed by perivascular astrocytes and the opposite pole of each astrocyte enters in contact with approximately 30,000 synapses (Bushong et al., 2002). Therefore, astrocytes are uniquely positioned to couple the uptake of nutrients from the intravascular space with changes in neuronal demands.

Glucose was considered for a long time to be the only source of energy for neurons (Simpson et al., 2007). However, a growing body of experimental evidence shows that lactate generated by astrocytes, via activation of the glycolytic pathway, is also a fuel for neuronal activity (Fox et al., 1988). These and other observations led to postulate the existence of an astrocyte–neuron lactate shuttle (ANLS) in which astrocytes metabolize glucose to lactate which is uptaken by neurons as an energy source (Pellerin and Magistretti, 1994) via the monocarboxylate transporter-2 (MCT-2), that is abundantly expressed in neurons (Simpson et al., 2007). Although lactate is able to sustain basal neuronal metabolism, glucose is a required substrate for synaptic activity (Bak et al., 2006), suggesting that while glucose is the main fuel for neuronal activity under physiological conditions, lactate synthesized and released by astrocytes is the principal source of energy when the supply of glucose is interrupted.

Adenosine monophosphate-activated protein kinase (AMPK) is an evolutionary conserved kinase that acts as an energy sensor, promotes cellular adaptation to metabolic stress, and couples cellular activity with energy consumption (Amato and Man, 2011). AMPK phosphorylation at Thr172 induces the uptake of glucose and turns on catabolic pathways that generate ATP such as glycogenolysis and glycolysis (Hardie, 2007). The uptake of glucose by endothelial cells and

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Abbreviations: 2-NBDG, 2-*N* (7-nitrobenz-2-oxa-1,3-diazol-4-yl-amino)-2-deoxyglucose; AMPK, adenosine monophosphate-activated protein kinase; BBB, blood–brain barrier; BCA, bicinchoninic acid; DAPI, 4′-6-diamidino-2-phenylindole; DH, 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; GLUT1, glucose transporter-1; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane sulfonic acid; MCT-2, monocarboxylate transporter-2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OGD, oxygen and glucose deprivation; RBMVEC, rat brain microvascular endothelial cells; tPA, tissue-type plasminogen activator.

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astrocytes in mediated by glucose transporter-1 (GLUT1), a member of the SLC2 family of transport proteins (Joost and Thorens, 2001; Joost et al., 2002) abundantly expressed in the luminal and abluminal surface of endothelial cells and in astrocytic end-feet processes. In the brain GLUT1 is detected in two different molecular weights: a 55-kDa form expressed only in the endothelial cells of the BBB, and a 45-kDa isoform found in perivascular astrocytes (Birnbaum et al., 1986). Variations in the concentration of glucose in the intravascular space induce changes in the expression of GLUT1 (Vannucci et al., 1997) and its redistribution from an intracellular pool to the luminal and abluminal membranes of endothelial cells, and to the end-feed processes of perivascular astrocytes (Simpson et al., 2007). Remarkably, astrocytes not only uptake glucose but also are the only cells in the brain able to store it as glycogen.

Tissue-type plasminogen activator (tPA) is a serine proteinase found in neurons, astrocytes and endothelial cells (Yepes et al., 2009). Our early work indicates that genetic overexpression of neuronal tPA or treatment with recombinant tPA (rtPA) protects neurons from the deleterious effects of ischemia and deprivation of oxygen and glucose, via a mechanism independent of tPA's ability to cleave plasminogen into plasmin (Echeverry et al., 2010; Wu et al., 2012, 2013). The results presented here show that oxygen and glucose deprivation (OGD) induces the rapid release of tPA from cerebral cortical neurons but not from astrocytes. This tPA activates AMPK in astrocytes and endothelial cells, promotes the recruitment of GLUT1 to their plasma membrane, and induces GLUT1-mediated uptake of glucose, followed by synthesis and release of lactic acid from astrocytes. Remarkably, we found that MCT2-mediated uptake of lactic acid promotes neuronal survival following exposure to OGD conditions. In summary, here we report that neuronal tPA mediates the metabolic coupling between neurons, astrocytes and endothelial cells. Based on our data, we propose a model where in response to an increase in metabolic demands tPA is released from neurons. This tPA activates a cell signaling pathway in perivascular astrocytes and endothelial cells that promotes the uptake of glucose and the activation of the glycolytic pathway with the synthesis and release of lactic acid that is used by neurons as a metabolic fuel.

EXPERIMENTAL PROCEDURES

Reagents

All experiments conformed to guidelines of the Institutional Animal Care Use Committee of Emory University, Atlanta, GA, as well as International Guidelines on the ethical use of animals and all efforts were made to minimize the number of animals used and their suffering. Recombinant murine tPA and an enzyme-linked immunosorbent assay (ELISA) kit that detects active tPA were acquired from Molecular Innovations (Novi, MI, USA). Other reagents were 2-*N* (7-nitrobenz-2-oxa-1,3-diazol-4-yl-amino)-2-deoxyglucose (2-NBDG), goat alexa-conjugated secondary antibodies and 4′-6-diamidino-2-phenylindole (DAPI; Invitrogen; Grand Island, NY, USA), antibodies against glial fibrillary acidic protein (GFAP), AMPK phosphorylated at Thr172 (Cell Signaling Technology; Denvers, MA, USA) and the glucose transporter GLUT1, lactic acid, and L-Lactate acid assay kit (Abcam, Cambridge, MA, USA), advasep-7, phloretin and cyano-4-hydroxycinnamic acid and 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride (DH) (Sigma; St Louis, MO, USA), the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide ((MTT) assay. ATCC; Manassas, VA, USA), the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific Inc., Waltham, MA, USA), 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (Cell Signaling Technology; Denvers, MA), and rat brain microvascular endothelial cells (RBMVEC) and attachment factor solution (Cell Applications, Inc., San Diego, CA, USA).

Cell cultures and determination of cell survival

RBMVEC were plated on T75 flasks coated with attachment factor solution and maintained in an humidified incubator at 37 °C and 5% CO_2 until they reached 80% confuency. Cells were used at passages 2–8. Astrocytes and cerebral cortical neurons were cultured from 1-day-old and E16–18 wild-type mice, respectively, as described elsewhere (Polavarapu et al., 2007; Echeverry et al., 2010). Briefly, the cerebral cortex was dissected, transferred into Hanks' balanced salt solution containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 mm HEPES, and incubated in trypsin containing 0.02% DNase at 37 °C for 15 min. Tissue was then triturated, and the supernatant was re-suspended in B27-supplemented neurobasal medium containing 2 mM L-glutamine and plated onto 0.1 mg/ml poly-L-lysine-coated wells. To study the effect of lactic acid on neuronal survival cerebral cortical neurons were maintained during 55 min in medium with no glucose and exposed in an anaerobic chamber to $<0.1\%$ oxygen (Hypoxygen; Frederick, MD, USA) in the presence of either vehicle (control), or 5 mM of lactic acid alone or in combination with 0.5 mM of cyano-4-hydroxycinnamic acid. Twenty-four hours later cell survival was quantified with the MTT assay following the manufacturer's instructions and as described elsewhere (Echeverry et al., 2010). Results are given as a percentage of cell survival compared to cultures maintained under physiological conditions. Each experiment was performed in cultures from three different animals and each observation was repeated 12 times.

TPA activity assay

The culture media of wild-type neurons, astrocytes and brain microvascular endothelial cells was sampled after 0, 1, 5, 15, 30 and 60 min of exposure to OGD conditions. The concentration of tPA was quantified with an ELISA kit following the manufacturer's instructions. Results were normalized to protein concentration in