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Targeting endogenous glucocorticoids in degenerative disease

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Introduction – is Cushing's syndrome only the tip of the iceberg?

Glucocorticoid hormones play a vital role in the adaptive response to acute stress. In the face of a threat to homeostasis, a rapid increase in circulating glucocorticoids, principally cortisol in humans, results in activation of corticosteroid receptors and altered transcription of a wide array of genes that represent as much as 5% of the genome. This variety is reflected in the diversity of the stress response, which includes mobilisation of energy stores, inhibition of 'vegetative' functions such as tissue repair and growth, counter-regulation of the inflammatory response, maintenance of blood volume and pressure, and neurocognitive adaptation. Together, this coordinated adaptive response helps maintain homeostasis in the presence of an acute stressor. However, should an excess of glucocorticoids persist, the effects of the stress response become maladaptive. This is exemplified by the well-known constellation of clinical features that occur in Cushing's syndrome, which is due to an excess of endogenous or exogenous glucocorticoids.

Although spontaneous (non-iatrogenic) Cushing's syndrome is rare, its clinical features are shared by a number of more common degenerative diseases (Fig 1), which has led many to question whether an excess of endogenous glucocorticoids plays a wider role in human disease than previously appreciated. Perhaps most striking is the similarity between Cushing's syndrome and the collection of features that define the 'metabolic syndrome': central obesity, insulin resistance, hypertension and dyslipidaemia. In metabolic syndrome, as with Cushing's syndrome, the risk of premature atherosclerotic disease is greatly increased; in fact, metabolic syndrome underlies much of the global burden of cardiovascular disease – the most common cause of death in the developed world. Cushing's syndrome is also characterised by debilitating effects on mood, cognitive function and musculoskeletal health (myopathy and osteoporosis). Accumulating evidence now suggests that a subtle chronic excess of endogenous glucocorticoids in a substantial proportion of the population may play a maladaptive role in accelerated ageing of the brain, musculature and skeleton.

Much of the literature that supports an association between an excess of glucocorticoids and these degenerative conditions relates to activation of the hypothalamic–pituitary–adrenal (HPA) axis and consequent increases in concentrations of cortisol in plasma. However, this provides little therapeutic opportunity, as interfering with the HPA axis risks preventing the cortisol response to stress, which is likely to be hazardous. More recently, increasing knowledge of the complex factors governing the action of glucocorticoids at the tissue and cellular levels has identified additional factors that contribute to their excess in degenerative diseases. This has led to the potential for novel therapies designed to reduce the action of cortisol in a targeted manner without interfering with the stress response. At least one such approach, involving inhibition of the cortisol-generating enzyme 11β-hydroxysteroid dehydrogenase type 1, has already translated into clinical trials. We review the evidence for the role of endogenous glucocorticoids in degenerative disease and progress to date in therapeutic targeting of this process.

Endogenous glucocorticoids in metabolic syndrome

Evidence from a number of sources indicates that subtle increases in levels of cortisol in plasma are associated with features of metabolic syndrome (including hyperinsulinaemia, hyperglycaemia, hyperlipidaemia and hypertension) and with cardiovascular disease.[1,2] The picture is potentially confused by the additional observation that levels of cortisol in plasma are

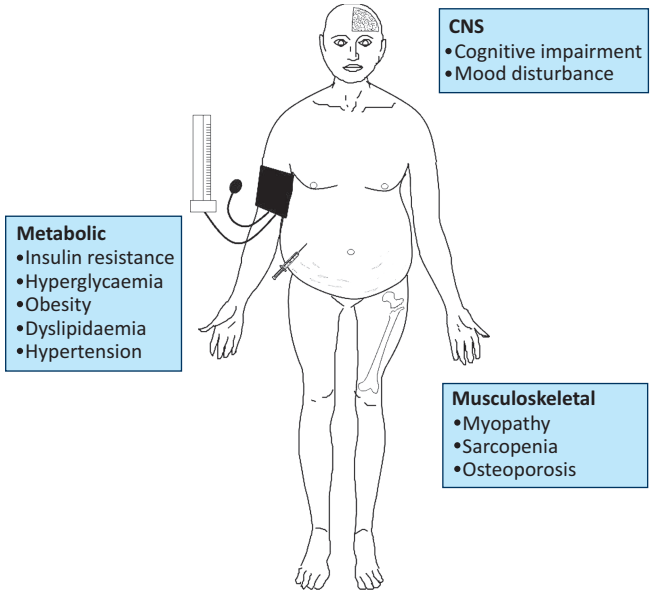

Fig 1. Shared clinical features of Cushing's syndrome and common degenerative diseases. The effects of an excess of cortisol on metabolism overlap with the key features of metabolic syndrome. Similarities also exist between the cognitive abnormalities seen in Cushing's syndrome and age-related cognitive decline. Muscle weakness and osteoporosis commonly seen in old age are characteristic features of Cushing's syndrome.

