Physiology & Behavior 126 (2014) 39–49

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Chronic stress modulates regional cerebral glucose transporter expression in an age-specific and sexually-dimorphic manner

Sean D. Kelly [a], Constance S. Harrell [a], Gretchen N. Neigh [a,b,*]

[a] Department of Physiology, Emory University, Atlanta, GA 30322, United States
[b] Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA 30322, United States

HIGHLIGHTS

- Sex differences in GLUT expression are greatest in the hypothalamus.
- The transition to adulthood decreases expression of specific hippocampal GLUTs.
- Stress alters cerebral GLUTs across development in a sex- and region-specific way.
- Cerebral GLUT expression differs based on region, sex, age, and stress exposure.

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form 8 November 2013
Accepted 12 December 2013

Keywords:
Stress
Age
Sex
Brain
Glucose transporter

ABSTRACT

Facilitative glucose transporters (GLUT) mediate glucose uptake across the blood–brain-barrier into neurons and glia. Deficits in specific cerebral GLUT isoforms are linked to developmental and neurological dysfunction, but less is known about the range of variation in cerebral GLUT expression in normal conditions and the effects of environmental influences on cerebral GLUT expression. Knowing that puberty is a time of increased cerebral plasticity, metabolic demand, and shifts in hormonal balance for males and females, we first assessed gene expression of five GLUT subtypes in four brain regions in male and female adolescent and adult Wistar rats. The data indicated that sex differences in GLUT expression were most profound in the hypothalamus, and the transition from adolescence to adulthood had the most profound effect on GLUT expression in the hippocampus. Next, given the substantial energetic demands during adolescence and prior demonstrations of the adverse effects of adolescent stress, we determined the extent to which chronic stress altered GLUT expression in males and females in both adolescence and adulthood. Chronic stress significantly altered cerebral GLUT expression in males and females throughout both developmental stages but in a sexually dimorphic and brain region-specific manner. Collectively, our data demonstrate that cerebral GLUTs are expressed differentially based on brain region, sex, age, and stress exposure. These results suggest that developmental and environmental factors influence GLUT expression in multiple brain regions. Given the importance of appropriate metabolic balance within the brain, further assessment of the functional implications of life stage and environmentally-induced changes in GLUTs are warranted.

1. Introduction

Adequate glucose transport is essential to brain function and survival. The adult brain accounts for 20% of total resting oxygen consumption in humans [1–3] and 4–6% in rats [1], which is almost entirely utilized for the oxidation of carbohydrates [4]. While the cerebral metabolic demand is substantial in the adult, the metabolic rate in children is much higher, calculating to as much as 50% of whole-body glucose utilization by the developing human brain [4]. Glucose transporters (protein symbol GLUT, gene symbol *Slc2a*) facilitate glucose transport across the blood–brain-barrier and the uptake of glucose into neurons and glia [5–7]. The crucial role of GLUTs is illustrated by the profound neurological deficits manifested in De Vivo disease, a rare genetic condition in which GLUT subtype 1 is not expressed [8]. Milder deficits in the expression and translocation of GLUTs have been linked to neuropathology including Alzheimer's pathology, post-ischemic/hypoxic brain function, and following traumatic brain injury [9–13]. Less is known about the range of variation in cerebral GLUT expression in normal conditions and the effects of environmental influences on cerebral GLUT expression.

The GLUT family is not fully characterized, however, current literature indicates at least nine GLUT isoforms are expressed throughout the brain [6,14], and five of the nine have a plausible role in development [15,16] and a potential role in the effects of chronic stress [16–19]. Here we focus on transporter isoforms GLUT 1, 3, 4, 5, and 8. The specific

* Corresponding author at: Department of Physiology, Emory University, 615 Michael Street, Suite 600, Atlanta, GA 30322, United States. Tel.: +1 404 727 9022.
E-mail address: gmccand@emory.edu (G.N. Neigh).

0031-9384/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.physbeh.2013.12.002

Table 1
Summary of cerebral glucose transporters potentially involved in neurological disease or stress responses.

Protein	Gene	Cerebral location	Involvement in neurologic disease and the stress response	References
GLUT1	*Slc2a1*	Astrocytes; neurons Endothelial cells	Astrocyte transporter (45 kDa); Blood brain barrier transporter (55 kDa) Deficiency in De Vivo disease Decreased expression in Alzheimer's disease, traumatic brain injury, ischemia	[8–10,12,59–62]
GLUT3	*Slc2a3*	Neurons; neurophil	Decreased expression in Alzheimer's disease, traumatic brain injury, ischemia Reduced expression in rat forebrain after chronic stress exposure	[9,10,12,13,16,59]
GLUT4	*Slc2a4*	Neurons; somatodendritic	Insulin sensitive glucose transporter Impaired insulin-induced translocation after corticosterone treatment in rat hippocampus	[5,7,63] [17,64]
GLUT5	*Slc2a5*	Microglia	Microglial localization; involvement in inflammatory response	[7,65]
GLUT8	*Slc2a8*	Neurons; somatodendritic	Depletion results in increased neurogenesis, hyperactivity, and reduction in risk assessment behaviors without memory alteration in mice	[66,67]

functions of these GLUTs and their involvement in neurologic and stress-related disorders are outlined in Table 1. Few studies have examined multiple cerebral GLUTs in the same animal, and little attention has been given to the influences of sex, age, or stress exposure on the expression of these essential transporters. (See Tables 2–4.)

