ADRENOCORTICAL FUNCTION IN GLUCOCORTICOID RECEPTOR-DEFICIENT MICE

Zaniani GM[1], Chapman KE[1], Richardson RV[1], Morley SD[2], Kenyon CJ[1]

[1]University/BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, and [2]Division of Health Sciences, Chancellor's Building, University of Edinburgh, EH16 4TJ, UK

Introduction

The hypothalamic-pituitary-adrenal axis is hyperactive in humans and rodents that are heterozygous for null mutations of the glucocorticoid receptor (GR+/-) (1).

GR+/- mice have increased renal mass and salt-sensitive hypertension perhaps due to excess mineralocorticoid activity (2).

Renal mechanisms of hypertension have been investigated but the adrenal phenotype of GR+/- mice has yet to be studied.

Methods

Adult GR+/- heterozygous (HET) male mice and their wild-type (WT) littermates were infused subcutaneously for 2 weeks with bromodeoxyuridine (BrdU) to monitor cell proliferation. Tissues were fixed in formalin for histology. All work was carried out following UK Home Office Guidelines.

Adrenal cell size, proliferation & apoptosis were quantified in fixed adrenal sections using Image J software.

Immunohistochemistry was used to detect Aldosterone synthase (AS) and 11-beta hydroxylase (11β-OH) expression.

Results

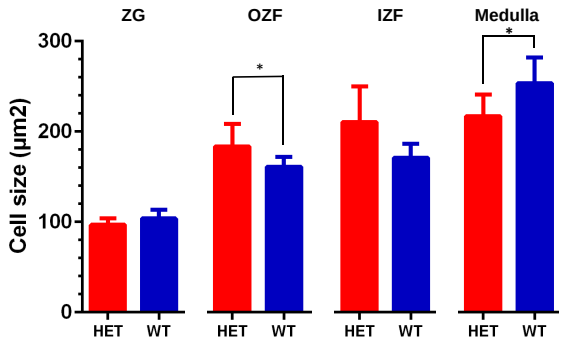


Figure 1: GR deficiency differentially affects cortical and medulla cell size. In H & E stained sections, the cross-sectional area (mean ± SE, n=5-6) of outer zona fasciculata cells (OZF) was increased in HET adrenals ($p<0.05$) but medullary cells were smaller ($p<0.05$). ZG and Inner ZF cells were not significantly affected. Adrenal mass was increased in HET mice, but this was not statistically significant in this cohort (data not shown).

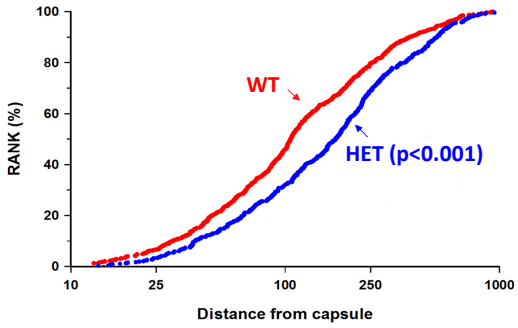


Figure 3: GR deficiency affects BrdU cell distribution. Cumulative distribution curves show distances from capsule of BrdU-positive cells in sections of WT and HET adrenals as they migrate inwards towards the medulla. There is a significant inward shift ($p<0.001$) in HET sections. However, this could reflect proportionately fewer proliferating cells in outermost ZG regions of HET adrenals.

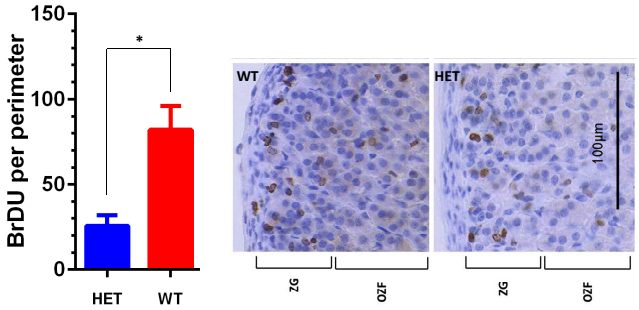


Figure 2: GR deficiency reduces cell proliferation in the adrenal cortex. Numbers of BrdU positive nuclei were reduced ($p<0.01$) in the cortex of HET mice, but were not significantly affected in the medulla.

Figure 4: GR deficiency does not affect apoptosis. TUNEL staining (blue, arrows) was variable in WT (A) and HET (B). There were no significant qualitative or quantitative differences between WT & HET mice.

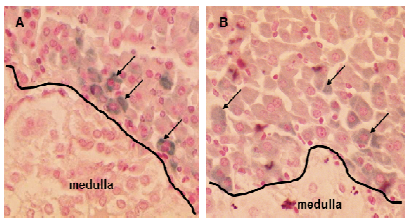
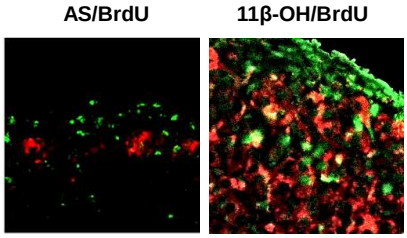


Figure 5: BrdU+ve cells express 11β-OH but not AS. BrdU (green nuclei) and 11β-OH (red) often co-localise in ZF cells but AS+ve ZG cells (red) are BrdU-negative. This pattern was seen in both HET and WT adrenals with perhaps greater 11β-OH intensity in HETs.



Conclusions

Impaired negative feedback of the hypothalamic-pituitary-adrenal axis in GR+/- mice may cause ACTH-dependent hypertrophy but not hyperplasia of the glucocorticoid-synthesising ZF cells.

Urinary aldosterone is increased in GR+/- mice but this does not appear to be due to ZG cell hypertrophy or hyperplasia. Steroid-dependent hypertension may explain the counter-regulatory decrease in medulla cell size.

References:

1. Michailidou Z, et al., GR haploinsufficiency causes hypertension and attenuates HPA axis & BP adaptations to high-fat diet. *FASEB J.* 2008; 22: 3896-3907.
2. Richardson R, et al., Salt-sensitive BP in glucocorticoid receptor deficient mice. *Endocrine Abstracts.* 2012: 28 P200.

Acknowledgements:

We thank Ruth Hamblin (http://surf.ed.ac.uk) for immunostaining, and Xiaofeng Zhao for advice on the TUNEL protocol. This work was supported by a Society for Endocrinology of Great Britain summer studentship award to GMZ.