Postnatal hypoxic-ischemic brain injury alters mechanisms mediating neuronal glucose transport

Ann Zovein, Judy Flowers-Ziegler, Shanthie Thamotharan, Don Shin, Raman Sankar, Khoi Nguyen, Sanjiv Gambhir, and Sherin U. Devaskar. Postnatal hypoxic-ischemic brain injury alters mechanisms mediating neuronal glucose transport. Am J Physiol Regul Integr Comp Physiol 286: R273–R282, 2004. First published October 2, 2003; 10.1152/ajpregu.00160.2003.—We examined the effect of hypoxic ischemia and hypoxia vs. normoxia on postnatal murine brain substrate transporter concentrations and function. We detected a transient increase in the neuronal brain glucose transporter isoform (GLUT-3) in response to hypoxic ischemia after 4 h of reoxygenation. This increase was associated with no change in GLUT-1 (blood-brain barrier/glial isoform), monocarboxylate transporter isoforms 1 and 2, synapsin 1 (neuronal marker), or Bax (proapoptotic protein) but with a modest increase in Bcl-2 (antiapoptotic mitochondrial protein) protein concentrations. At 24 h of reoxygenation, the increase in GLUT-3 disappeared but was associated with a decline in Bcl-2 protein concentrations and the Bcl2:Bax ratio, an increase in caspase-3 enzyme activity (apoptotic effector enzyme), and extensive DNA fragmentation, which persisted later in time (48 h) only in the hippocampus. Hypoxia alone in the absence of ischemia was associated with a transient but modest increase in GLUT-3 and synapsin 1 protein concentrations, which did not cause significant apoptosis and/or necrosis. Assessment of glucose transporter function by 2-deoxyglucose (2-DG) uptake using two distinct techniques, namely positron emission tomography (PET) and the modified Sokoloff method, revealed a discrepancy due to glucose uptake by extracranial Harderian glands that masked the accurate detection of intracranial brain glucose uptake by PET scanning. The modified Sokoloff method assessing 2-DG uptake revealed that the transient increase in GLUT-3 was critical in protecting against a decline in brain glucose uptake. We conclude that hypoxic-ischemic brain injury is associated with transient compensatory changes targeted at protecting glucose delivery to fuel cellular energy metabolism, which then may delay the processes of apoptosis and cell necrosis.

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Hypoglycemia has traditionally been shown to increase cellular glucose transport and metabolism, with specific increases in brain facilitative glucose transporter isoforms GLUT-1 and GLUT-3 (31, 40). However, hypoxic-ischemic brain injury at various ages has demonstrated a differing effect on GLUT-1 and/or GLUT-3 expression and concentrations (12, 27, 28, 42, 44, 52, 56, 57). These previously reported age-dependent discrepancies may be related to the degree of reliance on glucose by neurons that are adversely affected by hypoxic ischemia. Thus, while the adult brain is completely reliant on glucose as a substrate for oxidative metabolism, during the suckling phase, other substrates such as lactate and ketones are known to compensate for a relative deficiency in glucose that hinders fueling the cellular oxidative metabolism (15). Monocarboxylates, such as acetocetate and β-hydroxybutyrate, serve as secondary brain substrates when glucose is insufficient (35). Their transporter isoforms (of a family numbered monocarboxylate transporter (MCT)-1, -2, -3, -4, -5, -6, -7, -8, -9) mediate the transport of these substrates into the brain, and their expression is influenced by a variety of factors, including hypoxia and ischemia.

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-7, -8, and -9] are correlates to the glucose transporters such that MCT-1 isoform similar to GLUT-1 is located in the blood-brain barrier and astrocytes, while MCT-2 like GLUT-3 is predominantly a neuronal isoform (17, 24, 37).

We hypothesized that postnatal hypoxic ischemia will alter brain GLUTs and MCTs in an attempt to maintain the energy production necessary to fuel brain cellular oxidative metabolism and thereby transiently protect against cellular apoptosis. To test this hypothesis, we undertook the present study in postnatal day 11 (P11)–P13 mice employing nitrogen breathing and unilateral carotid artery ligation to effect hypoxic-ischemic brain injury. In addition, we assessed brain 2-DG uptake by a novel micro-postion tomography (PET) noninvasive assessment of \(^{18}\)F-labeled deoxyglucose uptake and compared it with the classical method of Sokoloff with modifications (49) as the previously described “gold standard.”

