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Hippocampal insulin resistance links maternal obesity with impaired neuronal plasticity in adult offspring

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ABSTRACT

Objective: Maternal obesity and a disturbed metabolic environment during pregnancy and lactation have been shown to result in many long-term health consequences for the offspring. Among them, impairments in neurocognitive development and performance belong to the most dreaded ones. So far, very few mechanistic approaches have aimed to determine the responsible molecular events.

Methods: In a mouse model of maternal diet-induced obesity and perinatal hyperinsulinemia, we assessed adult offspring's hippocampal insulin signaling as well as concurrent effects on markers of hippocampal neurogenesis, synaptic plasticity and function using western blotting and immunohistochemistry. In search for a potential link between neuronal insulin resistance and hippocampal plasticity, we additionally quantified protein expression of key molecules of synaptic plasticity in an *in vitro* model of acute neuronal insulin resistance.

Results: Maternal obesity and perinatal hyperinsulinemia result in adult hippocampal insulin resistance with subsequently reduced hippocampal mTor signaling and altered expression of markers of neurogenesis (doublecortin), synaptic plasticity (ampaloxO1, pSynapsin) and function (vGlut, vGAT) in the offspring. The observed effects are independent of the offspring's adult metabolic phenotype and can be associated with multiple previously reported behavioral abnormalities. Additionally, we demonstrate that induction of insulin resistance in cultured hippocampal neurons reduces mTor signaling, doublecortin and vGAT protein expression.

Conclusions: Hippocampal insulin resistance might play a key role in mediating the long-term effects of maternal obesity and perinatal hyperinsulinemia on hippocampal plasticity and the offspring's neurocognitive outcome.

1. Introduction

Obese pregnancies are on the verge of becoming the rule, not the exception in many developed countries. Only recently, neurocognitive impairments have emerged as a very unfavourable outcome in the offspring of obese pregnancies (Rivera et al., 2015). Specifically, human and animal studies have shown that maternal obesity is associated with numerous neurodevelopmental and psychiatric disorders, including intellectual deficits, anxiety, depression, attention deficit hyperactivity disorder, and autism (Edlow, 2017). Insulin has been identified as an important modulator of neuronal network development – mostly in the hypothalamus – during early phases of life (Dearden and Ozanne, 2015). In detail, hyperinsulinemia following maternal overnutrition has been shown to alter neuronal projections of feeding neurons located in the arcuate nucleus of the hypothalamus affecting the offspring's pancreatic parasympathetic innervation and glucose-stimulated insulin secretion (Vogt et al., 2014). Despite the undeniable relevance of neurocognitive long-term consequences, hippocampal neurogenesis, synaptic plasticity and function have not been the major focus of mechanistic studies on maternal obesity–related offspring pathology to date (Rivera et al., 2015; Dearden and Ozanne, 2015).

Previously, we have shown that maternal diet-induced obesity in the mouse results in transiently increased body weight and body fat content at the end of lactation, going along with profound

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hyperinsulinemia and impaired glucose tolerance at postnatal day (P) 21 (Janoschek et al., 2016; Bae-Gartz et al., 2016). After being weaned to standard chow, however, 70 day old offspring of obese mouse dams appear to be metabolically indistinguishable from control offspring (Janoschek et al., 2016; Bae-Gartz et al., 2016). In the current study, we aimed to determine the long-term effects of maternal obesity and the consecutive major changes in early metabolic environment on hippocampal insulin sensitivity and synaptic plasticity and function in the offspring at P70. Since insulin's intracellular signaling cascade involves activation of mTor (mammalian target of rapamycin), a well characterized serine/threonine kinase which has recently gained attention due to its involvement in neurocognitive function (Graber et al., 2013; Bergeron et al., 2014) and synaptic plasticity (Sosanya et al., 2015; Dwyer et al., 2015), we additionally set out to examine whether reduced mTor activation in the hippocampus might link hippocampal insulin resistance with altered plasticity of hippocampal neuronal networks in adult offspring of obese mothers.

2. Material and methods

2.1. Experimental design and animal procedures

All animal procedures were conducted in compliance with protocols approved by local government authorities (Land NRW) and were in accordance with National Institutes of Health guidelines. Mice (C57BL/6N) were bred locally at a designated animal unit of the University Hospital of Cologne (Cologne, Germany).

For detailed information on experimental design, animal housing, diet content, and mating scheme, see (Janoschek et al., 2016; Bae-Gartz et al., 2016). Briefly, control (CO) females, receiving standard chow at all times, and high fat diet (HFD)-fed females, receiving high fat diet starting upon their weaning at three weeks of age for the rest of the experiment, were mated at 12–14 weeks of age. All offspring studies were performed using male offspring. On P3, litter size was adjusted to six for each litter. At P21, in different subsets of only one animal per litter, either blood samples were taken, glucose tolerance testing (GTT) was performed, or animals underwent micro computed tomography (μCT) measurement. At P70, again, in subsets of one animal per litter, either blood samples were taken, GTT was performed, or animals were sacrificed. Hippocampus from both hemispheres was rapidly dissected and stored at −80 °C. Also, liver, skeletal muscle, and white adipose tissue were dissected, snap-frozen, and stored at −80 °C. The brain of one animal per litter was paraformaldehyde (PFA) fixated, mounted in tissue freezing medium, and stored at −80 °C for immunohistochemistry.

