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ACUTE HYPOGLYCEMIA RESULTS IN REDUCED CORTICAL NEURONAL INJURY IN THE DEVELOPING IUGR RAT

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Abstract

Background—Hypoglycemia (HG) is common in IUGR neonates. In normally grown (NG) neonatal rats, acute HG causes neuronal injury in the brain, cerebral cortex more vulnerable than the hippocampus (HPC). We hypothesized that the IUGR brain is less vulnerable to hypoglycemia-induced injury while preserving the regional variation in vulnerability.

Methods—We induced IUGR via bilateral uterine artery ligation on gestational day 19 (term 22d) rats. On postnatal day 14, insulin-induced HG of equivalent severity and duration (blood glucose <40mg/dl for 240 min) was produced in IUGR and NG (IUGR/HG and NG/HG) groups. Neuronal injury in the cortex and HPC was quantified 6-72 hr later using Fluoro-Jade B (FJB) histochemistry. The mRNA expression of monocarboxylate transporters, MCT1 and MCT2, and glucose transporters, GLUT1, and GLUT3 was determined using qPCR.

Results—There were fewer FJB+ cells in the cortex of IUGR/HG; no difference was observed in FJB+ cells in HPC. Core body temperature was lower in IUGR/HG compared with NG/HG. MCT2 expression was increased in the IUGR cortex.

Conclusion—Hypoglycemia-induced neuronal injury is decreased in the cortex of the developing IUGR brain. Adaptations including systemic hypothermia and enhanced delivery of alternative substrates via MCT2 might protect against hypoglycemia-induced neuronal injury in IUGR.

Introduction

Infants born with intrauterine growth restriction (IUGR) as a result of placental insufficiency are at an increased risk for hypoglycemia in the neonatal period due to decreased glycogen stores, increased brain to body weight ratio, and an increased sensitivity to insulin (1,2).

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Whether or not IUGR infants are at an increased risk for hypoglycemic brain injury is unknown. Neurodevelopmental deficits have been described in school-aged children who were born IUGR with respect to learning and memory, cognition, attention and behavior, suggesting functional deficits in the cerebral cortex and hippocampus (3-6). The cerebral cortex and hippocampus are particularly vulnerable to hypoglycemic brain injury in both humans and rodents (7-9) with the cortex being more susceptible to injury than the hippocampus in the developing rat brain (7,10,11).

Animal models of IUGR describe altered carbohydrate and energy metabolism, blunted glucoregulation, and ineffective ketone body response to hypoglycemia (3,12-14) suggesting that the IUGR brain may be at greater risk of injury during periods of low glucose availability. Conversely, there is evidence that fetal metabolic adaptations as a result of IUGR might be protective during periods of perinatal or postnatal nutrient insufficiency by prioritizing limited resources to the brain over less metabolically active peripheral tissues. This is supported by evidence of increased glucose transport into the brain versus the lung, liver, skeletal muscle and heart in the rat and sheep models of IUGR (1,15,16). Glucose enters the brain down a concentration gradient via the Glut 1 facilitative transport protein on the blood brain barrier (BBB) and is transported into neurons via Glut 3. Monocarboxylate transporters (MCT) are found on the plasma and mitochondrial membrane of a variety of tissues, including the brain, and transport lactate, pyruvate and ketone bodies. MCT 1 is found on the BBB, neurons, glia, astrocytes and retina, while MCT 2 is expressed almost exclusively in post-synaptic neurons, having a higher affinity for substrates than MCT 1 (17) and is essential for cerebral metabolism by coupling astrocyte and neuronal metabolism via the lactatepyruvate shuttle (17). This preferential substrate transport along with increased cerebral blood flow and more efficient utilization of non-glucose substrates (i.e., lactate, ketone bodies and amino acids) have been proposed as mechanisms behind the “brain-sparing” effect seen with asymmetric IUGR (1,15,16). However, given the neurologic deficits described above, it is likely that the entire brain is not “spared” and that some regions such as the hippocampus or cerebral cortex may be more vulnerable to injury than others.