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lower in the morning, rather than higher, in patients with obesity; this is explained by enhanced metabolic clearance of cortisol in patients with obesity.

The observation of increased single basal concentrations of glucocorticoids in plasma in patients with metabolic syndrome has stimulated more detailed investigation of the dynamic control of the HPA axis. Although increased responsiveness to exogenous adrenocorticotropic hormone (ACTH) simply confirms that HPA activity is increased and associated with subtle adrenocortical hyperplasia, the finding of impaired habituation of cortisol in plasma in response to the stress of repeated sampling points to dysregulation of central control, in which negative feedback suppression is key. Results of dexamethasone suppression tests are normal in patients with metabolic syndrome, but this reflects only glucocorticoid receptor-mediated negative feedback and fails to test the contribution to suppression of the HPA axis mediated by cortisol binding to the mineralocorticoid receptor (MR) in the feedback centres of the brain. Recently, a novel test – the combined receptor antagonist stimulation of the HPA axis (CRASH) test, which employs blockade of feedback mediated by both low-affinity (glucocorticoid receptor; GR) and high-affinity (MR) receptors – demonstrated impaired central negative feedback of the HPA axis in people with obesity.[3] The mechanisms underlying this remain to be established, but hypothesised explanations include psychosocial stress in adult life or 'programming' of the HPA axis due to adverse conditions in utero and in infancy. In support of the latter, a low birth weight consistently predicts increased levels of cortisol in plasma in adult life, accompanied by an adverse cardiometabolic profile.[4]

An important finding in recent years is that tissue responsiveness to endogenous glucocorticoids is also disrupted in people with metabolic syndrome. A key mechanism involves activation and inactivation of glucocorticoids within target tissues by the 11β-hydroxysteroid dehydrogenase enzymes (11β-HSDs).[5] Expressed predominantly in the kidney, 11β-HSD type 2 (HSD2) inactivates cortisol, which prevents glucocorticoid-mediated activation of MRs in the kidney. In doing so, inactive cortisone is released into the circulation (Fig 2). Cortisone then acts as a substrate for the isoenzyme 11β-HSD type 1 (HSD1), which catalyses the reverse reaction to regenerate active cortisol.

