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DIFFERENTIAL EFFECTS OF INTRAUTERINE GROWTH RESTRICTION ON THE REGIONAL NEUROCHEMICAL PROFILE OF THE DEVELOPING RAT BRAIN

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Abstract

Background—Intrauterine growth restricted (IUGR) infants are at increased risk for neurodevelopmental deficits that suggest the hippocampus and cerebral cortex may be particularly vulnerable.

Objective—Evaluate regional neurochemical profiles in IUGR and normally grown (NG) 7-day old rat pups using *in vivo* ¹H magnetic resonance (MR) spectroscopy at 9.4T.

Methods—IUGR was induced via bilateral uterine artery ligation at gestational day 19 in pregnant Sprague Dawley dams. MR spectra were obtained from the cerebral cortex, hippocampus and striatum at P7 in IUGR (N=12) and NG (N=13) rats.

Results—In the cortex, IUGR resulted in lower concentrations of phosphocreatine, glutathione, taurine, total choline, total creatine ($P<0.01$) and [glutamate]/[glutamine] ratio ($P<0.05$). Lower taurine concentrations were observed in the hippocampus ($P<0.01$) and striatum ($P<0.05$).

Conclusion—IUGR differentially affects the neurochemical profile of the P7 rat brain regions. Persistent neurochemical changes may lead to cortex-based long-term neurodevelopmental deficits in human IUGR infants.

Keywords

IUGR; brain; metabolism; magnetic resonance spectroscopy

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INTRODUCTION

IUGR affects 30–40% of low birth weight (<2500g) infants, and the majority of cases result from placental insufficiency (1,2). Placental insufficiency is characterized by decreased nutrient and oxygen delivery to the fetus and results in “asymmetric” IUGR. This growth pattern reflects sparing of brain growth relative to somatic growth (1) by prioritizing limited resources to the brain at the expense of metabolically less active peripheral organs. The fetal adaptations to limited resources that result in “brain sparing” are unknown.

Despite the relative brain sparing, IUGR infants remain at increased risk for cognitive, motor and intellectual deficits as children and adults (3–6). The nature of the deficits suggests that specific brain regions (the hippocampus and cerebral cortex) may be particularly vulnerable, relative to other brain regions (e.g., the striatum). The mechanisms for the region-specific vulnerability and sparing are not known; however, a regional effect of nutritional deficiency on the developing brain has been demonstrated in other conditions. In iron deficient neonatal rats, cytochrome c oxidase (an iron-containing terminal enzyme in oxidative phosphorylation) activity was reduced in the hippocampus, piriform cortex and cingulate cortex, but not in the striatum (7). Similarly, animal models of IUGR have demonstrated adverse effects on myelination and synaptogenesis in certain brain regions including the hippocampus suggesting that IUGR may have similar regional effects on cerebral metabolism as iron deficiency (8,9). Studies employing *in vivo* ^1H magnetic resonance spectroscopy (MRS) in both rodents and humans have shown reduced concentrations of neurochemicals responsible for energy metabolism, neurotransmission and neuronal development in the IUGR hippocampus (10) and preterm IUGR striatum (11). However, no study has simultaneously evaluated the effects of IUGR on the neurochemical profile of multiple brain regions. Such region-based assessment is crucial for understanding the mechanisms underlying cerebral metabolic adaptations to IUGR and risk for long-term region-specific neurodevelopmental deficits.

The objective of the present study was to evaluate the effects of IUGR on the neurochemical profile of the cerebral cortex, hippocampus and striatum in developing rats using ultra high-field *in vivo* ^1H MRS. *In vivo* ^1H MRS is a robust method for assessing multiple metabolites indexing neurotransmission, energy status, phospholipid synthesis and oxidative metabolism from distinct brain regions (12,13). We hypothesized that IUGR will negatively affect metabolic markers of energy metabolism, neurotransmission and myelination in the cerebral cortex and hippocampus while sparing the striatum. We used a well-described surgical model of IUGR that results in a 20–25% reduction in birth weight (14,15). Rat pups at postnatal (P) day 7 were studied due to their neurodevelopmental similarity to human near-term infants (16).