The objective of this study was to determine the vulnerability of the cerebral cortex and hippocampus of the developing IUGR rat brain to injury during acute insulin-induced hypoglycemia (AHG). Our purpose was to understand the role of fetal metabolic adaptations and not to model the human scenario of hypoglycemia in the IUGR neonate. We hypothesized that due to fetal metabolic adaptations the developing IUGR brain will be less vulnerable to injury from AHG than normally grown controls and that the regional distribution of injury will be preserved.

We determined neuronal injury after 4 hours of AHG in the hippocampus and cortex of postnatal day (P) 14 IUGR and normally grown (NG) pups using Fluoro-Jade B (FJB) histochemistry. FJB stains for dead or stressed cells and has been previously used to quantify hypoglycemia-induced neuronal injury in developing and adult rats (7,10,18). We studied the effects in P14 pups because of their neurodevelopmental similarities with the human term infant (19). We also measured fasting ß-hydroxybutyrate concentrations in

blood and the transcript expression of glucose and monocarboxylate transporters (Glut 1, Glut 3, MCT 1 and MCT 2) in the cortex and hippocampus.

Results

Animal weights

IUGR was induced via bilateral uterine artery ligation on gestational day (G) 19 as previously described (16). Body weights of the IUGR pups were 27% lower than normally grown (NG) pups (IUGR 4.5±0.01g vs. NG 5.9±0.01g $P<0.001$) at birth and this effect persisted through P14 (IUGR 29.8±0.8g vs. NG 32.7±0.5g, $P<0.01$). Brain weight measured at P14 did not differ between the groups (IUGR 0.95±0.01g vs. NG 0.92±0.01g).

Blood glucose and ß-hydroxybutyrate concentrations

We induced acute hypoglycemia (AHG) in NG and IUGR P14 rats using a combination of fasting and insulin administration (7). Littermates in the nonhypoglycemic control group (CON) were similarly fasted and received normal saline yielding 4 experimental groups: IUGR/HG (IUGR and insulin), IUGR/CON (IUGR and saline), NG/HG (NG and insulin), NG/CON (NG and saline). The control groups receiving normal saline (IUGR/CON and NG/CON) remained euglycemic (blood glucose > 50mg/dl) throughout the study period. After the overnight fast, blood glucose concentrations at baseline (time 0) were similar between groups (105.1±1.6 mg/dl NG vs. 106.8±1.8 mg/dl IUGR). Beginning 30 minutes after the administration of insulin and until the termination of hypoglycemia 240 min after the insulin administration (i.e., for 210 minutes), the mean blood glucose concentrations were lower in the IUGR/HG and NG/HG groups (Figure 1). The severity of hypoglycemia did not differ between the IUGR/HG and NG/HG groups. Blood glucose concentrations were measured in 7 IUGR/HG and 8 NG/HG pups 30 minutes after receiving the rescue dose of 10% dextrose (at approximately t=270 minutes). There was no difference between the groups (IUGR 124.7±14.9 mg/dL vs NG 159.3±21.6 mg/dL). There was no difference in fasting ß-hydroxybutyrate (BHB) concentrations in NG vs. IUGR (1.2±0.054 mmol/L NG vs. 1.45±0.11 mmol/L IUGR).

Core body temperature during acute hypoglycemia

Using a fiberoptic rectal probe, core body temperature (CBT) was measured every hour during the 4-hours of observation. The CBT was lower in IUGR/HG compared with the NG/HG (31.94±0.01°C vs 33.58±0.06°C, $P<0.05$).

Neuronal injury

Neuronal injury was assessed 24 hr after AHG using FJB histochemistry as in previous studies (7). There were rare FJB-positive (FJB+) cells in the cerebral cortex and hippocampus of the NG/CON and IUGR/CON groups. FJB+ cells were present in the NG/HG and IUGR/HG groups, primarily in the cerebral cortex. The FJB+ cells were generally symmetrical between hemispheres and were isolated with staining of the cell body and neuronal processes. When compared with the NG/HG group, fewer FJB+ cells were present in the IUGR/HG group in the cerebral cortex ($P<0.01$, Figure 2). There was no difference in FJB+ cells between NG/HG and IUGR/HG groups in the hippocampus (data

not shown). To determine whether the FJB differences between the IUGR and NG group were due to differences in the neuronal number, Nissl histochemical analysis was performed. The intensity of Nissl staining did not differ between IUGR and NG in either the cortex or HPC (data not shown).