Rapid developmental periods, such as puberty, are characterized by both increased cerebral plasticity and augmented demand for metabolic energy. Therefore, changes in GLUT during the adolescent developmental period could lead to longstanding changes in cerebral metabolism and neuron function. Although there is some evidence that stress can modify cerebral GLUT in the adult rat [16,19,20], it is unknown whether stress during adolescence can alter cerebral GLUT expression, or whether stress affects GLUT expression similarly in males and females. Thus, the present study examined the interactions among sex, age, and chronic mixed modality stress on cerebral GLUT mRNA abundance. Because of the dearth of information available regarding cerebral GLUTs during adolescent development and minimal information regarding sex differences, we initially assessed expression of the five GLUT subtypes outlined in four brain regions in male and female adolescent and adult rats. We hypothesized that developmental sex differences in GLUT mRNA abundance would exist in brain regions involved in regulating hormonal secretion and affective behavior, particularly the hypothalamus. In addition, given the substantial energetic demands during adolescence and previous demonstrations of the adverse effects of adolescent stress [21,22], we determined the extent to which chronic stress altered GLUT mRNA abundance in males and females in both adolescence and adulthood. Collectively, our data demonstrate that cerebral GLUTs are expressed differentially based on brain region, sex, age, and exposure to stress. Furthermore, the timing of the stress exposure interacts with sex to determine the changes in GLUT mRNA abundance. This study is one of the first reports of multiple cerebral GLUTs with attention to age, sex, environment, and brain region. Regional changes in GLUT expression may impact brain function and thereby behavior.

2. Materials and methods

2.1. Animals

Timed pregnant Wistar rats (Charles River, Wilmington, MA) arrived on gestational day 12. This timing of shipping stress is not associated with changes in developmental outcomes [23], whereas shipping stress during puberty can have enduring effects on behavior [24,25]. Rats were housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20 °C–23 °C). Rodent diet 5001 chow (Purina Mills, Richmond, IN) and water were maintained ad libitum throughout the study. Three days after birth litters were culled and weaned on postnatal day (PND) 23. On PND 36, rats were assigned groups and housed in same-sex pairs. Littermates were assigned to adolescent or adult control or stress groups with no more than two pups per litter assigned to each group. All groups contained between 10 and 12 rats. All animal experiments were approved by Emory University's Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering and to reduce the number of rats used.

2.2. Experimental design

For the cohort of rats used to assess regional differences in cerebral GLUT expression as a function of age and sex, rats were maintained in standard colony conditions and adolescent animals were euthanized

Table 2
Summary of qRT-PCR analysis addressing factors of age and sex.

Cerebral location	Sex	Age	*Slc2a1*	*Slc2a3*	*Slc2a4*	*Slc2a5*	*Slc2a8*
Hippocampus	Male	Adolescent	1.00 ± 0.18	1.00 ± 0.10	1.00 ± 0.12	1.00 ± 0.09	1.00 ± 0.09
		Adult	0.96 ± 0.11	0.95 ± 0.09	0.74 ± 0.09	1.20 ± 0.07	0.83 ± 0.10
	Female	Adolescent	1.12 ± 0.11	1.24 ± 0.12	0.98 ± 0.09	1.07 ± 0.06	1.17 ± 0.09
		Adult	0.89 ± 0.15	0.87 ± 0.08	0.82 ± 0.09	1.12 ± 0.09	0.89 ± 0.08
Hypothalamus	Male	Adolescent	1.00 ± 0.37	1.00 ± 0.19	1.00 ± 0.34	1.00 ± 0.15	1.00 ± 0.23
		Adult	2.13 ± 0.26*	0.98 ± 0.06	2.02 ± 0.16*	1.10 ± 0.11	1.39 ± 0.06
	Female	Adolescent	2.13 ± 0.19*	1.17 ± 0.07	2.11 ± 0.17*	0.88 ± 0.22	1.30 ± 0.09
		Adult	2.14 ± 0.19*	1.25 ± 0.12	1.95 ± 0.20*	0.91 ± 0.10	1.40 ± 0.12
Amygdala	Male	Adolescent	1.00 ± 0.13	1.00 ± 0.07	1.00 ± 0.14	1.00 ± 0.15	1.00 ± 0.11
		Adult	0.98 ± 0.07	1.17 ± 0.09	1.26 ± 0.22	1.33 ± 0.11	1.05 ± 0.12
	Female	Adolescent	1.00 ± 0.08	1.11 ± 0.09	1.22 ± 0.18	1.14 ± 0.15	1.02 ± 0.15
		Adult	1.00 ± 0.05	1.00 ± .09	1.33 ± 0.12	1.28 ± 0.12	1.09 ± 0.05
Prefrontal Cortex	Male	Adolescent	1.00 ± 0.11	1.00 ± 0.04	1.00 ± 0.11	1.00 ± 0.06	1.00 ± 0.10
		Adult	1.03 ± 0.07	0.99 ± 0.07	1.09 ± 0.11	0.88 ± 0.06	1.01 ± 0.07
	Female	Adolescent	1.09 ± 0.05	1.08 ± 0.06	1.01 ± 0.08	0.97 ± 0.08	1.14 ± 0.09
		Adult	0.9 ± 0.11	0.91 ± 0.09	0.74 ± 0.11	0.90 ± 0.05	1.18 ± 0.10

Data shown represent mean fold change ($2^{-\Delta CT}$) ± SEM. Significant main effects are shown only in corresponding graphs. Tukey's post hoc analysis is represented in the table by an asterisk (*), which indicates an increase in gene expression relative to the male adolescent cohort.