**MATERIALS AND METHODS**

**Animals**

Balb/c mice were purchased from Jackson Laboratories and housed in individual cages under 12:12-h light-dark cycles with free access to water and standard chow. The care and use of animals followed the guidelines established by the National Institutes of Health and were approved by the Magee-Womens Research Institutional Committee for Animal Care and Use and the UCLA Animal Research Committee.

**Experimental Model**

Male and female mice were mated, and the pregnant animals were allowed to deliver. The postnatal animals at P11 were chosen as this period occurred before the glucose transporter surge previously reported by us (20) and coincided with the peak period of synaptogenesis (30, 43, 50). These animals were subjected to unilateral carotid artery ligation under inhalational halothane or isoflurane anesthesia. After surgery, the animals were allowed to recover for 1 h, after which they were exposed to 8% oxygen for 2 h at 37°C (Fig. 1). This was achieved by bleeding nitrogen into room air. Controls were included that underwent sham surgery without carotid artery ligation and were exposed to room air (Fig. 1). A third group of animals underwent unilateral carotid artery ligation with subsequent exposure to room air to serve as a comparative ischemic model. After the hypoxic/normoxic exposure period, the ipsilateral (hypoxic-ischemic; ischemic) and contralateral (hypoxic; normoxic) hemispheres were collected separately, at varying periods of reoxygenation (4, 24, and 48 h), snap-frozen in liquid nitrogen, and stored at \(-70^\circ\)C until further analysis. In the case of the enzymatic assay, brain tissue was freshly assayed.

**Protein Assay**

Brain tissue was homogenized using a Tekmar tissuehomogenizer (Cincinnati, OH) and then sonicated (60 Sonic Disembrator, Fisher Scientific, Pittsburgh, PA) using two 50-s cycles of 5–7 W to ensure adequate homogenization of tissue. The resultant suspension was centrifuged at 10,000 g at 4°C for 10 min, and the supernatant was saved for Western blot analysis. Some of the normoxic control brains were subjected to subfractionation to isolate the plasma membrane-enriched fraction from the low-density microsomes as previously described (48, 51). Protein content was determined by the Bio-Rad protein dye binding assay (Bio-Rad, Richmond, CA) (4). Predetermined optimal protein concentrations of the homogenates (50 μg), plasma membranes, and low-density microsomes (5 μg) were solubilized in Laemmli buffer. The samples were then separated by a 10 or 14% discontinuous SDS-PAGE, the proteins were transferred to nitrocellulose filters by electroblotting, and the filters were subjected to Western blot analysis as described previously (20, 45, 47). Equality of loading and efficiency of transfer were assessed by Coomassie blue staining of the gel and the transfer of prestained markers. The nitrocellulose filters were incubated overnight at 4°C in 5% nonfat dry milk in PBS to decrease nonspecific binding of the antibody. This was followed by incubation for 1 h at 4°C with the primary antibodies. The primary antibodies used were affinity-purified rabbit anti-rat GLUT-1 (2 μg/ml at a 1:2,000 dilution) and anti-mouse GLUT-3 (3 μg/ml at a 1:7 dilution) that were produced, purified, and characterized by us (39). The secondary antibody consisted of a peroxidase-linked donkey anti-rabbit IgG (Sigma Chemical, St. Louis, MO) that was incubated for 1 h at room temperature. The blots used to detect MCT-1 and MCT-2 proteins were blocked in the Sea block solution (Pierce, Rockford, IL) at 1:7 dilution for 1 h at room temperature, before the primary antibody incubations. The primary antibodies consisted of affinity-purified chicken anti-rat MCT-1 and MCT-2 IgGs each at a 1:5,000 dilution that were produced by Dr. S. J. Korsemyer, Howard Hughes Medical Institute, Washington University, St. Louis, MO) that were incubated for 18 h at 4°C. The secondary antibody was a rabbit anti-chicken IgG that was linked to horseradish peroxidase conjugate (Sigma Chemical) that was incubated at 1:10,000 dilution for 1 h at room temperature.

