Functional consequences of germline mutations in a novel non-RET medullary thyroid cancer susceptibility gene

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DOI:
10.1530/endoabs.38.OC5.1

Citation for published version (Harvard):

Download date: 14. Sep. 2023
OC4.4 Hyperinsulinaemia due to inhibition of 5a-reductases is ameliorated by liver-selective glucocorticoid receptor antagonism in diet-induced obesity
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Background
5a-reductase 1 (5aR1) metabolises steroids such as glucocorticoids and androgens, and is highly expressed in murine liver. Genetic disruption of 5aR1 leads to adverse metabolic changes in mice. We hypothesised that dutasteride, a 5aR inhibitor, induces insulin resistance in mice, as in humans, and this effect is underpinned by increased hepatic glucocorticoid action; an experimental paradigm was set up using A-348441, a liver-selective glucocorticoid receptor (GR) antagonist, and then utilised to assessed the contribution of increased hepatic glucocorticoid action to the metabolic consequences of dutasteride.

Methods
C57BL/6J male mice (n=8-15/group; age 12 weeks) were given high fat (HF), HF with A-348441 (KarolBio), HF + dutasteride (Dut), or HF + Dut + A-348441 diet for 4 weeks. Glucose tolerance tests (GTT) were performed at week 3, with mice culled at week 4. Plasma insulin and corticosterone were measured by ELISA and plasma glucose spectrophotometrically. Data are mean±S.E.M., *P<0.05 vs HF diet and †P<0.05 vs HF+Dut diet.

Results
Plasma corticosterone concentrations were not changed by A-348441, supporting liver-selective GR antagonism. A-348441 improved metabolic health of mice receiving a HF diet, preventing HF-induced bodyweight gain (34.3±0.5 g vs 31.8±0.4 g), and total white adipose depot weight gain (2.46±0.1 g vs 1.58±0.1 g), and attenuating HF-induced elevations in fasting plasma insulin, fasting glucose and insulin response to GTT (lowered by 52%, 25%, and 44% respectively). Inhibition of 5aRs with dutasteride impaired insulin sensitivity, with increased insulin response to GTT but did not change body weight, total adipose depot weight, fasting insulin, fasting glucose, or glucose response to GTT; A-348441 reduced this hyperinsulinaemia (235.9±17 ng/ml per min vs 329.3±16 ng/ml per min vs 198.4±25 ng/ml per min). Conclusions
Liver-specific GR antagonism ameliorates the metabolic consequences of acute diet-induced obesity. Hyperinsulinaemia caused by inhibition of 5aRs was ameliorated by A-348441, suggesting that hepatic glucocorticoid action plays a substantial role in metabolic dysfunction caused by 5aR inhibition. Moreover, targeting hepatic GR may be beneficial in maintaining metabolic homeostasis in diet-induced obesity.

DOI: 10.1530/endoabs.38.OC4.4

OC4.5 Glucagon increases energy expenditure independently of brown adipose tissue activation in humans
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Background
Energy expenditure (EE) is a highly effective treatment approach to treat obesity but no current drugs can safely achieve this. Cold exposure potently increases EE through brown adipose tissue (BAT) thermogenesis in humans. Glucagon elevates EE via BAT in rodents but the mechanism in humans is unknown. We investigated for the first time the mechanism in humans.

Methods
Eleven volunteers underwent measurement of EE using an indirect calorimeter at the start and end of three interventions: i) cold exposure; ii) control (vehicle); and iii) glucagon infusion (23°C). On each visit thermal images of the neck were taken – an increase in temperature is a non-invasive measure of increased BAT activity. All 11 volunteers also underwent a FDG PET–CT scan with cold exposure. In those in which this confirmed cold-induced BAT activity (n=8), they had a second PET–CT scan with either vehicle (n=4) or glucagon (n=4) infusion (23°C).

Results
EE rose by 14% with cold exposure and 15% following glucagon infusion (P<0.05 vs control). BAT deposits identified on the cold scan had significantly (4×) higher metabolic activity than on the vehicle or glucagon infusion scans, which were not significantly different from each other. There was a 0.31°C rise (P<0.001) in neck temperature on thermal images after cold exposure in the BAT positive cohort but not after glucagon or vehicle infusion.

Conclusions
Glucagon and cold exposure have a similar effect in stimulating energy expenditure but glucagon has no effect on the metabolic activity of classical adult supraclavicular BAT compared with cold exposure. This information is of importance to the development of better targeted and safe treatments designed to combat obesity through upregulation of energy expenditure.

DOI: 10.1530/endoabs.38.OC4.5

OC4.6 Cardiac fibrosis and the balance between glucocorticoid and mineralocorticoid receptors signalling
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Specific variations in the human glucocorticoid receptor (GR) gene associate with increased cardiovascular disease risk. GR signalling is essential for cardiac maturation in utero and adult mice with cardiacmyocyte and vascular smooth muscle deletion of GR (SMGRKO mice) have cardiac hypertrophy, fibrosis, and impaired function. Intriguingly, levels of left ventricle (LV) mRNA encoding the mineralocorticoid receptor (MR), which is pro-fibrotic in heart, rise postnatally in SMGRKO mice in parallel with the development of cardiac fibrosis. Here, the benefit of MR antagonism in limiting cardiac fibrosis was assessed in SMGRKO mice.

