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Impaired development of fetal serotonergic neurons in intrauterine growth restricted baboons

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Abstract

We studied development of the fetal serotonergic central nervous system in a baboon, non-human primate model of intrauterine growth restriction (IUGR). Fetal (90% of full-term) IUGR brains were comparable in size to controls, but have reduced expression of serotonergic proteins and mRNAs, as well as having fewer serotonergic neurons.

Keywords

Serotonin; maternal nutrition; fetus; brain development; IUGR

Introduction

Human epidemiology studies show an increased risk of schizophrenia, mood disorders, and impaired cognition in adults whose mothers experienced under-nutrition during pregnancy and lactation [5, 15, 17]. Impaired serotonergic signaling is thought to play a role in the etiology of these conditions as well as other psychiatric disorders [1, 4, 6, 8, 11, 16]. We hypothesized that maternal nutrient reduction (MNR) and associated IUGR reduce the developmental expression of important components of the fetal baboon serotonergic central nervous system.

MATERIALS AND METHODS

Animals, Feeding and Breeding

The Southwest National Primate Research Center, San Antonio, Texas, USA, maintained 38 female baboons (*Papio hamadryas*) in group housing. All procedures were approved by the Texas Biomedical Research Institute Institutional Animal Care and Use Committee and

conducted in AAALAC approved facilities as described [13]. Briefly, females were maintained in social groups of 16, fed *ad libitum*. At 0.16G pregnant animals were randomly assigned to either the control (CTRL) *ad libitum* diet or MNR fed 70% of feed eaten by controls on a weight-adjusted basis [10]. Food consumption, weights and health status were recorded daily. Water was continuously available.

Brain preparation

Fetuses were delivered by cesarean section under isoflurane general anesthesia at 165 days gestation (90% of term, 0.9G) [12]. Fetal brains were immediately dissected longitudinally; the right half fixed in 4% paraformaldehyde and left half flash frozen in liquid nitrogen and stored at -80°C [2, 10].

Immunohistochemistry

Brain immunohistochemistry was performed in a randomly chosen sub-set of the larger cohort; $n=7$ controls (3 males, 4 females) and $n=6$ IUGR fetuses (3 males, 3 females). Paraffin-embedded tissues sectioned at $5\mu\text{m}$ were processed as described [9]. For immunostaining, sections were incubated in 10% normal serum for 10 minutes and then primary antibody for 24hrs before developing by the DAB method (Antibodies: TPH: Abcam #ab49686 dilution 1:50 and SERT: Santa Cruz #sc-1458 dilution 1–150). Images were collected at magnification of $20\times$. Sections ($5\mu\text{m}$) were cut throughout the pons and immunostained at $500\mu\text{m}$ intervals. Images from 4 slides/animal with 10 pictures/slide were assessed. The frontal cortex was sampled at $50\mu\text{m}$ intervals 3 slides/animal and 6 pictures/slide. Fraction of the field stained was quantified using NIH image-J [7].

Stereology

TPH immunoreactive cells were counted using the physical fractionator method [3, 14] (TPH antibody Abcam #ab40846 dilution 1:250). Data were analyzed using *Stereo Investigator* (MicroBrightField, Williston, VT) and the total number of serotonergic neurons was estimated.

Quantitative Real-time PCR

Total RNA was extracted from frozen tissue using the miRNeasy Mini kit (Qiagen) and cDNA was generated using the high capacity cDNA Reverse Transcription kit. Gene expression was assessed by qRT-PCR using gene-specific TaqMan primer/probe sets (Hs00984355-g1 for SERT, Hs00188220-m1 for TPH1 and Hs00998777-m1 for TPH2) and TaqMan universal PCR master mix (Applied Biosystems). HPRT1 (Hs99999909-m1) was used as an endogenous control. Assays were performed in triplicate at the cDNA synthesis and quantified using standard curve methods.

