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Neonatal overfeeding increases capacity for catecholamine biosynthesis from the adrenal gland acutely and long-term in the male rat

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ABSTRACT

A poor nutritional environment during early development has long been known to increase disease susceptibility later in life. We have previously shown that rats that are overfed as neonates (i.e. suckled in small litters (4 pups) relative to control conditions (12 pups)) show dysregulated hypothalamic-pituitary-adrenal axis responses to immune stress in adulthood, particularly due to an altered capacity of the adrenal to respond to an immune challenge. Here we hypothesised that neonatal overfeeding similarly affects the sympathomedullary system, testing this by investigating the biochemical function of tyrosine hydroxylase (TH), the first rate-limiting enzyme in the catecholamine synthesis. We also examined changes in adrenal expression of the leptin receptor and in mitogen-activated protein kinase (MAPK) signalling. During the neonatal period, we saw age-dependent changes in TH activity and phosphorylation, with neonatal overfeeding stimulating increased adrenal TH specific activity at postnatal days 7 and 14, along with a compensatory reduction in total TH protein levels. This increased TH activity was maintained into adulthood where neonatally overfed rats exhibited increased adrenal responsiveness 30 min after an immune challenge with lipopolysaccharide, evident in a concomitant increase in TH protein levels and specific activity. Neonatal overfeeding significantly reduced the expression of the leptin receptor in neonatal adrenals at postnatal day 7 and in adult adrenals, but did not affect MAPK signalling. These data suggest neonatal overfeeding alters the capacity of the adrenal to synthesise catecholamines, both acutely and long term, and these effects may be independent of leptin signalling.

1. Introduction

Poor nutrition during early development can alter hypothalamic-pituitary-adrenal (HPA) axis function acutely and long-term, leading to exacerbated responses to psychological and physical stress. Prenatally, maternal nutrition can influence foetal exposure to glucocorticoids, and excessive exposure can result in hippocampal mineralocorticoid expression and impaired glucocorticoid negative feedback to stressful events later in life (Lesage et al., 2002, 2006). In humans, unbalanced high-protein diet during late pregnancy exposes the foetus to high cortisol levels in the maternal environment and this subsequently leads to developmental programming of the HPA axis and increased cortisol release in response to psychological stress in adult offspring (Reynolds et al., 2007). In rodents, the first two postnatal weeks are also critical in hypothalamic development that occurs almost entirely prenatally in humans and non-human primates (Bouret, 2012), and in these species neonatal diet also influences HPA axis function (Spencer and Tilbrook, 2009; Cai et al., 2016). Rats that are overfed as neonates have faster maturation of their HPA axes, with basal circulating glucocorticoid and adrenocorticotropic hormone (ACTH) concentrations increasing to adult levels earlier and paraventricular nucleus of the hypothalamus (PVN) corticotropin-releasing hormone (CRH) mRNA progressively decreasing to adult levels earlier than in control rats (Boullu-Ciocca et al., 2005). These effects lead to long-term HPA axis alterations, including elevated glucocorticoid responses to psychological stress (Boullu-Ciocca et al., 2005).

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In addition to the HPA axis, a functional response to stress also includes the sympathomedullary system wherein hypothalamic activation leads to adrenaline and noradrenaline release from the adrenal medulla to elevate sympathetic and suppress parasympathetic nervous system activity (Ulrich-Lai and Herman, 2009). The immediate and long-term impacts of poor nutrition during early development on the sympathomedullary system have not yet been fully established. However, Conceicao and colleagues recently demonstrated that neonatal overfeeding in rats can lead to an increase, in adulthood, in aromatic L-amino acid decarboxylase (DOPA) decarboxylase and tyrosine hydroxylase (TH); key enzymes for catecholamine synthesis. As such, these neonatally overfed rats have increased adrenal catecholamine content and secretion under non-stimulated conditions *in vivo* and an exacerbated response to caffeine stimulation *in vitro* (Conceicao et al., 2013). Our own previous studies have demonstrated long-term susceptibility of adrenal TH function to a neonatal immune challenge (Sominsky et al., 2013). The findings of our work suggest that the long-term effects of early life immune challenge, at least, on the adrenal gland may be directly due to changes occurring in the neonatal phase (Sominsky et al., 2012a, 2012b, 2013; Ong et al., 2012). Whether neonatal overfeeding has a similar acute impact on adrenal function in the context of the sympathomedullary system has not yet been tested. This distinction is important given that neonatal overfeeding also induces a long-term overweight/obese phenotype (Stefanidis and Spencer, 2012) that could independently account for effects on catecholamine production. We therefore hypothesised that neonatal overfeeding would induce increases in catecholamine biosynthesis in the neonatal phase that would be reflected in exacerbated responses to immune challenge long-term.