EXPERIMENTAL PROCEDURES

Animal Preparation

Timed-pregnant Sprague-Dawley rats were received on gestational (G) day 13–15 (Harlan Laboratories, Madison, WI) and individually housed in a temperature and humidity-controlled animal care facility with a 12 hour light/dark cycle, and free access to standard

lab diet and water. On G19 (term = 22.5 days), pregnant rats were anesthetized with inhaled isoflurane (1.5%–3%) in a 50:50 mixture of N₂O and O₂. To induce fetal IUGR, a mid-line incision was made along the abdomen, the uterus was exposed and both uterine arteries were ligated (14,15). Normally grown (NG) control animals did not undergo surgery. A sham-operated group was not used, given previous evidence of lack of differences in the metabolic phenotype compared to non-operated controls after P4 (14,17). 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 and the 4 largest animals were euthanized, and litters were culled to a maximum of 8. NG control pups were treated in a similar manner. In total, 11 litters were used to generate 13 NG and 12 IUGR pups. All experimental procedures 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.

***In vivo* ¹H MRS**

IUGR and NG pups were studied using *in vivo* ¹H MRS on postnatal (P) day 7. Spectra were obtained from the hippocampus, striatum, and cerebral cortex in spontaneously breathing rats under inhalation anesthesia (gas mixture O₂:N₂O = 1:1, isoflurane 3% for induction, 1–1.5% for maintenance). All experiments were performed using a horizontal bore 9.4T/31 cm magnet (Varian/Magnex Scientific, Yarnton, UK) interfaced to a Varian INOVA console (Varian/Alto, CA, USA) (12,13). A quadrature surface RF coil with two geometrically decoupled single-turn coils (10 mm diameter) was used for transmission and reception. Coronal and sagittal brain images were acquired using multi-slice fast spin echo (FSE) MRI (slice thickness = 1mm). Magnetic field homogeneity was adjusted in each volume of interest (VOI) using FASTMAP shimming technique (18). ¹H MR spectra were acquired from 7.5–9.4μl VOI in the hippocampus, 6–12μl VOI in the striatum and a 11.2–18μl VOI in the cortex. These volumes were selected using the FSE images based on previous studies (12) measuring the same regions in P7 rat pups. All spectra were acquired from VOI using a LASER localization sequence (repetition time TR = 5s, echo time TE = 15ms, number of transients (NT)=128) (19) combined with VAPOR water suppression (20), as described previously (21).

Quantification of metabolites

In vivo ¹H MRS spectra were analyzed using LCModel with the spectrum of fast relaxing macromolecules included in the basis set (12,13,22). The model metabolite spectra were generated using density matrix simulations (23) with the MATLAB software (MathWorks) based on previously reported chemical shifts and coupling constants (24,25). Unsuppressed water signal was used as an internal reference. Brain water content of 87% was used in the calculations, based on a previous study (12). The following metabolites were quantified from the spectra: ascorbate (Asc), aspartate (Asp), creatine (Cr), phosphocreatine (PCr), γ -aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), lactate (Lac), *myo*-inositol (Ins), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau) and the sum of

glycerophosphocholine and phosphocholine (GPC+PC). Total creatine (PCr+Cr) as well as ratios of PCr/Cr and Glu/Gln were also determined.

Statistical analysis

Data are expressed as mean $\pm$ SD. One-way ANOVA was used to determine the effect of group, sex and brain region on individual metabolites. Intergroup differences in a region were determined using independent samples *t*-test. Significance was set at $\alpha < 0.05$.

RESULTS

Animal weights

IUGR was induced via bilateral uterine artery ligation on G19. Body weights of the IUGR pups were 20% lower than normally grown (NG) pups (4.8 ± 0.71 g vs. 5.9 ± 0.54 g, $p < 0.001$) at birth.