2.2. Intraperitoneal glucose tolerance test and analytical procedures

Glucose tolerance tests were performed as previously described (Janoschek et al., 2016; Bae-Gartz et al., 2016). Briefly, after overnight fasting (16 hrs), each animal received an intraperitoneal (ip) injection of 2 g glucose/kg body weight. Blood glucose levels were measured before glucose injection and after 15, 30, 60, and 120 min using an automatic glucose monitor (GlucoMen; A. Menarini Diagnostics, Berlin, Germany). Serum levels of insulin were measured by ELISA using mouse standards according to the manufacturer's guidelines (mouse insulin ELISA (EZRMI–13 K); Millipore CorpBillerica, MA).

2.3. Quantification of fat by μCT (micro computed tomography)

Whole mice were scanned post mortem with a μCT scanner (SkyScan 1176, Bruker, Belgium) with an isotropic voxel size of 35.26 μm^3. The x-ray settings for each scan were 45 kV and 475 μA using a 0.5 mm aluminum filter. All scans were performed over 360 ° with a rotation step of 0.6 ° and a frame averaging of 2. Images were reconstructed, analyzed and visualized using NRecon, CTAn and CTVox software, respectively (Bruker, Belgium). Images were segmented based on tissue density for both total volume and fat volume. Total fat volume was further segmented into visceral and subcutaneous fat using the abdominal muscular wall as orientation.

2.4. Cell culture experiments

HT22 cells were plated with a cell count of 10^6 per 10 cm dish and maintained in their feeding medium (DMEM (Gibco, 41966-029) + 10% FBS (Biochrom, S0615) + 1% Pen/Strep (Sigma Aldrich, P4458)) for 16 hrs. For 24hrs-induction of insulin resistance (Fig. 4 a, c, Suppl. Fig. 2 a, b), cells were exposed to fresh feeding medium either with (IR) or without (CO) 20 nM insulin for 24 hrs as described before (Kim et al., 2011). Following induction of insulin resistance, cells were washed with PBS and either acutely stimulated with fresh feeding medium containing 20 nM insulin for another 15 min or not. Cells were washed with PBS, harvested, and frozen at −80 °C for protein extraction and Western blotting. For 72hrs-induction of insulin resistance (Fig. 4 a, b, Suppl. Fig. 2 c), cells were exposed to fresh feeding medium either with (IR) or without (CO) 20 nM insulin every 24 hrs for 72 hrs. After 72 hrs, cells were washed with PBS and either acutely stimulated with fresh feeding medium containing 20 nM insulin for another 15 min or not. Cells were again washed with PBS, harvested, and frozen at −80 °C for protein extraction and Western blotting.

2.5. Western blotting

Frozen tissue or cells were homogenized in lysis buffer as previously described (Bae-Gartz et al., 2016). Protein concentration was determined with a BCA-Protein Assay Kit (Thermo Scientific, Waltham, USA). Lysates resolved on a 10% reducing SDS-PAGE gel were transferred to a PVDF membrane. Blots were probed with the antibodies (Suppl. Table 1).

2.6. Immunohistochemistry

For immunohistochemistry, 20 μm thick coronal cryosections were washed in PBS and permeabilized with 0.3% Triton-X-100 (Sigma-Aldrich, T8532) in PBS for 30 min. After blocking of non-specific binding components with Sea Block blocking Buffer (ThermoFisher, 37527) for 2 h, samples were incubated with primary antibodies (Suppl. Table 1) dissolved in antibody diluent (Dako, #S202230). After an overnight incubation at 4 °C and intensive washing in PBS, samples were incubated with secondary antibodies at room temperature for 2 hrs. Slices were rinsed in PBS and cover-slipped in Fluoroshield Mounting Medium containing 4′,6-Diamidin-2-phenylindol (DAPI) (Abcam. ab104139). For morphometric analysis, fluorescence images were taken by Olympus BX43F with cellSens Dimension software (DP80 dual CCD Camera, cellSens Dimension (V1.8)) and analyzed with the aid of ImageJ software supplemented with Olympus viewer plugin. All analyses are performed in a double-blind setting by at least two independent researchers.

2.7. Statistics

Values are reported as mean +/− SD. All statistical analysis was performed in GraphPad Prism 6 software. After testing for normality, we performed an unpaired t-test or a Mann-Whitney t-test (for non-parametric distribution). Significance was set at $p < 0.05$.

3. Results

3.1. Offspring metabolic phenotype at P21 and P70

Offspring of obese mouse dams show increased body weight, body fat content, serum insulin levels, and significantly increased 15 min