RESULTS

Prior to becoming pregnant, female baboons were randomly assigned to the control (CTL) group ($n=24$) or maternal nutrient reduction (MNR) group ($n=14$). Initial maternal weights did not significantly differ between the groups ($p>0.05$). Within these pregnancies, MNR fetuses had lower body weights ($n=14$) 715 ± 21.0 g vs. CTR ($n=24$), 795 ± 23.5 g ($p<0.03$)

[10]. When fetuses were compared by sex, the percent decrease in MNR fetal weights was comparable for both male and female fetuses (11.0% in males and 11.4% in females), demonstrating that MNR reliably produces intrauterine growth restriction (IUGR) fetuses [10] and thus MNR exposed fetuses will be referred to as IUGR. The studies presented here were performed on a subset of the 38 fetuses: CTL male (n=3), 784 $\pm$ 46.2g; CTL female (n=4), 714 $\pm$ 41.6g; IUGR male (n=3), 711 $\pm$ 55.2g; and IUGR female (n=3), 626 $\pm$ 27.1g. Body weights of these animals fell within values observed in the full cohort [10].

Fetal brain weights were similar between groups (CTL 77.69 $\pm$ 2.49g vs. IUGR 77.17 $\pm$ 3.07g. Growth restriction in IUGR fetuses was asymmetric as indicated by an increase in brain weight as a percentage of total body weight compared to CTL fetuses (IUGR: 11.69 $\pm$ 0.39% vs. CTL: 10.43 $\pm$ 0.48%, $P=0.07$).

To test the hypothesis that the IUGR offspring have deficits in neural development, we assessed expression of genes controlling key proteins expressed in midbrain raphe serotonergic neurons using quantitative real-time PCR (qRT-PCR). Decreased tryptophan hydroxylase 1 (TPH1), TPH2, and serotonin transporter (SERT) mRNA expression was detected in IUGR compared to CTL midbrains ($p<0.05$) (Fig 1A).

We performed stereological analysis to determine whether the reduced serotonergic mRNA expression observed in IUGR midbrains is due to reduced serotonergic neuron numbers. IUGR midbrains contained fewer TPH positive neurons relative to CTL brains (435.8 $\pm$ 83.7 vs. 731.5 $\pm$ 118.7 cells, $p<0.06$, $n=3$) (Fig. 1B).

TPH immunoreactivity was measured in the pons, which contains a portion of the median and dorsal raphe. Fraction and density of TPH immunostaining was reduced in the IUGR pons (Fig. 2A). SERT immunoreactivity was measured in serotonergic terminal fields (medial pre-frontal cortex). Fraction and density of SERT immunostaining was reduced in the IUGR cortex (Fig 2B).

Discussion

IUGR is a known risk factor for the development of psychiatric disease [5, 15, 17]. This study is the first to examine potential molecular underpinnings of these elevated risks in nonhuman primates. Importantly, impaired function of serotonergic neurons is implicated in the etiology of numerous psychiatric conditions [1, 4, 6, 8, 11, 16]. We have shown significant disruption of central nervous system serotonergic neurons in a nonhuman primate model of IUGR. Disruptions in serotonergic development were evident at three functional levels: reduced mRNA expression, reduced protein expression and reduced serotonergic neuron numbers in the median and dorsal raphe. The decreases in mRNA and protein expression appear to be greater than the decrease in neuronal numbers, suggesting that the remaining neurons are less active in serotonergic signaling. More studies are needed to address the impact of IUGR on development of psychiatric disease, how these developmental changes impact life-long behavior of IUGR offspring and potential interventions to prevent adverse outcomes.