To further confirm that the differences in the severity of injury between IUGR/HG and NG/HG groups was not due to variations in the onset of injury, brain sections were obtained from subsets of animals in the IUGR/HG and NG/HG groups 6 hr and 72 hr post-hypoglycemia and stained for FJB histochemistry. At both time points, IUGR/HG had fewer FJB+ cells in the cerebral cortex than NG/HG (Table 1). Similarly there was no difference in the number of FJB+ cells in the hippocampus at either time point.

There was no relationship between the number of FJB+ cells and blood ketone concentration, CBT or post-recovery glucose concentration (data not shown). However, all the animals with > 40 FJB+ cells had ketone concentrations <1.5mmol/L.

Substrate Transporter Transcript Expression

The expression of Glut 1, Glut 3, MCT 1 and MCT 2 transcripts in the cortex and hippocampus was determined using qPCR in P14 IUGR and NG pups under euglycemic conditions. The expression of MCT 2 mRNA was significantly increased in the cerebral cortex of the IUGR group, relative to the NG group (Figure 3b). There was no difference in hippocampal MCT 2 gene expression between the two groups (Figure 3d). There was no difference between the groups with respect to Glut 1, Glut 3, and MCT 1 mRNA expression in either brain region (MCT 1 Figure 3a,c; Glut data not shown, $P>0.25$ for all comparisons).

Discussion

Asymmetric IUGR or growth restriction with head sparing is a growth pattern often seen as a result of placental insufficiency (2,14,15). The mechanism of brain sparing is unknown; however, increased cerebral blood flow prioritizing essential nutrients and substrates to the brain at the expense of metabolically less active peripheral tissues, such as the liver and skeletal muscle may in part be responsible for this growth pattern (1,2,15,20). Although brain size may be spared at birth, it is unknown whether the brain function is similarly spared and whether individual brain regions are protected equally during common metabolic perturbations in the postnatal period, such as hypoglycemia. Understanding the effects of postnatal hypoglycemia on the developing IUGR brain is clinically relevant since many IUGR neonates experience recurrent or prolonged episodes of hypoglycemia well beyond the immediate newborn period (21-23).

Our objective in this study was to better understand how adaptations that result from IUGR might be protective or harmful in the setting of a metabolic insult such as acute hypoglycemia. We used a well-established rat model of placental insufficiency (12, 16) to determine whether the developing IUGR brain is more or less vulnerable to secondary injury from postnatal hypoglycemia than the normally grown rats. We chose P14 because our group has shown previously that the normally grown developing rat brain is resistant to

neuronal injury from AHG until approximately P14 (7). Using FJB immunohistochemistry, we demonstrate that 4 hours of acute, insulin-induced hypoglycemia leads to neuronal injury in the cerebral cortex of IUGR rats. However, compared with the normally grown rats, the injury was less severe. There was no difference in the severity of neuronal injury in the hippocampus. This is consistent with previous work from our group and others who have found an age-dependent regional vulnerability to hypoglycemia, such that the cerebral cortex is more vulnerable to neuronal damage than the striatum or hippocampus (7,10,11,24). The severity of injury as determined by the number of FJB+ cells in the cortex and hippocampus of NG/HG was similar to our previous reports (7,25). Thus, IUGR protects the developing cortex during AHG, but does not alter the regional distribution of injury previously demonstrated in this model of AHG (7,10,11). Interestingly, core body temperature was lower in the IUGR pups and systemic hypothermia is known to be neuroprotective in many settings (40). Furthermore, iIncreased MCT 2 transcript expression in the IUGR cerebral cortex suggests that increased delivery of alternative substrates (lactate, pyruvate and ketone bodies) into the cerebral cortex might also play a role in protecting against neuronal injury during AHG.

