

The effect of maternal separation stress and high fat diet on tyrosine hydroxylase regulation in the rat adrenal gland

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Background: Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of catecholamines. In the short term TH activity is regulated by its phosphorylation at specific serine residues most particularly Ser40, whereas in the longer term TH is regulated by increases in TH protein. Previous studies *in vivo* have shown altered expression of TH mRNA and TH protein in adrenal gland in response to different types of stress. Maternal separation stress is known to exert lasting effects on the HPA axis but the impact on the adrenomedullary hormonal system is not known.

Aims: In this study we aimed to investigate the effects of maternal separation stress and palatable high fat diet on the adrenomedullary TH protein and TH phosphorylation at Ser40 in male and female rats.

Methods: Male and female Sprague-Dawley rats were exposed to maternal separation for 15 minutes per day (S15, control non-stressed group), 180 minutes per day (S180, stressed group) or left non-handled (NH, non-handled control group) from postnatal days 2-14. At weaning, postnatal day 21, half of pups of each gender from each treatment group were assigned to standard laboratory chow (11 kJ/g, energy 12% fat, 21% protein, 65% carbohydrate- NH, S15, S180 groups) and half to high fat diet (15.3 kJ/g, energy 32% fat, 18% protein, and 50% carbohydrate-NH+HFD, S15+HFD, S180+HFD groups). At 19 weeks of age the pups were anaesthetised by halothane exposure and sacrificed. Adrenal glands were rapidly removed and TH phosphorylation at Ser40 and TH protein were analysed by western blotting.

Results: TH protein was not significantly changed between the treatment groups in male rats. However, pSer40TH was significantly increased in the S180+HFD group relative to the S180 group (2 fold, $p < 0.05$) in male rats. In contrast, in female rats TH protein was significantly increased (1.7 fold, $p < 0.01$) in the NH+HFD group relative to the NH group while pSer40TH was significantly reduced (by 60%, $p < 0.05$) in the S180 group relative to the NH group.

Conclusions: We provide evidence for the first time that the maternal separation stress decreases pSer40TH in female rats but not in male rats. We also show that high fat diet increases TH protein content in non-stressed female rats and pSer40TH in stressed male rats. These data suggest that in female rats the maternal separation stress leads to the decreased capacity of the adrenal gland to synthesise catecholamines by inhibiting pSer40TH. High fat diet leads to the increased capacity of the adrenal medulla to synthesise catecholamines by increasing TH protein content (in non-stressed female rats) or by increasing pSer40TH (in stressed male rats). The gender differences observed in these studies may suggest that gonadal steroids contribute to the regulation of TH in the adrenal gland in response to the maternal separation stress and high fat diet.