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Research Report

Sex-specific basal and hypoglycemic patterns of in vivo caudal dorsal vagal complex astrocyte glycogen metabolic enzyme protein expression

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ABSTRACT

Astrocytes contribute to neurometabolic stability through uptake, catabolism, and storage of glucose. These cells maintain the major brain glycogen reservoir, which is a critical fuel supply to neurons during glucose deficiency and increased brain activity. We used a combinatory approach incorporating immunocytochemistry, laser microdissection, and Western blotting to investigate the hypothesis of divergent expression of key enzymes regulating glycogen metabolism and glycolysis during in vivo normo- and/or hypoglycemia in male versus female hindbrain astrocytes. Glycogen synthase (GS) and glycogen phosphorylase (GP) levels were both enhanced in dorsal vagal complex astrocytes from vehicle-injected female versus male controls, with incremental increase in GS exceeding GP. Insulin-induced hypoglycemia (IIH) diminished GS and increased glycogen synthase kinase-3-beta (GSK3β) expression in both sexes, but decreased phosphoprotein phosphatase-1 (PP1) levels only in males. Astrocyte GP content was elevated by IIH in male, but not female rats. Data reveal sex-dependent sensitivity of these enzyme proteins to lactate as caudal hindbrain repletion of this energy substrate fully or incompletely reversed hypoglycemic inhibition of GS and prevented hypoglycemic augmentation of GSK3β and GP in females and males, respectively. Sex dimorphic patterns of glycogen branching and debranching enzyme protein expression were also observed. Levels of the rate-limiting glycolytic enzyme, phosphofructokinase, were unaffected by IIH with or without lactate repletion. Current data demonstrating sex-dependent basal and hypoglycemic patterns of hindbrain astrocyte glycogen metabolic enzyme expression imply that glycogen volume and turnover during glucose sufficiency and shortage may vary accordingly.

1. Introduction

In the brain, neuroglial metabolic coupling involves cell-type compartmentation of glucose metabolism with provision of oxidizable substrate fuel to neurons by astrocytes. Despite high energy needs, nerve cells are ironically devoid of energy stores and exhibit a truncated glycolytic pathway that favors pentose phosphate metabolism and antioxidative protection

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over energy production (Barros, 2013). The astrocyte-neuron lactate shuttle hypothesis (ANLSH) postulates that glucose, the primary energy source to the brain, is acquired from the circulation by astrocytes and either stored as glycogen, a complex branched polymer, or catabolized to lactate for trafficking to neurons (Pellerin and Magistretti, 1994; Pellerin et al., 1998). Brain glycogen is the principal alternative to blood-derived glucose as a source of energy. Astrocytes maintain the main glycogen repository in the mammalian brain (Phelps, 1972; Koizumi, 1974). This glycogen reservoir is dynamic during normal brain activity and metabolic stasis, and is an important reserve of lactate equivalents during states of heightened activity or glucose deficiency (Stobart and Anderson, 2013). Unlike neurons, astrocytes maintain a high rate of glycolysis; internal glycogen stores thus favor glial energetic stability as glucose serves as a rapid cytosol source of ATP (Obel et al., 2012).

Sex differences in bodily energy metabolism and endocrine disruptor-induced obesity reflect, in part, potent regulatory effects of the ovarian steroid hormone, estradiol, on cellular energy-producing pathways (Chen et al., 2009). So far, insight on estrogen involvement in neuroenergetics pertains to known effects of this hormone on mitochondrial oxidative respiration and ensuing benefits to nerve cell function (Nilsen et al., 2007). Much less is known about potential gender effects on astrocyte energy metabolism, e.g. glucose storage and catabolism. Glycogen metabolism is governed by opposing actions of glycogen synthase (GS) and glycogen phosphorylase (GP), which respectively catalyze glycogen synthesis and depletion. Glycogen synthase kinase-3-beta (GSK3β) inactivates GS, whereas phosphoprotein phosphase-1 (PP1) stimulates GS and deactivates GP. During glycogen formation, glycogen branching enzyme (GBE) promotes addition of glucose unit side chains at intervals of 10–14 glucose units; branching of the glycogen polymer is advantageous in providing large numbers of terminal residues for rapid degradation and increasing glycogen solubility. Glycogen debranching enzyme (GBE) facilitates GP-mediated glycogen breakdown by cleaving linkages at branch points. Brain glycogenolysis is stimulated under conditions where energy is insufficient relative to demand, including seizure, sleep deprivation, and hypoglycemia (Gruetter, 2003; Brown, 2004). Iatrogenic insulin-induced hypoglycemia is a persistent worrisome