In vivo ^1H MRS

The representative ^1H MRS spectra acquired from the hippocampus, striatum and cerebral cortex of IUGR and NG rat pups on P7 are shown in Figure 1. The achieved spectral quality (Figure 1) enabled reliable quantification (Cramér Rao lower bounds CRLB $< 30\%$) of 16 metabolites in each of the 67 measured spectra. The average signal to noise ratio for each region was similar between groups (Cortex: NG= 40.1 ± 5.8 vs. IUGR= 40.9 ± 5.7 ; hippocampus NG= 20.3 ± 2.3 vs. IUGR= 22.2 ± 6.1 ; striatum NG= 14.9 ± 5.7 vs. IUGR= 14.3 ± 2.7). Average line width measurements were also similar between groups for the cortex and hippocampus (Cortex NG= 13.0 ± 2.9 Hz, IUGR= 14.5 ± 1.6 Hz, hippocampus NG= 8.1 ± 0.6 Hz, IUGR= 8.3 ± 0.4 Hz). Only the striatum had a significant difference in line width between the groups (striatum NG= 7.7 ± 0.3 Hz vs. IUGR= 8.1 ± 0.4 Hz, $P=0.02$). The neurochemical profile consisted of 16 neurochemicals, one neurochemical sum and two ratios ([PCr]/[Cr] and [Glu]/[Gln]). Overall, the neurochemical profiles were obtained from the cortex of 13 NG and 10 IUGR rats, from the hippocampus of 8 NG and 12 IUGR rats and from the striatum of 13 NG and 9 IUGR rats.

There was a main effect of group and brain region ($P < 0.05$ for each), but not sex on the neurochemical profile. Hence, data from both sexes were grouped together. The neurochemical differences between the regions within a group were similar in the NG and IUGR groups except for GSH and PCr+Cr in the IUGR group. IUGR affected the neurochemical concentrations more in the cerebral cortex than in the hippocampus or striatum at P7 (Figure 2). In the cortex, IUGR resulted in lower concentrations of PCr, GSH, Tau, GPC+PC and PCr+Cr ($P < 0.01$ for each, Figure 2) while only lower concentrations of Tau were seen in the hippocampus ($P < 0.01$) and striatum ($P < 0.05$) as a result of IUGR. The [Glu]/[Gln] ratio was lower in the IUGR cortex ($P < 0.05$) but unchanged in the hippocampus and striatum (Figure 2).

DISCUSSION

The results of this *in vivo* ^1H MRS study clearly demonstrate that IUGR differentially affects the neurochemical profiles of rat brain regions on postnatal day 7. While the cerebral cortex showed greater alterations, the hippocampus and striatum were minimally affected. Out of all the neurochemicals quantified in this study, we found that in the cerebral cortex, IUGR resulted in significantly lower concentrations of 5 metabolites and one metabolite ratio, while in the hippocampus and striatum, only the concentration of Tau was reduced due to IUGR. This suggests that early in development the cerebral cortex is more vulnerable to IUGR than other brain regions. Based on the functional role of the metabolites involved, the neurochemical changes in the cerebral cortex as a result of IUGR suggest an underlying alteration in basal energy metabolism.

This is the first study to simultaneously quantify the neurochemical profile of three distinct brain regions in the IUGR rat using ^1H MRS at 9.4T. Previously, our group described the regional effect of age on the neurochemical profile in the normally grown Sprague Dawley rat and our control data in this study is similar to what was previously reported (12). Specifically, as in our previous study, the concentrations of Tau were the highest of all metabolites measured with no differences seen between the regions. In contrast, concentrations of Ins and GPC+PC have been shown to differ between the three regions at P7 (striatum>hippocampus>cortex) and our data here are consistent with this finding. As shown by the representative spectra in Figure 1 for both NG and IUGR brain regions, our data were of high quality as illustrated by high signal to noise ratio and spectral resolution and absence of unwanted coherences.

The concentrations of PCr and PCr+Cr were lower in the IUGR cerebral cortex compared with the NG controls (Figure 2). PCr, stored in the cytosol, is a source of high-energy phosphate used for ATP production during times of increased energy demand (13). Thus, decreased PCr concentrations might reflect lower energy reserve in the IUGR cerebral cortex. These findings are consistent with Somm et al who found decreased concentrations of PCr and PCr +Cr in the hippocampus of P7 IUGR rat pups using ^1H MRS (10). It is noteworthy that the model of IUGR used by Somm et al differs from ours in that IUGR was generated via maternal administration of dexamethasone. The developing hippocampus contains an abundant amount of glucocorticoid receptors rendering it particularly sensitive to the effects of dexamethasone (10), which might explain why the hippocampus was more affected in that study.

We did not observe a statistically significant difference in the PCr/Cr ratio that has been shown in previous studies from our lab examining chronic hypoxia and fetal and neonatal iron deficiency (26). In both conditions, an elevated PCr/Cr ratio was observed and was postulated to be a compensatory mechanism to conserve energy in the setting of poor ATP production (26). Compared with those studies, perinatal hypoxia and nutrient deficiencies were of much shorter duration in the present study, which may explain the discrepant results between the studies.

