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Coupling of glutamate and glucose uptake in cultured Bergmann glial cells

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ABSTRACT

Glutamate, the main excitatory neurotransmitter in the vertebrate brain, exerts its actions through specific membrane receptors present in neurons and glial cells. Over-stimulation of glutamate receptors results in neuronal death, phenomena known as excitotoxicity. A family of sodium-dependent, glutamate uptake transporters mainly expressed in glial cells, removes the amino acid from the synaptic cleft preventing neuronal death.

The sustained sodium influx associated to glutamate removal in glial cells, activates the sodium/potassium ATPase restoring the ionic balance, additionally, glutamate entrance activates glutamine synthetase, both events are energy demanding, therefore glia cells increase their ATP expenditure favouring glucose uptake, and triggering several signal transduction pathways linked to proper neuronal glutamate availability, *via* the glutamate/glutamine shuttle. To further characterize these complex transporters interactions, we used the well-established model system of cultured chick cerebellum Bergmann glia cells.

A time and dose-dependent increase in the activity, plasma membrane localization and protein levels of glucose transporters was detected upon D-aspartate exposure. Interestingly, this increase is the result of a protein kinase C-dependent signaling cascade. Furthermore, a glutamate-dependent glucose and glutamate transporters co-immunoprecipitation was detected. These results favour the notion that glial cells are involved in glutamatergic neuronal physiology.

1. Introduction

Glutamate (Glu) is the main excitatory amino acid neurotransmitter in the Central Nervous System (CNS) (Fonnum, 1984). A high percentage (80–90%) of brain synapses use it as their neurotransmitter (Braitenberg et al., 1998), therefore Glu is involved in almost every function of the brain, from sensory motor information and coordination to emotions and cognition. Glu exerts its actions through the activation of specific membrane receptors and transporters that are expressed both in neurons and glia cells. Glu receptors are divided into two main subtypes: ionotropic (iGluRs) and metabotropic receptors (mGluRs) (Hollmann and Heinemann, 1994).

Removal of Glu from the synaptic cleft relays in a family of sodium-dependent Glu transporters, known as excitatory amino acid transporters (EAATs) (Danbolt, 2001). Five different EAAT

Abbreviations: Glu, L-glutamate; D-Asp, D-aspartic acid; Gln, glutamine; iGluRs, ionotropic glutamate receptors; mGluRs, metabotropic glutamate receptors; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*- methyl-D-aspartate; EAAT, excitatory amino acid transporter; BGC, Bergmann glia cell; GLAST, Glutamate Aspartate Transporter; GLT1, Glutamate transporter 1; ALS, Amyotrophic Lateral Sclerosis; SNATs, sodium dependent neutral amino acid transporters; GS, Gln synthetase; GAP, phosphate-activated glutaminase; [H^3]2DOG, [^{3}H]2-deoxy-D-glucose; VGLUTS, vesicular Glu transporters; GLUTs, Glucose transporters; TTBS, Tris Buffered Saline, 0.1% Tween 20; GLUT1, Glucose transporter 1; GLUT3, Glucose transporter 3; GLUTs, glucose transporters; LDH, lactate dehydrogenase; ANLS, Astrocyte Neuron Lactate Suttle.

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subtypes have been described, EAAT1/GLAST and EAAT2/GLT1 are preferentially expressed in glial cells and carry more than 80% of the total brain Glu uptake activity (Swanson, 2005). Within the cerebellar cortex, most of the Glu uptake takes place in Bergmann glial cells (BGC), these cells express exclusively EAAT1/GLAST, also known as Na^+- glutamate/aspartate transporter (GLAST). In contrast, in most of all other CNS structures, EAAT2 or Glutamate transporter 1 (GLT-1) is the major Glu carrier, in fact, it is known that this transporter represents roughly 2% of total brain protein (Danbolt et al., 1998). It should be noted that this transporter is also present in presynaptic neurons in certain brain areas such as the hippocampus (Danbolt, 2001).

Glu extracellular levels are tightly controlled in order to prevent its toxicity to neurons and oligodendrocytes. Neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's disease are related to an un-regulated excess of extracellular Glu (Sheldon and Robinson, 2007; Paul and de Belleroche, 2014).

It has long been proposed that glutamatergic transmission depends on a metabolic and energetic coupling between neurons and glia cells (Fatemi, 2008; Rojas, 2014; Volk et al., 2015). The neuronal availability of releasable Glu involves the proper function of glial proteins, Glu transporters, the enzyme glutamine (Gln) synthetase (GS) and the sodium dependent neutral amino acid transporters (SNATs). An accepted model of this coupling is the *so-called* Glu/Gln shuttle (Shank and Campbell, 1984). After an action potential, Glu released from the presynaptic terminal activates post-synaptic receptors and diffuses to the neighbouring glia cells, despite of the fact of presence of neuronal Glu transporters of the EAAT3 subtype, mainly due to their low expression, high affinity/low capacity characteristics (Danbolt, 2001). The abundance and capacity of glia Glu transporters is sufficient to clear extracellular Glu micromolar concentrations. Once internalized, Glu is mainly metabolized to Gln by means of the glia-enriched GS (Farinelli and Nicklas, 1992), accumulated Gln is released to the extracellular media through the inverse function of Gln transporters of the N family (SNAT 3,5). Presynaptic neurons take up Gln *via* the A subtype of Gln transporters (SNAT 1,2) and through the action of phosphate-activated glutaminase (GAP) Gln is converted back to Glu that is packed in the presynaptic vesicles through the vesicular Glu transporters (VGLUTS) completing the cycle. Needless to say, inhibition of any of these proteins impairs glutamatergic synaptic transmission (Broer and Brookes, 2001).