Subsequently, the peroxidase-linked IgG-treated filters were extensively washed and exposed to a chemiluminescence reagent (Amer sham Life Science, Little Chalfont, Buckinghamshire, UK). The chemiluminescence was captured on X-ray film over varying periods (1–5 min) to determine the optimal exposure time. The GLUT or MCT protein band concentrations were assessed by densitometry of the X-ray films. The optical density was corrected for interlane loading using an internal control (45), and the data are expressed as a percentage of the mean of the respective normoxic control group.

**Mitochondrial Proteins Underlying the Apoptotic Process**

To assess the extent of apoptosis, we examined the concentrations of the cell death promoter Bax and cell death protector Bcl-2 proteins. In addition, we quantified the activity of the effector enzyme caspase-3 as well. Bcl-2 and Bax protein levels were assessed by Western blot analysis as described above. The primary antibodies consisted of a hamster anti-mouse Bcl-2 IgG (4 μg/ml) (Phar mingen, San Diego, CA) and a rabbit anti-mouse Bax IgG (13 μg/ml; a gift from Dr. S. J. Kor semyer, Howard Hughes Medical Institute, Washington University, St. Louis, MO) that were incubated for 1 h at room temperature. Jurkat lymphoma cells exposed to UV light served as the positive control for Bax. Appropriate secondary antibodies were employed that consisted of peroxidase-linked rabbit anti-hamster for...
Bcl-2 and donkey anti-rabbit IgG for Bax (1:2,500 dilution over 1 h at room temperature).

**Synapsin I Protein: A Neuronal Marker**

To detect synapsin I, rabbit anti-mouse synapsin I antibody (Chemicon International) was used at a 1:1,000 dilution as the primary antibody in Western blot analysis.

**Caspase-3 Enzyme Activity**

Caspase-3 enzyme activity was assessed by using the Apoalert CPP 32/caspase-3 assay (Clontech, Palo Alto, CA). The Apoalert caspase-3 colorimetric assay kit detects the proteolytic cleavage of the chromophore p-nitroanilide (pNA) from a DEVD tetrapeptide sequence. Frozen brain tissue was homogenized in a chilled lysis buffer provided in the kit, incubated over ice for 15 min, and centrifuged at 3,000 rpm at 4°C for 20 min to remove cellular debris. The supernatant was recentrifuged twice, at 14,000 rpm at 4°C for 15 min each. Fifty microliters of the sample was incubated with 1 mM conjugated substrate at 37°C for 1 h and 15 min. In addition to noninduced tissue samples and an induced tissue sample inhibited by the caspase-3 inhibitor DEVD-fmk that served as negative controls, Jurkat lymphoma cells exposed to UV light served as the positive control. Absorbances were read at 405 nm. The standard curve consisted of varying concentrations of the CPP32 chromogenic substrate.

**TUNEL Staining of Brain Sections**

Mice were euthanized with pentobarbital sodium (100 mg/kg) and perfused with PBS (pH 7.4) followed by 4% paraformaldehyde. The brains were removed from the cranium and post-fixed for 4-6 h and then cryopreserved in 30% sucrose. Rostrocaudal coronal and sagittal brain sections (30 μm) were mounted onto slides as previously described (45). TdT-mediated dUTP nick end labeling (TUNEL) was performed using a commercially available kit (NeuroTACS II, Trevigen, Gaithersburg, MD). Briefly, sections were incubated with proteinase K followed by terminal deoxynucleotidyl transferase and biotinylated-16-dUTP. Endogenous peroxidase was quenched with 30% hydrogen peroxide (Sigma Chemical). The reaction product was visualized with diaminobenzidine (Sigma Chemical) and counter-stained with 1% methyl green (Sigma Chemical).