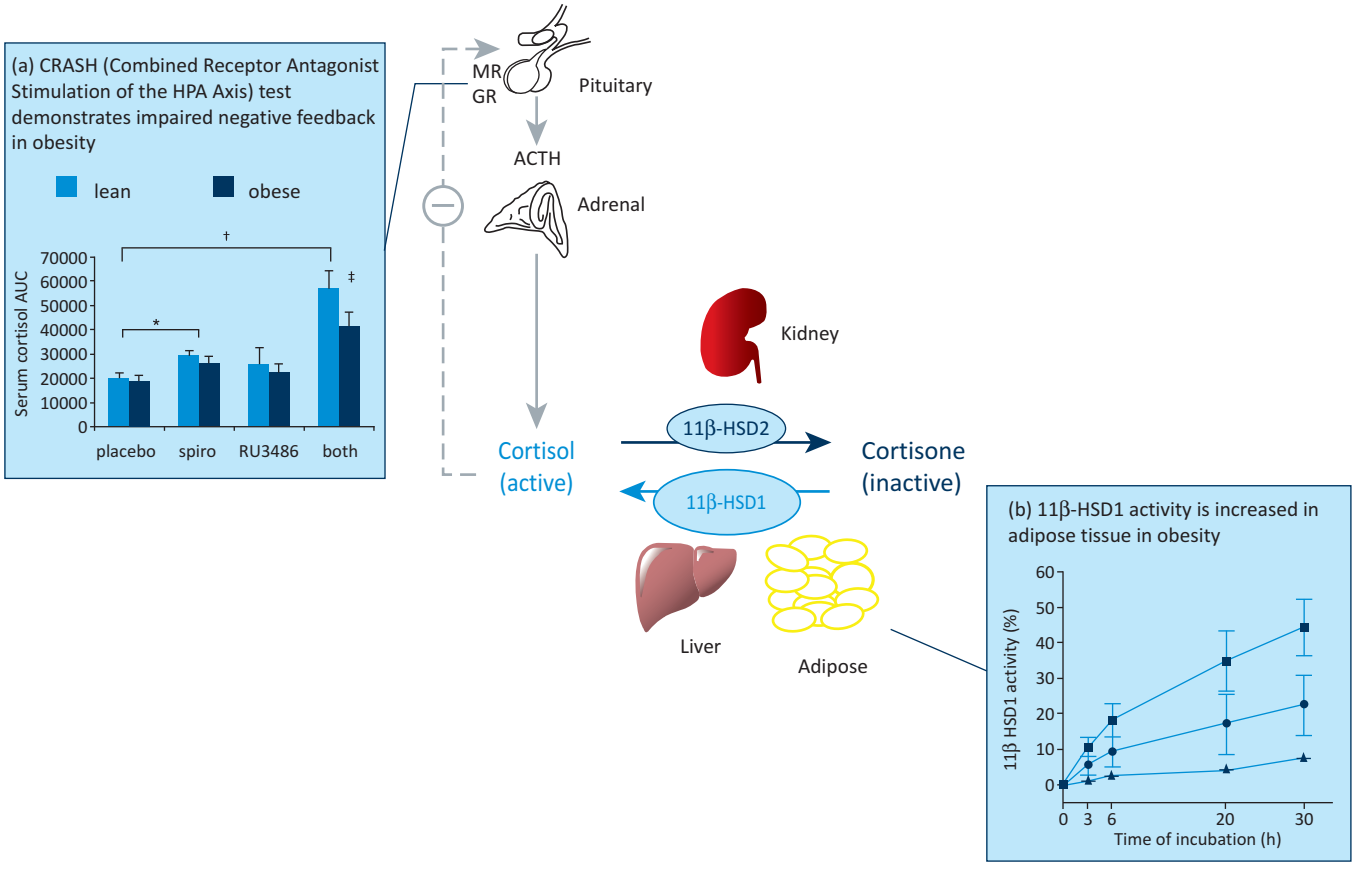

Fig 2. Central and peripheral regulation of cortisol action and its alteration in obesity. (a) Differences in lean and obese men in response to combined receptor antagonist stimulation. Data are mean area under the curve (AUC) ± standard error margin (SEM) in lean (n=15) and obese (n=16) men after placebo, spironolactone (mineralocorticoid receptor (MR) antagonist), RU38486 (glucocorticoid receptor (GR) antagonist) or spironolactone plus RU38486. *$p<0.001$; †$p<0.0001$ for responses to drug treatment in all subjects; ‡$p=0.002$ for difference in response to drug between lean and obese.[3] (b) Tissue-specific dysregulation of 11β-hydroxysteroid dehydrogenase type 1 (HSD-1) in obese men (n=16). Data are from otherwise healthy men from the lowest (triangle), middle (circle) and highest (square) tertiles of body mass index. Data are mean ± SEM. Adipose tissue 11β-HSD1 was measured by incubation of homogenised subcutaneous adipose tissue obtained by biopsy.[7]