It has been demonstrated that glia Glu uptake is linked to Gln release (Billups et al., 2013; Martinez-Lozada et al., 2013). Furthermore, an aspartate-dependent EAAT1-SNAT3 physical and functional interaction has been reported in BGC (Martinez-Lozada et al., 2013). Central to this coupling is the Na^+/K^+ ATPase, which also has been shown to associate with Glu transporters. The ATPase activity is needed to extrude the excess of Na^+ ions that enter the cell together with Glu *via* EAAT1/GLAST (Rose et al., 2009). The energetic coupling between astrocytes and neurons was suggested from experiments using cultured cortical glia exposed to Glu for short time periods. It became evident that astrocytes release lactate after an increased sodium-dependent Glu uptake (Pellerin and Magistretti, 1994). An Astrocyte-Neuron Lactate Shuttle (ANLS), was proposed and described in a number of systems (Jakoby et al., 2014).

We hypothesize that within the cerebellar cortex, BGC could be energetically coupled to the glutamatergic synapses surrounded by them. In such a scenario, glucose uptake would be favoured. To gain insight into this possibility, we decided to characterize [^{3}H]2-deoxy-D-glucose ([^{3}H]2DOG) uptake in primary cultures of chick cerebellar BGC. These cells completely enwrap the most abundant glutamatergic synapse in the encephalon, the one formed by the parallel fibers and the Purkinje cells in the cerebellar cortex (Somogyi et al., 1990).

The glucose transporter protein family (GLUTs) is integrated by fourteen members, from which class I isoforms have been better described in brain (Augustin, 2010). Tissue and cell specificity have been established for GLUTs. The major glucose transporter expressed in granular cells and neuronal cell lines is GLUT3, mainly in the plasma membrane. Interestingly, low levels of GLUT3 have been identified in rat cortical astrocytes (Maher et al., 1991). In general terms, it is quite accepted that GLUT1 is mainly found in glial cells and GLUT3 in the neuronal counterpart (Brekke et al., 2015).

Upon treatment of BGC with its specific EAAT ligand, D-aspartate, a time and dose-dependent increase in [^{3}H]2-Deoxy-D-glucose (DOG) uptake is observed. An increase of available glucose transporters in the plasma membrane is likely to be the responsible for the enhanced uptake. Moreover, an interaction between Glu and glucose transporters could be detected. These results support the involvement of glia/neuronal interactions in glutamatergic transmission.

2. Methods

2.1. Materials

Tissue culture reagents were obtained from GE Healthcare (Carlsbad, CA, USA). Deoxy-D-glucose, 2-[1,2-^{3}H (N)] (specific activity 20 Ci/mmol) was from Perkin Elmer (Boston, MA, USA). L-Gln, D-Asp, Glu and 2-Deoxy-D-glucose (2DOG) were obtained from Sigma-Aldrich, Mexico. Bisindolylmaleimide I (Bis I) from Calbiochem, (EMD Millipore, Darmstadt, Germany), H-9 dihydrochloride, KB-R7943 mesylate, DL-threo-b-Benzyloxyaspartic acid (TBOA), threo-b-hydroxyaspartate (THA) were from Tocris-Cookson (St. Louis, MO, USA).

Goat polyclonal anti-GLUT1 (sc-1605) and anti-GLUT3 (sc-7581) were from Santa Cruz Biotechnology, Dallas, TX USA. Monoclonal anti-calbindin D (C-8666) was from Sigma-Aldrich, St. Louis, MO, USA), anti-kainate binding protein (KBP) and anti-GLAST antiserum were produced and characterized in our laboratory (Martinez-Lozada et al., 2013). Monoclonal anti-actin antibody was kindly donated by Prof. Manuel Hernández (Cinvestav-IPN). Protein A and G coupled to agarose particles, horseradish peroxidase-coupled anti-mouse, anti-goat, anti-rabbit antibodies, and the Enhanced Chemiluminescence reagent, were obtained from GE Healthcare Life Sciences (Mexico). EZ-Link® Sulfo-NHS-LC-Biotin and agarose-coupled Streptavidin were from Thermo Scientific. All other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture and stimulation protocol

Primary cultures of cerebellar BGC were prepared from 14-day-old chick embryos, with subtle adjustments from our previously described protocol (Ortega et al., 1991). Briefly, trypsin and mechanically dissociated cells were sedimented consecutively two times, then plated in 24-well and 6-well plastic culture dishes in OPTIMEM containing 4% foetal bovine serum (FBS), gentamicin (50 μg/ml) and used on the 3rd to 5th day after plating. Before any treatment, assay buffer solution containing 25 mM HEPES-Tris, 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 5 mM glucose and 1 mM NaH_2PO_4 at pH 7.4 was replaced on the confluent monolayers. Experiments with different ionic conditions in the assay buffer solution included Tris-HCl for NaCl replacement and 0.1 mM EDTA addition for without calcium condition. Inhibitors and blockers were incubated 20 min before stimulation.