[Glu]/[Gln] ratio was significantly lower in the IUGR cerebral cortex compared with the NG cerebral cortex (Figure 2). A lower [Glu]/[Gln] ratio in a brain region can be a marker of a metabolic defect (12). Glu-Gln cycling between neurons and glia is an energy-demanding process and is closely linked to cerebral energy consumption (12,26). We speculate that the lower [Glu]/[Gln] ratio may suggest altered glutamatergic neurotransmission and neuronal/glia cycling in the energy-compromised IUGR cerebral cortex.

The concentration of the antioxidant glutathione (GSH) was also significantly lower in the IUGR cortex. GSH is one of the most important antioxidants in the central nervous system (27,28). Reduced concentrations of GSH indicate potential injury during oxidative stress (28, 29–31,32). Neurons are particularly vulnerable to oxidative stress due to the high content of unsaturated lipids in the membranes (28,33,34). Neuronal survival is dependent on the glutathione redox potential (33). Other animal models of IUGR have also shown disruptions in regional brain GSH concentrations (28). Newborn IUGR piglets had decreased concentrations of GSH after 90 minutes of hypoxia in the temporoparietal, frontal, occipital, basal ganglia and cerebellum (28). Furthermore, other studies have also shown that IUGR results in oxidative stress and mitochondrial dysfunction in skeletal muscle (35), β -cell (36), liver (37) and brain (8). The role of oxidative stress and mitochondrial function as it relates to the brain is important especially because these same mechanisms have been associated with adult neuropsychiatric disorders such as depression, Alzheimer's disease and schizophrenia that are also now being seen in adults who were formerly IUGR (38–41). The role of antioxidants including GSH and mitochondrial function in the IUGR brain requires further investigation; however, the lower concentration of GSH in the IUGR cerebral cortex observed in our study suggests that at P7, the cortex may be more vulnerable to injury due to oxidative stress than other regions and this may have long-term neurodevelopmental implications.

Choline containing compounds GPC+PC were lower in the IUGR cerebral cortex than controls. These compounds are precursors of membrane phospholipids phosphatidylcholine and sphingomyelin (42). Thus, lower GPC+PC indicates potential for decreased phospholipid synthesis and possibly myelination. PE, a metabolite linked directly with myelination was not affected by IUGR in this study. However, the onset of myelination occurs later than P7 (43). Thus, it is not surprising that no changes in PE were observed. Our results do support previous findings using the same model of IUGR where significant delays in oligodendrocyte maturation and myelination occurred in the hippocampus (8).

Of note, Tau was the only neurochemical that was lower in all 3 regions in the IUGR brain than controls. Tau is an essential amino acid for the fetus and neonate and has a variety of physiologic functions (44). Decreased fetal and neonatal concentrations of Tau have been shown in several animal models of IUGR including the one used in the present study (45–47). Recently, Li et al used a maternal low protein diet to generate IUGR rat pups and showed that the concentrations of Tau in the brain were significantly lower than normally grown rat pups. It is noteworthy that antenatal supplementation with Tau was found to improve both growth and brain Tau concentrations in the pups (47).

In addition to being an essential amino acid, brain Tau concentration reflects oxidative metabolism in the brain regions and there is a positive correlation between Tau concentrations and cerebral metabolic rate (48). Tau is released into the extracellular space as an osmoregulatory response to brain edema and osmotic changes during hypoglycemia (49–51). Extracellular release of Tau is considered a feedback mechanism for preventing excessive calcium influx during glutamate- and aspartate-mediated excitotoxicity (50).