SMGRKO mice (generated via SM22α-Cre mediated deletion of GR) and control (Cre−) littermates were treated from birth with vehicle or 20 mg/kg per day spironolactone, an MR antagonist, administered in the drinking water to lactating dams until weaning then to offspring (n=10–13/group). At 8 weeks of age, hearts were collected for histology and mRNA profiling. Data were analysed by two-way ANOVA with Tukey’s multiple comparisons test.

Heart weight in male SMGRKO mice was higher than controls irrespective of spironolactone treatment (P<0.01). Interestingly, spironolactone modestly reduced heart weight in both genotypes (P<0.05). PicroSirius Red staining showed greater collagen levels in LV of SMGRKO mice (P<0.001); spironolactone treatment reduced the magnitude of this genotypic difference. Although spironolactone did not prevent the increase in LV levels of mRNA encoding MR or profibrotic factors (connective tissue growth factor, collagen1α2 and collagen3α1) in SMGRKO mice, it did attenuate collagen1α2 mRNA increases (P<0.05).

In conclusion, the modulatory effects of spironolactone on pro-fibrotic signalling suggest that elevated MR contributes to the pro-fibrotic cardiac phenotype discovered in SMGRKO mice. Consequently, MR antagonism may benefit individuals with particular variants of the GR gene. Spironolactone effects on heart weight indicate a role for MR in early life cardiac growth and SMGRKO mice are, potentially, a useful new model to investigate MR-dependent cardiac fibrosis.

DOI: 10.1530/endoabs.38.OC4.6

Thyroid and parathyroid

OC5.1 Functional consequences of germline mutations in a novel non-RET medullary thyroid cancer susceptibility gene
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Whilst the majority of familial medullary thyroid cancer (MTC) is caused by germline mutations of the RET proto-oncogene, there are families and individuals
OC5.3
Use of 11C-methionine PET to localise parathyroid adenoma/hyperplasia: a single centre experience
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Introduction
It is established practice to localise parathyroid lesions preoperatively using ultrasound (US) and sestaMIBI (MBI). Whilst these imaging techniques have good sensitivity/specify, there are patients in whom imaging does not localise a parathyroid lesion. 11C-Methionine PET (MET PET) is an imaging modality where 11C-methionine, a radioactive tracer, is taken up at sites of protein/peptide synthesis and has been demonstrated to be effective in localising parathyroid lesions. We therefore investigated the clinical utility of this imaging technique at our centre.

Methods
All patients had biochemistry prior to imaging thought to be consistent with primary hyperparathyroidism. Criteria to undergo PET imaging were inability of conventional imaging to identify a parathyroid lesion, potential intrathyroidal parathyroid lesion, and three patients where mediastinal disease was suspected. Twenty patients underwent MET PET over an 18-month period.

Results
MET PET identified a parathyroid lesion in 14/20 patients. Three out of three of these were demonstrated to be mediastinal lesions, leading to a parathyroid adenoma being successfully resected by sternotomy. 11/20 demonstrated disease in the neck. Of these 3/11 parathyroid lesions were very deep in the neck adjacent to vertebral/oesophagus and not seen with US/sestaMIBI. In 2/11 patients MET PET demonstrated intrathyroidal parathyroid lesions and patients underwent hemithyroidectomy. All parathyroid lesions were confirmed on histology (13 adenoma and one hyperplasia). Of the 2/20 who had negative imaging, one now has a diagnosis of sarcoidosis with elevated 1,25-dihydroxycholecalciferol, one underwent bilateral neck exploration and histology demonstrated parathyroid hyperplasia. The remaining four patients are still being investigated with working diagnoses of PBH in three patients.

Discussion
MET PET is a useful additional functional imaging technique when conventional imaging fails to localise a lesion, where mediastinal disease is suspected or intrathyroidal disease needs confirmation. This can particularly helpful when deciding to refer patients for major surgery.

Examples

OC5.4
A novel modulator of cellular invasion and metastasis in endocrine cancer
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Metastasis is a multistep process responsible for the majority of endocrine cancer deaths. Central to the ability of cells to move is the recruitment of actin fibres at the periphery of the cell by key proteins, especially the cortical actin binding protein cortactin. A full understanding of cortactin function is required in order to address metastatic cell activity within endocrine cancer. We used IP-MS to discover protein binding partners, and now identify the proto-oncogene PBF as a new functional binding partner of cortactin, whose expression has recently been correlated with thyroid and breast cancer metastasis, and with colon cancer extra-mural vascular invasion. We show that cortactin and PBF interact and co-localise through immunofluorescence and Proximity Ligation Assays, and that this occurs particularly in more aggressive tumours, and significantly correlated with PBF expression. We also demonstrate the interaction between PBF and cortactin through co-immunoprecipitation assays and reveal that artificially targeting PBF to the plasma membrane results in increased cortactin binding, entirely blocking endogenous cellular invasion. Thus, we identify a new modulator of cortactin

Examples