We defined hypoglycemia as a blood glucose concentration of <40 mg/dl, a value known to be associated with decreased brain glucose concentration in developing rats (20,26). There was no difference in blood glucose concentrations after an overnight fast between the IUGR and NG groups. Furthermore, the sensitivity to insulin was similar in both the IUGR/HG and NG/HG groups (Figure 1). Although studies employing a variety of IUGR animal models, including the bilateral uterine artery surgery used here, have shown fetal hypoglycemia and disrupted glucoregulation at birth (1,3,14), the blood glucose concentrations and hepatic gluconeogenic enzyme activity normalize by 10 days of age in the rat (12) consistent with our findings of normal fasting blood glucose concentrations in P14 IUGR rat pups. Likewise, in our experimental groups receiving saline (IUGR/CON and NG/CON) there was no difference in blood glucose concentrations during the 4 hr study period (Figure 1). This finding further supports that postnatal glucose metabolism and regulation are intact in IUGR.

However, despite normal fasting glucose concentrations and apparent equivalent insulin sensitivity at P14, our results show that the IUGR cerebral cortex is more resistant to injury than NG pups. The mechanisms underlying these observations currently remain unexplained. It has been demonstrated in animal and human studies that as a result of placental insufficiency, the fetus undergoes metabolic adaptations that promote survival in the short term, which however may prove to be deleterious in the long-term (1,2,16,27). It is possible that these beneficial metabolic adaptations extend after birth, and the IUGR pup is able to more efficiently utilize glucose-sparing substrates such as ketones and lactate than normally grown pups. It is also possible that the energy requirement of the IUGR brain is low due to a decrease or delay in the ontogeny of energy-demanding developmental processes, such as synaptogenesis and myelination, after birth (28-30). Therefore brain injury might not occur when glucose concentrations are low.

To investigate the role of alternative substrates in IUGR further, we measured fasting blood ß-hydroxybutyrate (BHB) concentrations in both IUGR and NG prior to the administration

of insulin. Although the difference in fasting BHB concentrations was not significant between the two groups, we found increased gene expression of MCT 2 in the cortex of IUGR pups at baseline (non-fasting, euglycemic) compared with the NG pups suggesting that increased transport of alternative substrates prior to the onset of acute hypoglycemia might be protective against neuronal injury during glucose deprivation. Consistent with this possibility, previous studies have demonstrated that ketonemia, either innate or induced using a ketogenic diet prevents neuronal injury during acute hypoglycemia in developing rats (18). Furthermore, increased expression of MCT2 mRNA has been shown in the brainstem of female rats in response to food deprivation, supporting increased utilization of ketone bodies as a respiratory fuel under nutrient restricted conditions (31). Other studies have reported increased MCT2 expression in response to noradrenalin (32) and IGF-1 and insulin via the mTOR pathway (33). In our study, there was no difference with respect to MCT1 in either the cerebral cortex or hippocampus (Figure 3a, c). The differences observed in our study between the expression of MCT1 and MCT2 might be due to the species-specific affinities of the MCTs. In rats, MCT1 and 2 are both expressed in neurons; however, MCT2 has a 5-10 fold higher affinity for pyruvate, lactate, acetoacetate and BHB (17). Therefore, the magnitude of elevation in MCT2 here is likely to be clinically significant for this species. To our knowledge this is the first study to report the effect of IUGR on MCT1 and MCT2 mRNA expression in the brain regions.

In addition to monocarboxylate transport, we also analyzed gene transcripts of the facilitative glucose transporters Glut 1 and Glut 3. Using a variety of animal models of IUGR, several groups have reported elevated (absolute and relative) brain Glut 1 expression prenatally and/or soon after birth, suggesting that this is an adaptation to prioritize glucose delivery to the fetal brain over peripheral tissues in IUGR (1,15,16). We found no difference in Glut 1 or Glut 3 expression in either the cortex or hippocampus between IUGR and NG groups. This is not surprising given that our studies were performed at P14 when the pups were no longer hypoglycemic and had established normal feeding patterns. The similar plasma glucose concentration after overnight fasting in the two groups is consistent with this possibility. Sadiq et al in their study also found lower Glut 1 expression on P14, relative to the expression on P1, supporting postnatal recovery (15). Since the focus of our study was regional vulnerability of the brain to AHG, we did not measure peripheral glucose transport. Hence, it is unknown whether the IUGR pup continues to prioritize glucose centrally over the periphery on P14 and whether glucose metabolism has normalized in all organ systems.