**2-DG Uptake Studies**

**Modified Sokoloff method.** Cerebral glucose uptake (CGU), which includes glucose transport and phosphorylation, was measured as previously described by us (20) using 2-deoxy-[14C]glucose (2-[14C]DG) as the tracer. The study was performed at 4, 24, and 48 h of reoxygenation in all experimental groups. Animals received an intraperitoneal injection of 1 μCi/g body wt of the tracer, and at 90 min (optimal time) post injection, brains were quickly rinsed at 4°C and immediately placed in liquid nitrogen to stop intermediary metabolism. The brains were weighed and powdered under liquid nitrogen. Perchloric acid brain extracts were prepared and neutralized with 2 M KHCO3 to a pH of 6.8. KClO4 was then removed by centrifugation at 5,000 g. From the final supernatant, 10 μl was used to measure radioactivity by a scintillation spectrophotometer, whereas 20 μl was used to determine glucose concentration by the glucose oxidase method (20). Simultaneously, blood was also collected on ice and centrifuged at 3,000 g for 5 min to separate plasma from the red blood cells, and glucose concentrations were measured as described above, allowing derivation of the specific activity of glucose in circulation and brain tissue at the 90-min time point. CGU was calculated in micro- moles per 100 g per minute and expressed as a percentage of control using the following formula:

\[
\frac{\text{cerebral } [\text{14C}] \text{deoxyglucose (disintegrations min}^{-1} \text{g}^{-1})}{\text{cerebral glucose concentration (μmol/g)}} \times 100
\]

**PET assessment of 18F-labeled 2-DG uptake.** Initially a developmental profile was established to validate this methodology in suckling mice. Ninety minutes before whole body micro-PET (8) scanning, 18F-deoxyglucose (18FDG) (100–200 μCi) was intraperitoneally injected in mice at different developmental ages (P7, P11, P13, P15, P21, and P60), and the P11 mice were subjected to hypoxic ischemia, or normoxia with sham surgery, as described above. The mice were anesthetized immediately before scanning with 1–2 mg/kg of xylazine (20 mg/ml) and 5–10 mg/kg of ketamine (100 mg/ml) intraperitoneally. All micro-PET with effective resolution of ~2 mm3 was used to acquire scan data that were reconstructed via the maximum a posteriori algorithm previously described (6). 18FDG concentrations were measured by using region of interest (ROI) analysis over averaged multiple brain coronal sections (29). ROIs were drawn both manually and using a circular tool enclosing a constant area of each hemicranial field within each postnatal group. After correcting for the amount of radioactivity injected and the projected decay, 18FDG concentrations were expressed as a percentage of total activity per gram of tissue (assuming tissue density of 1 ml = 1 g) as previously described (29). To ensure that the 18FDG activity was emanating from the brain and not cranial or extracranial tissues, in certain animals the brains were removed from the cranium, and both the isolated brain and the cranium without the brain were separately subjected to micro-PET.

**Data Analysis**

All results are expressed as means ± SE. Differences when comparing more than two groups or time points were determined by one-way ANOVA followed by the post hoc Newman Keuls test. When comparing two groups, the Wilcoxon rank test (nonparametric) was used.

**RESULTS**

Figure 2A demonstrates brain GLUT-1 and GLUT-3 in hypoxic-ischemic cortexes vs. normoxic controls and compared with the respective mitochondrial proteins, namely Bcl-2 and Bax proteins. There were no differences in GLUT-1 or GLUT-3 between P11, P12, and P13 in the normoxic controls; hence all age-matched controls have been represented as 100%. Hypoxic ischemia led to a 2.8-fold increase in brain GLUT-3 during the suckling phase with no statistically significant difference in GLUT-1 at 4 h after reoxygenation. At this same time point, a 50% increase in brain Bcl-2 concentrations is noted with no change in Bax. Twenty four hours after reoxygenation, both GLUT-1 and GLUT-3 are no different in the hypoxic-ischemic group compared with the normoxic group, while Bcl-2 declined by 40% with no change in Bax. However, at 48 h after reoxygenation, all four proteins, namely GLUT-1, GLUT-3, Bcl-2, and Bax, decreased. In contrast, in the hemisphere exposed to hypoxia alone with 4 h of reoxygenation, only a 60% increase in brain GLUT-3 concentrations with no change in GLUT-1, Bcl-2, or Bax was noted. While GLUT-3 concentrations were similar to that found in the normoxic hemisphere at 24 h, no other changes were observed both at 24 and 48 h of reoxygenation (Fig. 2B). Figure 2, C and D, depicts the synapsin I concentrations along with GLUT-3 levels. As can be observed, hypoxic ischemia was associated with no change in synapsin I at 4 or 24 h; however, a decline consistent with the GLUT-3 decline was observed at 48 h.
Secondary to hypoxia alone, however, a 50% increase in synapsin I was noted at 4 h of reoxygenation. Overall, ischemia alone was no different from the normoxic group (data not shown).