Neonatal overfeeding leads to pronounced hyperleptinaemia, as early as P7 (Stefanidis and Spencer, 2012; Sominsky et al., 2016). Leptin receptors are expressed in the adrenal cortex and medulla (Glasow and Bornstein, 2000), and leptin has been implicated in the direct regulation of catecholamine production at the adrenal medulla, including early in life (Takekoshi et al., 1999). Thus, excess leptin at this time is likely to hyper-stimulate the immature adrenal medulla leading to excess catecholamine production, and may be one mechanism by which neonatal overfeeding causes long-lasting changes in the sympathomedullary axis and its responses to stress. We therefore further hypothesised that neonatal overfeeding disrupts leptin regulation of catecholamine production.

To test these hypotheses, we assessed the protein synthesis, phosphorylation and activity of adrenal TH, the rate-limiting enzyme in catecholamine biosynthesis, in the neonatal and adult periods after neonatal overfeeding. In adults, we investigated the capacity for catecholamine biosynthesis both under basal conditions and in response to challenge with LPS. TH can be phosphorylated and activated by mitogen-activated protein kinase (MAPK) signalling (Dunkley et al., 2004), and leptin induces catecholamine synthesis by activating the MAPK cascade, specifically through extracellular signal-regulated kinase 1/2 (ERK1/2) signalling (Shibuya et al., 2002). We therefore also assessed the effects of neonatal overfeeding on ERK1/2 phosphorylation in adult adrenal glands, under basal and LPS-stimulated conditions.

2. Methods

2.1. Animals

Wistar rats were sourced from the Animal Resources Centre, WA, Australia. They were timed-mated at this facility and transported to the RMIT University Animal Facility on gestational day 13. The dams (and offspring) were housed at 22 °C on a 12 h light/dark cycle (0700–1900 h). They were given *ad libitum* standard pelleted rat chow and water. Chow composition was as described in (Kenny et al., 2014). We conducted all procedures in accordance with the National Health and Medical Research Council Australia Code of Practice for the Care of Experimental Animals and RMIT University Animal Ethics Committee approval. Other tissues from these rats have been used in previously published experiments (Cai et al., 2016).

2.2. Litter size manipulation

As described in (Cai et al., 2016), on the day of birth (P0) we removed all pups from their dams and randomly fostered them to new dams in litters of 12 (control litter; CL) or four (small litter, SL, neonatally overfed). Dams did not receive any of their own pups and each new litter was made up of 50% males and 50% females. Excess pups were culled. This litter size manipulation results in neonatally overfed pups being significantly heavier by as early as P7 and heavier throughout life (Spencer and Tilbrook, 2009; Stefanidis and Spencer, 2012; Smith and Spencer, 2012).

We used the offspring in experimentation either as neonates (P7: n = 6 CL and n = 6 SL, or P14: n = 6 CL and n = 6 SL) or allowed them to grow into adulthood; approximately P70 (CL: n = 7 saline 30 min; n = 8 LPS (*Escherichia coli*, serotype 026:B6; L-3755; Sigma, St Louis, MO, USA; 100 μg/kg) 30 min; n = 8 LPS 24 h. SL: n = 6 saline 30 min; n = 5 LPS 30 min; n = 5 LPS 24 h). For those in the adult experiments, we separated the pups into same-sex littermate pairs upon weaning at P21 and left them undisturbed until experimentation, expect for the usual animal husbandry. We used only males in these experiments, keeping the females for use in other studies (Sominsky et al., 2016). We derived all experimental groups from three or more litters per group, using a maximum of two pups from the same litter for an experimental treatment (Spencer and Meyer, 2017). We culled all the rats in the beginning of the light phase of the 12 h light/dark cycle to minimize the potential effects of circadian variation.

2.3. Adrenal preparation

After deeply anaesthetising the rats with Lethabarb (150 mg/kg pentobarbitone sodium, i.p.), we excised the adrenal glands and stored these samples at −80 °C until assay as previously described (Ong et al., 2014). For TH Western blotting and tritiated water release assays the right adrenals were thawed and sonicated in 500 mL of homogenizing buffer (2 mM potassium phosphate buffer, 1 mM EGTA, 1 mM EDTA, one protease inhibitor cocktail tablet per 50 mL, one PhosStop tablet per 50 mL, 1 mM DTT, 80 mM ammonium molybdate, 1 mM sodium pyrophosphate, 1 mM sodium vanadate, 5 mM b-glycerolphosphate, 2 mM microcystin, pH 7.4) with a microsonicator (UP50H, Hielscher Ultrasonics GmbH, Teltow, Germany) for 30 s pulses at 4 °C. We then centrifuged the samples at 14 000 g for 20 min at 4 °C and collected the clear supernatant. From these we determined protein concentrations using Pierce BCA Protein Assay kits, according to the manufacturer's instructions. We then diluted the samples with homogenizing buffer to equalize protein concentrations to 1.5 mg/mL, separated the diluted samples into two equal volumes and stored them at −80 °C. One aliquot of each sample was mixed with sample buffer (2% sodium dodecyl sulfate, 50 mM Tris, 10% glycerol, 1% DTT, 0.1% bromophenol blue, pH 6.8) and this was used for Western blotting. The second aliquot from the same sample was used for the tritiated water release assay.

2.4. Western blotting

For TH Western blotting, samples (30 mg of total tissue protein)

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