Archival Report

Biological Psychiatry

Sex-Specific Neurodevelopmental Programming by Placental Insulin Receptors on Stress Reactivity and Sensorimotor Gating

Stefanie L. Bronson, Jennifer C. Chan, and Tracy L. Bale

ABSTRACT

BACKGROUND: Diabetes, obesity, and overweight are prevalent pregnancy complications that predispose offspring to neurodevelopmental disorders, including autism, attention-deficit/hyperactivity disorder, and schizophrenia. Although male individuals are three to four times more likely than female individuals to develop these disorders, the mechanisms driving the sex specificity of disease vulnerability remain unclear. Because defective placental insulin receptor (InsR) signaling is a hallmark of pregnancy metabolic dysfunction, we hypothesized that it may be an important contributor and novel mechanistic link to sex-specific neurodevelopmental changes underlying disease risk.

METHODS: We used Cre/loxP transgenic mice to conditionally target InsRs in fetally derived placental trophoblasts. Adult offspring were evaluated for effects of placental trophoblast-specific InsR deficiency on stress sensitivity, cognitive function, sensorimotor gating, and prefrontal cortical transcriptional reprogramming. To evaluate molecular mechanisms driving sex-specific outcomes, we assessed genome-wide expression profiles in the placenta and fetal brain.

RESULTS: Male, but not female, mice with placental trophoblast-specific InsR deficiency showed a significantly increased hypothalamic-pituitary-adrenal axis stress response and impaired sensorimotor gating, phenotypic effects that were associated with dysregulated nucleotide metabolic processes in the male prefrontal cortex. Within the placenta, InsR deficiency elicited changes in gene expression, predominantly in male mice, reflecting potential shifts in vasculature, amino acid transport, serotonin homeostasis, and mitochondrial function. These placental disruptions were associated with altered gene expression profiles in the male fetal brain and suggested delayed cortical development.

CONCLUSIONS: Together, these data demonstrate the novel role of placental InsRs in sex-specific neurodevelopment and reveal a potential mechanism for neurodevelopmental disorder risk in pregnancies complicated by maternal metabolic disorders, including diabetes and obesity.

Keywords: Epigenetic, Insulin, Prefrontal cortex, Prenatal, Serotonin, Sex

http://dx.doi.org/10.1016/j.biopsych.2016.12.025

Diabetes, obesity, and overweight during pregnancy are prevalent risk factors for offspring neurodevelopmental disorders, including autism, attention-deficit/hyperactivity disorder (ADHD), and schizophrenia (1–8). Such pregnancy complications confer significant risk to offspring in the United States in particular, where one-third of reproductive-aged women are obese and more than 9% of pregnancies are affected by gestational diabetes (9–11). Male offspring are especially at risk because they are more vulnerable to prenatal insults than female offspring and are three and four times more likely to develop ADHD and autism, respectively (12,13). Animal models have identified fetal sex as a key determinant of lifelong outcomes; however, the molecular mechanisms mediating such sex-specific programming remain unclear (14–18). Impaired insulin action is common to these maternal metabolic conditions, where reduced insulin production is a hallmark of type 1 diabetes mellitus and impaired cellular responses to insulin are characteristic of type 2 diabetes, gestational diabetes, and obesity. Insulin dysfunction has been demonstrated in placental tissue from pregnancies complicated by diabetes, preeclampsia, intrauterine growth restriction, and inflammation (19–27). Critically, then, placental insulin signaling may serve as a novel mediator of neurodevelopmental programming by maternal adversity contributing to disease risk.