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References

1. Albert PR, Benkelfat C. The neurobiology of depression--revisiting the serotonin hypothesis. II. Genetic, epigenetic and clinical studies. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2013; 368:20120535.
2. Antonow-Schlorke I, Schwab M, Cox LA, Li C, Stuchlik K, Witte OW, Nathanielsz PW, McDonald TJ. Vulnerability of the fetal primate brain to moderate reduction in maternal global nutrient availability. *Proc Natl Acad Sci U S A*. 2011; 108:3011–3016. [PubMed: 21252306]
3. Ciavarrro GL, Calvaresi N, Botturi A, Bendotti C, Andreoni G, Pedotti A. The densitometric physical fractionator for counting neuronal populations: application to a mouse model of familial amyotrophic lateral sclerosis. *Journal of neuroscience methods*. 2003; 129:61–71. [PubMed: 12951233]
4. Cook EH Jr, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, Haas R, Courchesne E, Leventhal BL. Evidence of linkage between the serotonin transporter and autistic disorder. *Molecular psychiatry*. 1997; 2:247–250. [PubMed: 9152989]
5. Hoek HW, Brown AS, Susser E. The Dutch famine and schizophrenia spectrum disorders. *Social psychiatry and psychiatric epidemiology*. 1998; 33:373–379. [PubMed: 9708024]
6. Homberg JR, Lesch KP. Looking on the bright side of serotonin transporter gene variation. *Biological psychiatry*. 2011; 69:513–519. [PubMed: 21047622]
7. Kamat A, Nijland MJ, McDonald TJ, Cox LA, Nathanielsz PW, Li C. Moderate global reduction in maternal nutrition has differential stage of gestation specific effects on {beta}1- and {beta}2-adrenergic receptors in the fetal baboon liver. *Reproductive sciences*. 2011; 18:398–405. [PubMed: 21079239]
8. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996; 274:1527–1531. [PubMed: 8929413]
9. Li C, Levitz M, Hubbard GB, Jenkins SL, Han V, Ferry RJ Jr, McDonald TJ, Nathanielsz PW, Schlambitz-Loutsevitch NE. The IGF axis in baboon pregnancy: placental and systemic responses to feeding 70% global ad libitum diet. *Placenta*. 2007; 28:1200–1210. [PubMed: 17707905]
10. Li C, McDonald TJ, Wu G, Nijland MJ, Nathanielsz PW. Intrauterine growth restriction alters term fetal baboon hypothalamic appetitive peptide balance. *The Journal of endocrinology*. 2013; 217:275–282. [PubMed: 23482706]
11. Mulder EJ, Anderson GM, Kema IP, Brugman AM, Ketelaars CE, de Bildt A, van Lang ND, den Boer JA, Minderaa RB. Serotonin transporter intron 2 polymorphism associated with rigid-compulsive behaviors in Dutch individuals with pervasive developmental disorder. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2005; 133B:93–96.
12. Schlambitz-Loutsevitch N, Ballesteros B, Dudley C, Jenkins S, Hubbard G, Burton GJ, Nathanielsz P. Moderate maternal nutrient restriction, but not glucocorticoid administration, leads to placental morphological changes in the baboon (*Papio sp.*). *Placenta*. 2007; 28:783–793. [PubMed: 17382997]
13. Schlambitz-Loutsevitch NE, Howell K, Rice K, Glover EJ, Nevill CH, Jenkins SL, Bill Cummins L, Frost PA, McDonald TJ, Nathanielsz PW. Development of a system for individual feeding of baboons maintained in an outdoor group social environment. *Journal of Medical Primatology*. 2004; 33:117–126. [PubMed: 15102068]
14. Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. *Journal of microscopy*. 1984; 134:127–136. [PubMed: 6737468]
15. Susser E, Hoek HW, Brown A. Neurodevelopmental disorders after prenatal famine: The story of the Dutch Famine Study. *American journal of epidemiology*. 1998; 147:213–216. [PubMed: 9482494]

16. Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, Jiang L, Li C, Folstein SE, Blakely RD. Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *American journal of human genetics*. 2005; 77:265–279. [PubMed: 15995945]
17. Xu MQ, Sun WS, Liu BX, Feng GY, Yu L, Yang L, He G, Sham P, Susser E, St Clair D, He L. Prenatal Malnutrition and Adult Schizophrenia: Further Evidence From the 1959–1961 Chinese Famine. *Schizophrenia Bulletin*. 2009; 35:568–576. [PubMed: 19155344]

Figure 1. Expression of serotonergic mRNAs and stereological assessment of serotonergic neurons

A) Quantitative Real-Time PCR was used to assess the relative expression of TPH1, TPH2 and SERT mRNA in the midbrains of control (CTL) and IUGR fetal baboons. B) Stereological assessment of the number and density of TPH positive neurons in the medial and dorsal raphe nuclei, within the first 600 μ m of the fetal midbrain. The n is indicated by the numeral just above the x-axis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CTL, one-tail t-test.

Figure 2. Immunohistochemical analysis of protein expression in serotonergic cell bodies and terminals

A) Representative images of SERT and TPH immunoreactivity in the frontal cortex and pons respectively. B) Quantitative analysis of the density of immunostaining staining and fraction of the field stained for SERT or TPH. The n is indicated by the numeral just above the x-axis. * $p < 0.05$ compared to CTL, two-tailed t-test.