Another possible explanation for our findings of reduced neuronal injury in the P14 IUGR cortex could be related to delayed neurodevelopment in IUGR. The developing rat brain has a very low glucose utilization rate from birth until approximately 10 days of life when it increases in a sigmoidal fashion (34). From P10-17, the rat begins to acquire audition, vision, play and locomotion and this corresponds to an increase in glucose utilization rates in the brain regions sub-serving those functions (35,36). Hence the demand of glucose by the brain region increases concurrent with its rapid phase of structural and functional development. In our previous study, we showed that postnatal age greatly affected the degree of neuronal injury after AHG in the normally grown developing rat such that prior to P14, there was no significant injury in any brain region (7). This suggests that as critical brain functions begin to develop, the demand for glucose increases and when the supply

cannot meet the demand, the brain is at a higher risk for injury. To what extent IUGR delays brain maturation is not well understood; however, functional neurodevelopmental delays have been noted in infants and children who were born IUGR (4). Furthermore, other studies investigating the effect of fetal nutritional deficiencies on neurodevelopment have shown shifts in the critical period to a later time point along the developmental axis (37). Specifically, in perinatal iron-deficient (ID) rat pups, the critical window of hippocampal development is later and shorter than in iron-sufficient pups. This is also associated with lower plasticity and altered long-term potentiation in the adult formerly ID rat (37). Therefore, it is plausible that the reduced neuronal injury observed after AHG in IUGR may in fact be related to a lower demand for glucose at that developmental age due to a delay in neurodevelopment. This is further supported by observations by Ogata et al in P21 IUGR rats where 48 hr of fasting resulted in significant reductions in plasma glucose, insulin and alanine concentrations and elevated BHB concentrations suggesting that the period for peak cerebral metabolism and glucose demand is offset in IUGR occurring closer to P21 and outside of the normal P10-17 window (12). The cerebral cortex may particularly benefit from this developmental shift as cortical development peaks later than all other brain regions (38).

One novel observation in this study is that the IUGR/HG pups were noted to have systemic hypothermia during the study period. Although ambient temperature was controlled uniformly between the two groups, IUGR CBTs were significantly lower than NG at baseline and throughout the duration of the study. The average CBT for the IUGR/HG group was 31.94±0.01°C compared to 33.58±0.06°C in the NG/HG. Central hypothermia (CBT <34°C) is known to be neuroprotective in both neonates and adults after severe hypoxic insults. Although it is unclear from this study to the extent at which central hypothermia may provide neuroprotection, it might also be indicative of a lower resting metabolism seen as protective adaptation to IUGR. Interestingly, the control group (NG/HG) had an average CBT <34°C yet still displayed significant injury in the cortex. When analyzed using linear regression, there was no relationship between core body temperature and number of FJB+ cells. The exact role of hypothermia in this setting needs to be explored further.

Our study has some limitations. We used Nissl histochemical analysis of limited number of brain sections to determine neuronal structure and number in the NG and IUGR groups, instead of the stereologic assessments. Our group has previously demonstrated that Nissl histochemical assessment is a valid method for assessing neuronal density after hypoxia-ischemia (39).

Another limitation of this study is that we only included follow-up through 72 hours and therefore cannot rule out late injury occurring beyond that time point. However, our group has shown that peak neuronal injury after AHG occurs at or before 24hrs (7). Further, we observed similar patterns and severity of injury at 6, 24 and 72 hr. post-hypoglycemia in IUGR/HG vs. NG/HG suggesting variations in the onset of injury in the NG and IUGR groups as the cause for the discrepant results is unlikely (Figure 2, Table 1). Lastly, we did not evaluate long-term functional or behavioral studies in this pilot study. Functional outcomes would greatly strengthen the clinical significance of our findings.

In summary, using a rodent model of IUGR we found that the cerebral cortex of the IUGR rat pup is less vulnerable to neuronal injury during postnatal hypoglycemia than normally grown controls. We also found a significant reduction in the core body temperature and increased gene expression of the neuronal MCT2 in the cerebral cortex of the IUGR rats. We speculate that adaptations such as systemic hypothermia or enhanced production and delivery of alternative substrates to the cerebral cortex might be neuroprotective in the setting of postnatal hypoglycemiametabolic perturbations in IUGR.