Throughout pregnancy, the placenta is important for fetal support because it delivers nutrients and growth factors, maintains a protective barrier, and initiates adaptive responses to intrauterine status signals (28–31). Insulin dynamically regulates placental function across gestation, promoting placental growth, angiogenesis, metabolism, and hormone secretion, especially during early pregnancy (32–34). Perturbation of

SEE COMMENTARY ON PAGE 81

ISSN: 0006-3223

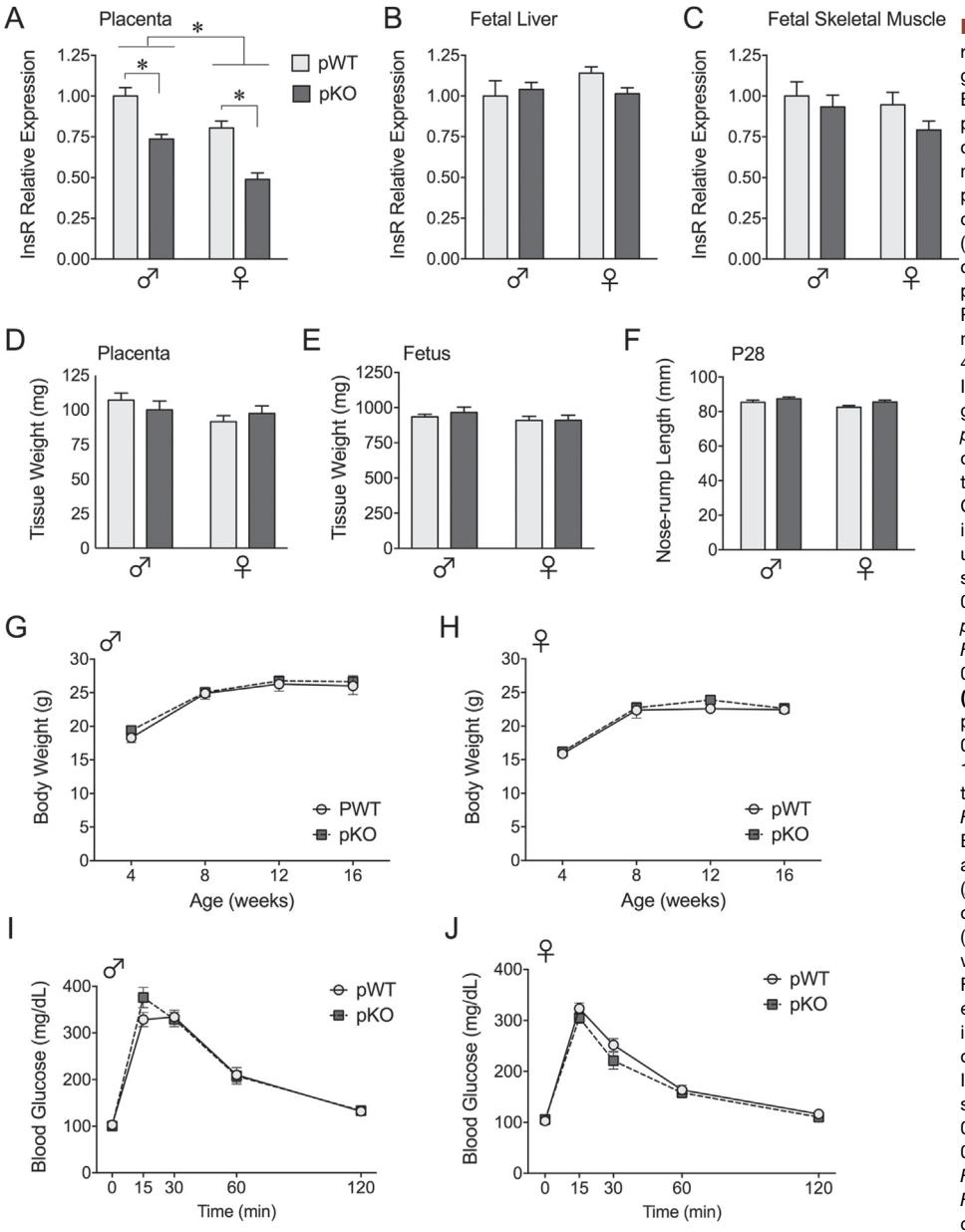

Figure 1. Placental-specific insulin receptor (InsR) targeting preserves growth and metabolic function. **(A–C)** Effective InsR targeting in fetally derived placental trophoblasts was confirmed by quantitative reverse transcription polymerase chain reaction in placental trophoblast-specific InsR deficiency (pKO) conceptuses and their littermate controls (pWT). **(A)** As expected, in the fully differentiated embryonic day (E) 12.5 placenta (n = 4/group), InsR messenger RNA was significantly reduced in pKO male and female mice (genotype: $F_{1,11}$ = 47.21, $p < .0001$). Male mice had higher InsR expression in the placenta at this gestational stage (sex: $F_{1,11}$ = 27.64, p = .0003); however, the effect of sex did not interact with genotype (interaction: $F_{1,11}$ = 0.38, p = .55). **(B, C)** Confirming tissue-specific InsR targeting, InsR messenger RNA remained unaltered in fetal insulin-responsive tissues, including liver (genotype: $F_{1,16}$ = 0.54, p = .47; interaction: $F_{1,16}$ = 2.056, p = .17) and skeletal muscle (genotype: $F_{1,14}$ = 2.11, p = .17; interaction: $F_{1,14}$ = 0.34, p = .57) at E17.5 (n = 5/group). **(D, E)** No differences in preparturition placenta weights (genotype: $F_{1,34}$ = 0.012, p = .91; interaction: $F_{1,34}$ = 1.42, p = .24) and fetus weights (genotype: $F_{1,34}$ = 0.30, p = .59; interaction: $F_{1,4}$ = 0.27, p = .61) were detected at E17.5 (n = 7–12 litters/group, weights averaged within litter). **(F)** In pKO mice (n = 8–10/group), there was a main effect of genotype on body length at weaning ($F_{1,30}$ = 4.83, p = .036); however, no within-sex differences were detected by Fisher's protected least significant difference test. **(G, H)** Analysis of body weight in these mice at 4, 8, 12, and 16 weeks of age indicated no impact of placental InsR deletion on growth across the lifespan of male mice (genotype: $F_{1,16}$ = 0.27, p = .61; genotype × age: $F_{3,48}$ = 0.32, p = .81) or female mice (genotype: $F_{1,10}$ = 2.81, p = .12; genotype × age: $F_{3,30}$ = 0.80, p = .50). **(I, J)** As further confirmation that metabolic processes were intact, pKO male and female mice showed no differences in their rate of glucose clearance as fasted adults (genotype: $F_{1,42}$ = 0.057, p = .81; genotype × sex: $F_{1,42}$ = 1.60, p = .21; genotype × sex × time: $F_{4,39}$ = 1.69, p = .17). Values are mean ± SEM. *$p < .05$.

these processes can elicit distinct responses in male and female individuals that can influence neurodevelopment throughout the entire course of gestation and may therefore underlie the sex-biased outcomes reported in animal studies (35). These unique strategies are likely mediated, in part, by X- and Y-linked gene expression by the fetally derived trophoblasts comprising the majority of the placenta (36,37).

To determine a novel mechanistic link between placental trophoblast-specific insulin receptor (InsR) dysfunction and sex-biased neurodevelopmental programming, we used the Cre/loxP system to conditionally ablate the InsR gene in fetally derived placental trophoblasts (38). Male and female mice were evaluated during adulthood for effects of placental trophoblast-specific InsR deficiency (pKO) on stress sensitivity, cognitive function, sensorimotor gating, and prefrontal cortical reprogramming, critical end points related to neurodevelopmental disorders. We hypothesized that sex differences in placental response to InsR deficiency would promote greater deficits in male mice than in female mice. To evaluate potential molecular mechanisms driving sex-specific programming, we assessed genome-wide expression profiles in the placenta and corresponding fetal brain.