Figure 2E depicts the subcellular distribution of GLUT-3, which is predominantly distributed to the cellular plasma membranes. Figure 2F demonstrates the brain caspase-3 enzyme activity. While no change was evident with exposure to hypoxia alone compared with the normoxia group, a sharp 2.5-fold increase was observed in caspase-3 enzyme activity at 24 h in response to hypoxic-ischemic brain injury, this increase reverting to normal by 48 h.

Figure 3A demonstrates the TUNEL labeling of normoxic (Fig. 3Aa), ischemic (Fig. 3Ab), and hypoxic (Fig. 3Ac) vs. hypoxic-ischemic (Fig. 3Ad, Ac, and Af) brain sections depicting the hippocampal region obtained at 24 and 48 h of reoxygenation. Extensive DNA fragmentation is observed in the granule cells of the dentate gyri (Fig. 3Ad) and the CA1 region (Fig. 3Ac) of the hippocampus at 24 h (Fig. 3Ac, Ad and Af) that persists at 48 h (Fig. 3Aa) as well. Figure 3B demonstrates the TUNEL labeling of normoxic (Fig. 3Ba), ischemic (Fig. 3Bb), and hypoxic (Fig. 3Bc) cortical hemispheres vs. the hypoxic-ischemic cortical hemisphere with reoxygenation for 24 h (Fig. 3Bd), and hypoxic (Fig. 3Be) and hypoxic-ischemic hemispheres with reoxygenation over 48 h (Fig. 3Bf). While normoxia, ischemia, and hypoxia demonstrated few scattered TUNEL-positive cells, 24 h of hypoxic ischemia (Fig. 3Bb) led to extensive TUNEL labeling that diminished at 48 h (Fig. 3Bf).

Figure 4 demonstrates the changes in MCT-1 and MCT-2 protein concentrations. Figure 4A reveals no change in both MCT-1 and MCT-2 at 4 and 24 h in response to hypoxic ischemia. However, at 48 h, a 30–40% decline in both the MCT isoforms was detected. Exposure to hypoxia alone led to a mild decrease only in MCT-1 at 24 and 48 h of reoxygenation with no change in the neuronal MCT-2 (Fig. 4B).

Figure 5, A and B, demonstrates the actual representative scans of the whole body (Fig. 5A) and cranial regions at various developmental stages (Fig. 5B). Figure 5C depicts the corresponding quantification of the micro-PET assessment of brain 18FDG uptake at differing developmental ages. A peak increase in brain glucose uptake is detected at postnatal age P15 with a decline by P21 and P60 compared with earlier
stages ranging from P7 to P13 (Fig. 5C). Figure 6A demonstrates the quantification of brain $^{18}$FDG uptake as assessed by micro-PET in response to hypoxic ischemia compared with the normoxic control. This method demonstrates that CGU trends toward an increase to a maximum at 48 h postreoxygenation after a hypoxic-ischemic injury compared with normoxic controls. In contrast, Fig. 6B demonstrates CGU as assessed by a modified Sokoloff method employing 2-$[^{14}$C]$DG as the tracer.
No change in CGU was observed secondary to hypoxic ischemia and hypoxia alone compared with the normoxic control on reoxygenation for 4 or 24 h. Forty eight hours of reoxygenation led to a 70% decline in CGU, reflecting the decline in brain GLUT-3 concentrations (Fig. 2A).