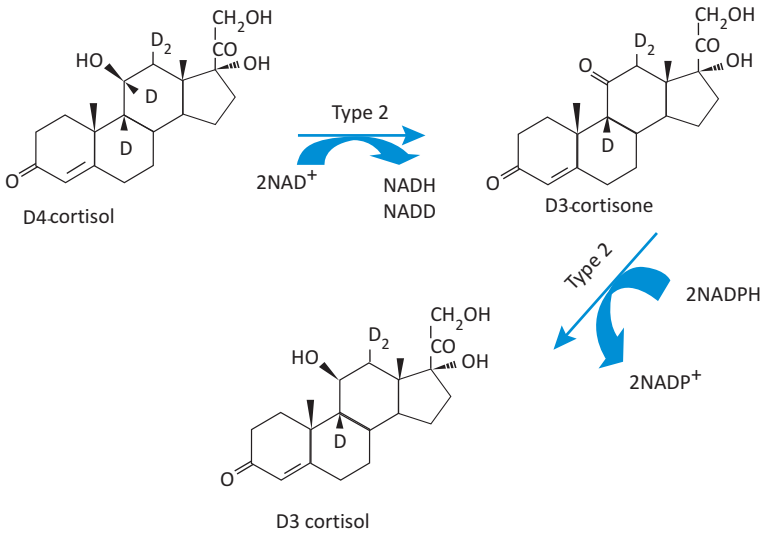

Fig 3. Schematic of metabolism of deuterated cortisol tracer by 11β-hydroxysteroid dehydrogenase type 1 (HSD-1). NAD^+ = reduced form of nicotinamide adenine dinucleotide; NADH = oxidised form of nicotinamide adenine dinucleotide; $NADP^+$ = reduced form of nicotinamide adenine dinucleotide phosphate; NADPH = oxidised form of nicotinamide adenine dinucleotide phosphate. Adapted from Andrew *et al*. 2002.[13]

Tissue-specific expression of 11β-HSD1 amplifies glucocorticoid action in selected tissues. Although 11β-HSD1 is expressed in most tissues, its levels are highest in adipose tissue and the liver. It has been possible to quantify the regeneration of cortisol by 11β-HSD1 using infusion of stable isotope tracers (Fig 3) in combination with arteriovenous sampling. Peripherally infused cortisol labelled with four deuterium atoms (D4-cortisol) is converted in a predictable manner to D3-cortisone through removal of one of the deuterium atoms by 11β-HSD2 (Fig 3). Subsequent conversion of D3-cortisone to D3-cortisol can be carried out only by 11β-HSD1. The dilution of infused D4-cortisol by newly formed D3-cortisol, detected by liquid chromatography mass spectrometry, thus quantifies 11β-HSD1 activity. In this fashion, generation of D3-cortisol – that is, the activity of 11β-HSD1 – has been quantified in the liver and adipose tissue in humans.[6] This mechanism may exist in these tissues so that locally regenerated active glucocorticoids can act in an autocrine manner to maintain activation of GRs and regulate metabolic function when plasma cortisol levels are low – for example, during sleep. Moreover, evidence of regulation of 11β-HSD1 expression by inflammatory and nutritional signals suggests that local levels of cortisol are physiologically regulated in a subtle, tissue-specific manner over and above the more crude influence of the HPA axis.

The 11β-HSD1 system is dysregulated in people with metabolic syndrome. In the subcutaneous fat of obese individuals, 11β-HSD1 is upregulated 2–3-fold – and more so in the presence of cardiometabolic complications of obesity including diabetes mellitus[7] – which predicts increased glucocorticoid activity in the adipose tissue and consequent exacerbation of metabolic dysfunction. Whether or not this 'Cushing's of the adipose tissue' is a primary or secondary phenomenon in obesity remains unclear. The pattern is more complicated in the liver, with downregulation of cortisol regeneration by 11β-HSD1 in obesity likely responsible for the increased metabolic clearance of cortisol in obesity discussed above, which is then reversed with the addition of insulin deficiency in type 2 diabetes.

Is it possible to develop therapies against the chronic endogenous excess of glucocorticoids in metabolic syndrome? Any such approach would need to be tissue specific, as attempts to reduce global synthesis of glucocorticoids – for example, using inhibitors of adrenal steroidogenesis – would result in compensatory upregulation of the HPA axis to maintain circulating levels of glucocorticoids. Moreover, it is critical to ensure that the integrity of the stress response is maintained. For these reasons, 11β-HSD1 seems an obvious target, as tissues in which the enzyme is upregulated, such as adipose tissue in people with obesity, would be predicted to have enhanced sensitivity to an 11β-HSD1 inhibitor.