MATERIALS AND METHODS

Animal Preparation

Timed-pregnant Sprague-Dawley rats were received on day 13-15 of gestation (Harlan Laboratories, Madison, WI) and individually housed under standard laboratory conditions with free access to standard rat chow and water. On gestational (G) day 19 (term 22 d), pregnant rats were anesthetized with inhaled isoflurane (1.5%-3%) in a 50:50 mixture of N_2O and O_2, and both uterine arteries were ligated as previously described (12,16). Normally grown (NG) control animals did not undergo surgery since previous studies have shown no differences in metabolic phenotype between sham-operated control group and non-operated controls after P4 (12,16). Rats recovered within an hour of surgery and were provided standard postoperative care. Dams were allowed to deliver spontaneously. Pups were weighed within 24 hours of birth, the 4 largest animals were euthanized, and litters were culled to a maximum of 8. Dams and pups were housed in a temperature and humidity-controlled animal care facility with 12-hr: 12-hr light:dark cycle and allowed food and water *ad libitum*. All experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee at the University of Minnesota approved all experimental protocols.

Induction of Hypoglycemia

On postnatal day (P) 14, IUGR and NG rats were subjected to a single episode of acute hypoglycemia (AHG) as previously described (7). In brief, AHG was induced using a 6 IU/kg intraperitoneal (i.p) injection of human regular insulin (Novo Norkisk Inc., Clayton, NC) after a period of overnight fasting. The target blood glucose was <40 mg/dL. Four experimental groups were created including normally grown control pups which remained euglycemic (NG/CON), NG pups with hypoglycemia (NG/HG), and IUGR/CON and IUGR/HG. Littermates in the CON groups were similarly fasted and were subcutaneously (s.c.) injected with an equivalent volume of 0.9% saline. Glucose concentrations were measured in whole blood collected from tail vein at baseline using a blood glucose meter (Accu-Check Compact, Roche Diagnostics, Indianapolis, IN). Whole blood BHB concentration was measured at 0 minutes using a blood ß-ketone monitoring system (Precision Xtra, Abbott Laboratories, Oxon, United Kingdom). Blood glucose concentrations were determined every 30 minutes in the HG groups and every 60 minutes in the CON groups throughout the duration of the study. If blood glucose concentrations dropped below 20 mg/dL, 10% dextrose was administered in a dose of 200 mg/kg, i.p. and s.c. in order to prevent seizures (7). No pups in either group experienced seizure activity

throughout the study. AHG was terminated 240 minutes after the insulin administration by injecting 200mg/kg of 10% dextrose i.p. and s.c. before reuniting the pups with their respective dams. Control pups not receiving insulin were similarly injected with an equal volume of 0.9% normal saline. Post-rescue glucose concentrations were measured 30 minutes after the administration of 10% dextrose. In a subset of IUGR/HG and NG/HG pups, rectal temperature was measured every hour during AHG using a Fiberoptic Rectal Temperature Module (SA Instruments, INC, Stony Brook, NY).

Histochemical Analysis

Histochemistry was performed as previously described (7) using Fluoro-Jade B (FJB) immunohistochemistry (Abcam, Cambridge, MA). In brief, brains were harvested 6, 24 and 72 hours after the conclusion of AHG. Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) before undergoing *in situ* transcardial perfusion with 0.9% saline followed by 4% formaldehyde and 5% sucrose in PBS. Brains were removed and post-fixed overnight at 4°C in 4% formalin, followed by serial overnight passages in 20% and 30% sucrose in PBS at 4°C for cryoprotection. Brains were then flash-frozen on dry ice and acetone bath and embedded in a tissue-freezing medium (Triangle Biomedical Sciences; Durham, NC) for sectioning. Serial 20μm coronal sections were obtained using a cryostat (Model CM1900; Leica Instruments GmbH; Nussloch, Germany) at −22°C. FJB staining and analysis were performed as described in our previous study (7). All FJB+ cells in the brain regions of each brain section were counted and group means were determined. To assess differences in neuronal structure and cellular intensity, Nissl staining was performed as previously described (39).