Figure 7 demonstrates micro-PET scans of the intact brain within the cranium (Fig. 7, A and D), the cranium alone without the brain (Fig. 7, B and E), and the brain only (Fig. 7, C and F). 18FDG uptake was noted in the cranium and soft tissues that lacked the brain (Fig. 7, B and E). This uptake was by 10.220.32.246 on August 15, 2017 http://ajpregu.physiology.org/ Downloaded from

Fig. 4. Quantification of Western blot analysis assessing brain MCT-1 and MCT-2 protein concentrations at 4, 24, and 48 h of reoxygenation after hypoxic-ischemic brain injury effected by unilateral carotid artery ligation on the ipsilateral cortex (A) or hypoxia on the contralateral cortex (B). The horizontal dotted line represents the normoxic sham-operated respective time-matched controls; n = 6 per group and time point. Insets: representative Western blots.

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DISCUSSION

We have demonstrated in the P11-P13 suckling mouse that hypoxic ischemia and hypoxic injury were associated with certain transient compensatory changes that are distinct from those previously reported in the adult (52) or an earlier developmental stage of the fetal/postnatal rat (42, 56). These differences may stem from the fact that a time point when synaptogenesis (30, 43, 50) begins was chosen for the present study. This time point in brain development corresponds to a term human infant (14, 54). Furthermore, the P11-P13 time point represented a period in development before peak expression of brain GLUT-3 concentrations in the mouse brain (20) is observed. Despite some controversy (28), previous studies in the adult rat (52) and juvenile rabbit (12) demonstrated an increase in brain GLUT-3 concentrations in response to focal ischemia or global hypoxic ischemia, respectively. However, previous fetal and postnatal studies (P7) in the rat revealed contradictory results with respect to a change in brain GLUT-3 concentrations in response to focal ischemia or global hypoxic ischemia, respectively. However, previous fetal and postnatal studies (P7) in the rat revealed contradictory results with respect to a change in brain GLUT-3 concentrations in response to focal ischemia or global hypoxic ischemia, respectively. However, previous fetal and postnatal studies (P7) in the rat revealed contradictory results with respect to a change in brain GLUT-3 concentrations in response to focal ischemia or global hypoxic ischemia, respectively.

The postnatal rat investigations undertaken at P7 of age demonstrated major changes in brain GLUT-1 protein concentrations with minimal changes in GLUT-3 (56, 57). This is perhaps related to the time in development when brain GLUT-3 expression is relatively low and the process of synaptogenesis may or may not have begun. The establishment of synaptogenesis is important when investigating GLUT-3, because this protein is spatially distributed to neuronal processes that constitute the synapses (16). Furthermore, most of these studies focused primarily on GLUT-3 mRNA rather than on the protein. For example, the hypoxic studies in the fetus involved semiquantitative RT-PCR assays (42), while the postnatal rat studies demonstrated differences in spatial distribution...
in neuronal GLUT-3 could serve as a marker for a window in time when protective interventions focused on decreasing the metabolic demand by the brain can prevent the cascade of events leading to neuronal death and finally neuronal loss. Similarly, the hypoxia-induced transient increase in neuronal GLUT-3 can be the speculated mechanism involved in the previously reported hypoxia-induced preconditioning that is neuroprotective early in development during exposure to a second hypoxic-ischemic insult.

In an attempt to assess the corresponding function, namely brain glucose uptake, we employed the classical method of Sokoloff with modifications as the previously described "gold standard" (20, 49) and attempted to validate the brain 18FDG uptake as assessed by micro-PET scanning in the mouse. This was important, because brain imaging in humans has proved to be highly useful in determining structural and metabolic aberrations (9). Particularly PET measures of 18FDG uptake have served as a surrogate for neuronal activity (36). Hence in both normal and abnormal human developmental stages, PET scans have revealed vitally important information regarding brain metabolism and thereby neuronal activity. The recent advent of the micro-PET with a greater resolution has made this possible in small animals such as rats and mice (8). The power of micro-PET is that it can assess brain glucose uptake in vivo while the animal is sedated, thus allowing time-dependent longitudinal measurements in the same animal either through development or reoxygenation after the initial hypoxic-ischemic insult. Such assessments in both wild-type and genetically manipulated mice could potentially prove to be a powerful tool with wide application. Thus brain 18FDG uptake assessed by micro-PET scanning would potentially allow in vivo quantification of neuronal activity in the developing mouse and could parallel in vitro measurements of glucose transporter expression. This validation of the micro-PET technique could perhaps overcome the future need for assessing glucose transporter function, namely glucose transport (uptake) in vitro, which cannot be undertaken in the same animal longitudinally.