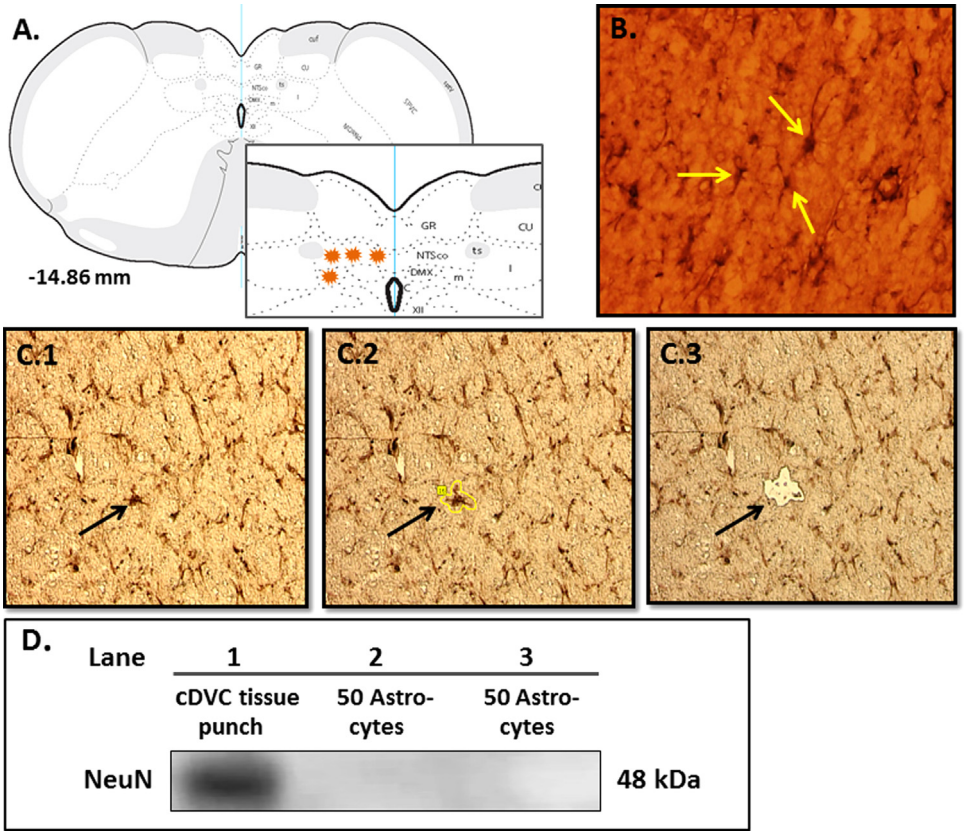

Fig. 1 – Astrocyte laser-catapult microdissection from rat caudal hindbrain. The brain map (−14.86 mm) and inset in panel A illustrate the location of astrocytes [represented by orange stars] harvested from the caudal dorsal vagal complex (cDVC) by laser microdissection after in situ identification by labeling for glial fibrillary acidic protein (GFAP)-immunoreactivity (-ir). Yellow arrows in the photomicrograph in panel B denote representative GFAP-ir-positive cells in the cDVC. The area in panel C.1 was re-photographed after positioning of a continuous laser cut (depicted in yellow) around a single astrocyte [panel C.2] and subsequent ejection of that cell by laser pulse [panel C.3]. Note that this microdissection technique causes negligible destruction of surrounding tissue and minimal inclusion of adjacent tissue. The immunoblot in Panel D shows that the neuron marker, Neu N, is present in cDVC tissue sample (lane 1), but absent from pooled astrocyte lysates (lanes 2 and 3). *Abbreviations in Panel A*: **NTSco,m,l: commissural, medial and lateral components of the nucleus of the solitary tract; ts: solitary tract; C: central canal; CU: cuneate nucleus; XII: hypoglossal nucleus.**