We only examined the regional effect of IUGR on neurochemistry on P7. This period in brain development is relatively quiescent. The period of active growth, myelination and increased energy metabolism in the rat brain occurs between P10–17 (12,43,52). Specifically in the hippocampus, the critical period for development occurs between P15 and P30 when dendritogenesis, synaptogenesis, energy production and utilization are at peak levels (43,53). During this time the rat acquires specific brain functions such as audition, locomotion and vision (52,54). Studies examining the effect of IUGR on neurochemistry throughout development are needed in order to determine how the IUGR brain adapts to increased energy needs and whether the changes observed on P7 persist throughout development. However, given the changes observed in this study at P7 we speculate that more robust adverse effects on biological processes will occur over time. A second limitation is that although we showed decreased concentrations of key neurochemicals particularly in the cerebral cortex, the areas sampled consist of heterogeneous populations of neurons and glia. As such, we cannot determine the most vulnerable cell type in a given region; however it is known that certain neurochemicals are more prominent in some cells versus others. For instance, glial cells contain 2–4 times more Cr than neurons (55). Furthermore, as mentioned previously, the reduced [Glu]/[Gln] ratio observed in the IUGR cerebral cortex in this present study suggests that neuron-glial neurotransmission might be altered. One final consideration when interpreting our data is the effect of IUGR on brain volume or cell density within a given region. Several groups have reported structural brain changes in IUGR that might affect the neurochemical profile for a given region (3,56–58). However, if the changes we observed in this study were due to a reduced brain volume or cell density alone, we would have expected a uniform reduction in neurochemical profiles in all 3 IUGR brain regions. Instead, we observed changes in only 5 neurochemicals and one neurochemical ratio and these differed between the cerebral cortex, hippocampus and striatum demonstrating that overall structural or volumetric changes due to IUGR are not responsible for the changes we observed here.

This study provides valuable information regarding the effect of IUGR on the developing brain, specifically as it relates to energy metabolism and oxidative stress. We hypothesized that the cerebral cortex and hippocampus would be more affected by IUGR than the striatum; however we found that the most profound alterations in neurochemistry were in the cerebral cortex alone. Future studies will focus on examining the effect of IUGR on the regional neurochemical profile throughout development. However, the identification of several key metabolites disrupted because of IUGR has the potential to serve as biomarkers for future studies examining interventions to improve neurodevelopmental outcomes. The non-invasiveness of *in vivo* ¹H MRS suggests the potential extension of this method for assessment in human infants.

In summary, we demonstrate that IUGR differentially affects the neurochemical profile of the P7 rat brain regions with the cerebral cortex resulting in greater alterations than the hippocampus or striatum. The neurochemical differences in the cerebral cortex suggest disruptions in 1) energy reserves and neurotransmission (PCr, PCr+Cr, [Glu]/[Gln] ratio) 2) oxidative metabolism (Tau, GSH), 3) amino acids (Tau), 4) antioxidants (GSH) and 5) phospholipid synthesis (GPC+PC). Persistent neurochemical changes may lead to cortex-based long-term cognitive and motor deficits in human IUGR infants.

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Figure 1. Representative spectra from the postnatal day 7 rat cerebral cortex, hippocampus and striatum of normally grown (NG) control and IUGR brains

All spectra were acquired from VOI using a LASER localization sequence (repetition time TR = 5s, echo time TE = 15ms, NT=128, VOI = 6–18 μ l) combined with VAPOR water suppression.

Abbreviations: macromolecules (Mac), ascorbate (Asc), aspartate (Asp), creatine (Cr), phosphocreatine (PCr), γ -aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), lactate (Lac), myo-inositol (Ins), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau), the sum of glycerophosphocholine and phosphocholine (GPC+PC).

Figure 2. Neurochemical profile of the cerebral cortex, hippocampus and striatum in the P7 NG and IUGR rat brains

NG is represented by the black bars, IUGR by the white bars. IUGR affected more neurochemicals in the cortex than in the hippocampus or striatum. * $P < 0.01$, # $P < 0.05$. All data are represented as mean $\pm$ SD. All metabolite concentrations are expressed in $\mu\text{mol/g}$. Macromolecule content is expressed in a.i. and the [Glu]/[Gln] ratio is dimensionless. Cortex (NG $n=13$, IUGR $n=10$), hippocampus (NG $n=8$, IUGR $n=12$) and striatum (NG $n=13$, IUGR $n=9$).

Abbreviations: macromolecules (Mac), ascorbate (Asc), aspartate (Asp), creatine (Cr), phosphocreatine (PCr), γ -aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), lactate (Lac), myo-inositol (Ins), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau), the sum of glycerophosphocholine and phosphocholine (GPC+PC), total creatine (PCr+Cr), glutamate/glutamine ratio ([Glu]/[Gln]).