Although we observed cranial 18FDG uptake at all ages examined with a peak at P15, this peak in uptake failed to correspond with the peak increase at P35 noted when radiolabeled 2-14C DG uptake was noted predominantly in extracranial structures by micro-PET scanning compared with the isolated brain. Thus the extracranial structures masked the brain within the cranium, making accurate noninvasive assessment of brain glucose uptake difficult. The discrepancy between the two methodologies was related to the fact that glucose uptake was noted predominantly in extracranial structures by micro-PET scanning compared with the isolated brain. Thus the extracranial structures masked the brain within the cranium, making accurate noninvasive assessment of brain glucose uptake difficult. The power of micro-PET scanning compared with the isolated brain. Thus the extracranial structures masked the brain within the cranium, making accurate noninvasive assessment of brain glucose uptake difficult. The brain glucose uptake separate from extracranial structures is accurately detected only when the brain is removed from the cranium and assessed separately by PET scanning. Thus our studies revealed that micro-PET assessment of 18FDG uptake by the developing mouse has significant limitations that lead to inaccuracies in assessing brain glucose metabolism and neuronal activity in vivo.

Similar to the methodological discrepancy observed during development, a discrepancy was also noted in response to hypoxic-ischemic brain injury. While 2-14C DG uptake decreased at 48 h of reoxygenation after a hypoxic-ischemic
injury, thereby paralleling the changes observed with brain GLUT-3 concentrations, the micro-PET method assessed brain 18FDG uptake did not reflect these changes longitudinally. The latter methodology reflects uptake predominantly by the extracranial Harderian glands. This observation in mice is similar to a previous report in rats (23, 59), where the predominant glucose uptake is noted in the extracranial Harderian glands, which in turn mask the assessment of brain glucose uptake with any accuracy. Hence, both in rats (23, 59) and mice, PET scanning at the current resolution does not allow for noninvasive measurement of brain glucose uptake at all developmental stages, limiting its usefulness as a tool in these animal species. The micro-PET used in our current study is the highest resolution small animal PET imaging system currently available. Perhaps the next-generation systems approaching a resolution of ~1 mm may prove to distinguish between the brain and Harderian glands (8, 10). Thus previous studies in either the developing or adult rat or mouse that have assessed brain 18FDG uptake by currently available micro-PET scanners should be cautiously interpreted and reevaluated in light of our present findings.

Contrary to our hypothesis, we did not find any change in neuronal monocarboxylate transporter (MCT-2) concentrations due to hypoxia or hypoxic ischemia during the early compensatory phase. Although this does not rule out an increase in monocarboxylate transport, no compensatory change similar to that of the glucose transporter (GLUT-3) was evident.

We conclude that hypoxic-ischemic brain injury during the suckling phase of development, particularly when synaptogenesis peaks, leads to a transient compensatory increase in neuronal GLUT-3. We speculate that this increase may serve to protect glucose uptake and thereby cellular energy stores. This possible compensation, albeit temporary, may either precondition or protect the brain against cellular apoptosis and death, providing a window in time during which introduction of opportune interventions targeted at decreased cellular metabolism can potentially reverse the trajectory set in motion by the initial insult. Our current studies in wild-type mice also set the stage for future investigations in mice carrying genetically modified glucose transporter isoforms and the impact of such modifications on the hypoxic-ischemia-induced injurious cascade of events in neurons. In addition, we have demonstrated that micro-PET noninvasive assessment of brain 18FDG uptake at the currently available resolution is not accurate in the developing mouse and does not parallel in vitro assessments of brain glucose uptake. This inaccuracy is due to 18FDG uptake by extracranial Harderian glands that overlap key areas of the brain, masking the actual brain 18FDG uptake. This poses a distinct disadvantage for undertaking developmental studies in mice that have been either genetically manipulated or subjected to injury as in our present study to examine the function of certain brain-specific genes.

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