Quantitative RT-PCR (qPCR)

Total RNA was isolated from dissected hippocampus and cortex using an RNA-isolation kit (Life Technologies, Grand Island, NY) and was used to generate cDNA by reverse transcription. All qPCR experiments were performed with 4μl of diluted cDNA, 5μl FastStart Universal Probe Master Mix (Roche Diagnostics, Indianapolis, IN) and 0.5μl DEPC H_2O, and 0.5 μl 20X Taqman Gene Expression Assay primer/probe (Agilent Technologies, Santa Clara, CA).

Statistical analysis

Group means were compared using ANOVA with group (NG/CON, NG/HG, IUGR/CON, IUGR/HG) and time of assessment (6 hr, 24 hr and 72 hr) as fixed factors. Intergroup differences were determined using unpaired *t* tests. Data are presented as mean ± SEM. Significance was set at $P<0.05$. The relationship between core body temperature and number FJB+ cells, ketone concentration and number of FJB+ cells and post-rescue glucose concentration and number of FJB+ cells was analyzed by linear regression.

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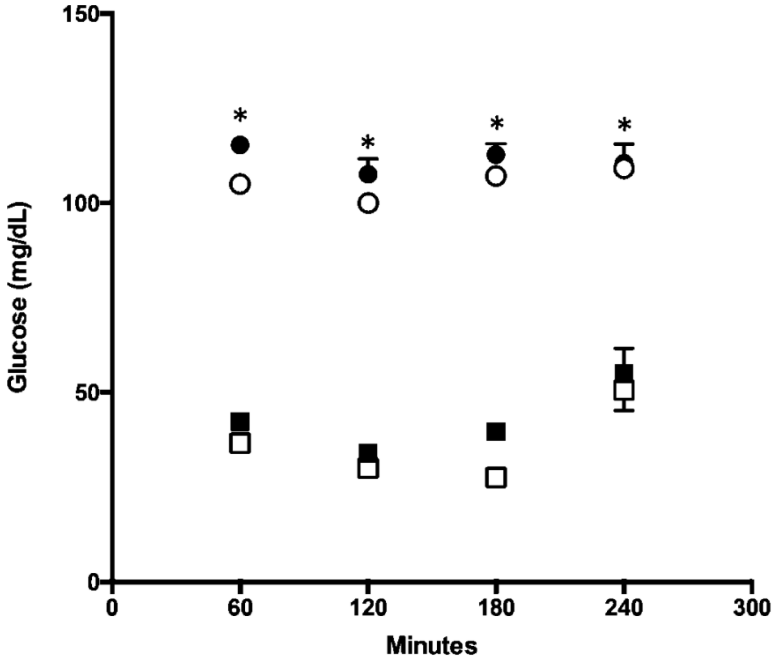

Figure 1. Blood glucose concentrations in control and hypoglycemia groups in NG and IUGR P14 rat pups

Blood glucose concentrations were lower in IUGR/HG and NG/HG compared to NG/CON and IUGR/CON (treatment effect, **P*<0.01). The mean blood glucose concentrations were similar between IUGR/HG and NG/HG. IUGR/CON black circles, IUGR/HG black squares; NG/CON open circles, NG/HG open squares. Values are mean ± SEM. N=20-40 rats/group at each time point.

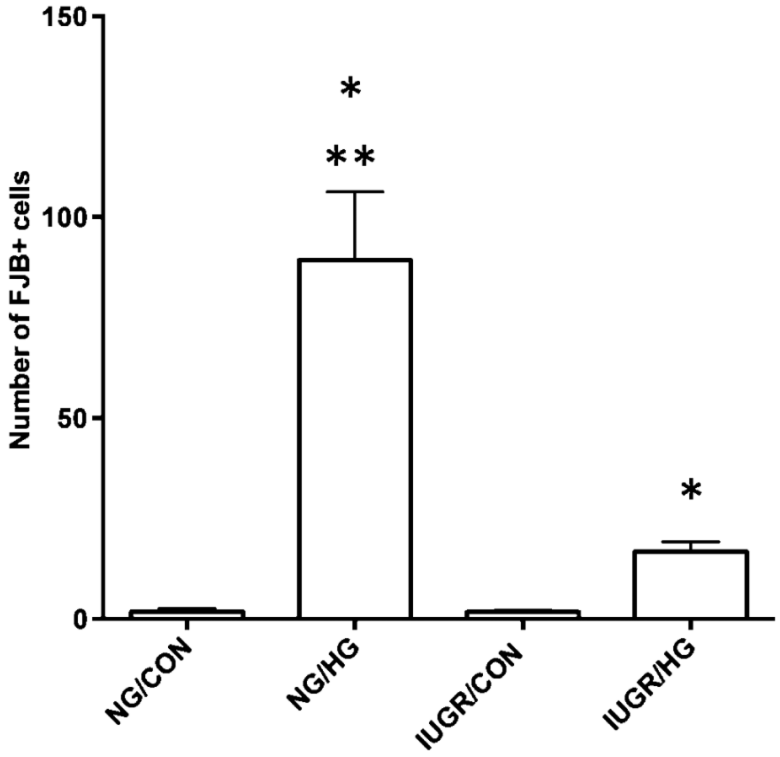

Figure 2. FJB+ cells in the cerebral cortex of P14 rat pups in the NG and IUGR groups at 24 hr post-hypoglycemia

The number of FJB+ cells in the IUGR/HG cortex was significantly lower compared with the number of FJB+ cells in the NG/HG cortex (group effect, ***P*<0.01). The number of FJB+ cells in the IUGR/HG and NG/HG cortex were elevated compared to IUGR/CON and NG/CON, respectively (treatment effect, **P*<0.05). Values are mean ± SEM FJB+ cells per brain section. N= 4-5 rats/group.

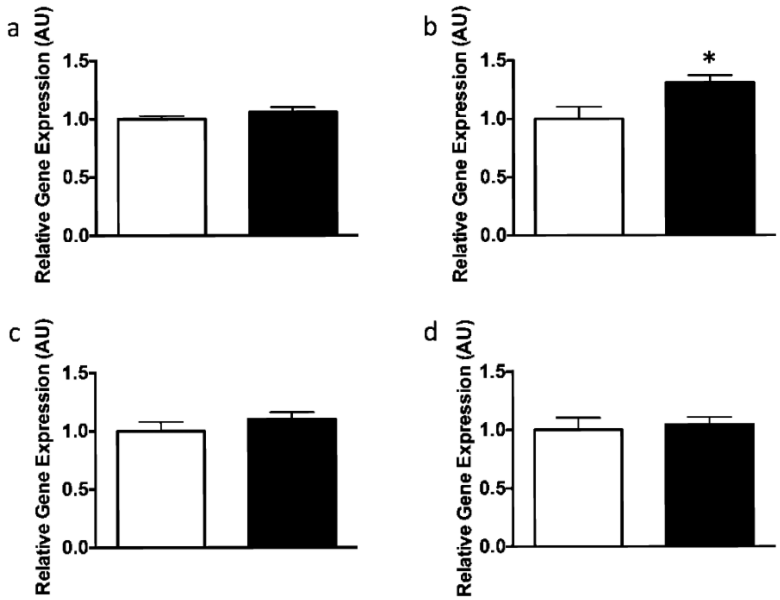

Figure 3. Monocarboxylate transporter (MCT) mRNA expression in P14 rat cortex and hippocampus under basal conditions. NG open box, IUGR black box

Panel A) MCT 1 gene expression in the cortex is similar between both groups. Panel B) MCT2 expression is elevated in the IUGR cortex (**P*< 0.03). Hippocampal MCT 1 (Panel C) and MCT 2 (Panel D) gene expression is similar in both groups. Values are mean ± SEM relative gene expression in arbitrary units (AU). N=6 pups per group.

Table 1

FJB Positive Cells in the Cerebral Cortex and Hippocampus of IUGR and NG Hypoglycemic Rat Pups

	Cortex		Hippocampus	
	6 hr	**72 hr**	**6hr**	**72 hr**
IUGR/HG	34.3±10.4*	13.4±2.0*	3.0±0.6	1.3±0.5
NG/HG	77.0±9.3	56.1±14.6	1.75±0.6	3.8±0.9

Values are means ± SE. Number of FJB+ cells in the region from 4-6 brain sections. For 6 hr, n=4 per group; 72 hr n=7 IUGR/HG and n=8 NG/HG

*$P<0.05$ IUGR/HG vs. NG/HG