Studies in animal models support the concept of 11β-HSD1 as a therapeutic target in metabolic syndrome, with knockout or inhibition of 11β-HSD1 in mice conferring protection against obesity, insulin resistance, dyslipidaemia and atherosclerosis.[8] These promising findings led to clinical investigation of 11β-HSD1 inhibitors in metabolic syndrome. Non-selective inhibitors, directed against both 11β-HSD1 and 11β-HSD2, include the liquorice-derived compound carbenoxolone, which has been licensed for some time as a treatment for peptic ulcer disease. Unfortunately, although carbenoxolone was effective in improving insulin sensitivity in the liver, it is not very potent and may not readily access the adipose tissue, so its overall benefit was small.[9] Subsequent selective inhibitors of 11β-HSD1, which avoid the potential for hypertension due to inhibition of 11β-HSD2, have demonstrated greater efficacy in clinical trials. For example, in a phase 2b trial involving 302 patients with type 2 diabetes and inadequate glycaemic control on metformin alone, the addition of 200 mg of Incyte's 11β-HSD1 inhibitor INCB13739 for 12 weeks resulted in a 0.6% decrease in glycosylated haemoglobin (HbA_{1c}), along with improvements in lipid profile and weight.[10] The drug was well tolerated, with reported adverse events similar to placebo. Whether this modest effect on HbA_{1c} will justify further commercial development of 11β-HSD1 inhibitors for type 2 diabetes in competition with existing more potent therapies is a moot point, and it is likely that additional benefits (on blood pressure, lipid profile, body weight and cardiovascular risk) will be the basis for progression of 11β-HSD1 inhibitors as a treatment of metabolic syndrome as a whole. At present, however, metabolic syndrome is not a therapeutic indication recognised by regulatory authorities for licensing of new agents.

An important question remains regarding the effects of 11β-HSD1 inhibitors on the HPA axis. Reduced contributions of the liver and adipose tissue to the circulating pool of cortisol would be expected to reduce the negative feedback signal to the axis. Indeed, ACTH was increased following 11β-HSD1 inhibition in

the study of INCB13739 described above, with unchanged concentrations of cortisol in the circulation indicating that this is a compensatory response. However, this enhanced ACTH drive means that adrenal androgen concentrations are increased, albeit within the reference range. Longer term studies should help to determine the clinical importance of such observations, but it is likely that 11β-HSD1 inhibitors will not be used in premenopausal women.

Targeting glucocorticoid action following vascular injury

Recent evidence suggests that glucocorticoids not only influence the development of atherosclerosis but may also determine its consequences by affecting the local response to acute vascular injury. In this context, a number of potentially beneficial effects of glucocorticoid action have been proposed, including induction of nitric oxide, anti-inflammatory effects and antiproliferative effects.[1] The latter may be of particular importance in preventing neointimal restenosis following intravascular injury during revascularisation procedures. Reassuringly, however, loss of 11β-HSD1 activity has not been associated with worsening of responses to vascular injury or inflammation. On the other hand, angiogenesis, which is crucial to tissue recovery following acute ischaemia, is inhibited by glucocorticoids. This angiostatic effect seems to be amplified by 11β-HSD1 generating cortisol within the vessel wall, as 11β-HSD1 knockout mice exhibit increased angiogenesis. This is advantageous, for example, in reperfusion following tissue ischaemia, as occurs in myocardial infarction; indeed 11β-HSD1 knockout mice have improved collateral revascularisation and are protected from left ventricular dysfunction following myocardial infarction. This observation raises the possibility that reducing endogenous glucocorticoid activity selectively in the ischaemic tissue may be therapeutically useful in patients with acute complications from cardiovascular disease.

Endogenous glucocorticoids and the brain

In Cushing's syndrome, chronic glucocorticoid excess can cause cognitive, mood and even psychotic disorders. This reflects the widespread role of glucocorticoids in the central nervous system (CNS), with effects on biochemistry; neurotransmission; and cell structure, birth and death. Could subtle glucocorticoid excess, similar to that seen in metabolic syndrome, also affect brain function?

Evidence in support of this hypothesis comes from a number of animal models. Animals with low glucocorticoid levels throughout life, either as a result of upregulation of GR and MR in HPA axis feedback sites or adrenalectomy with low-dose glucocorticoid replacement, display resilience to age-related cognitive decline. Similarly, increased levels of cortisol in plasma in humans have been associated with numerous CNS disorders and with accelerated age-associated cognitive impairment. By analogy with cardiometabolic disease, however, any therapeutic opportunity likely lies in understanding tissue-specific control of cortisol action in the brain.

11β-Hydroxysteroid dehydrogenase type 1 is widely expressed in the brain, potentially modulating glucocorticoid levels locally. In general, 11β-HSD1 null mice show normal cognitive function at a young age but are protected against cognitive decline with ageing. Clearly, this may be due in part to a favourable cardiometabolic profile, but tissue-specific manipulations suggest an additional direct effect of 11β-HSD1 in the brain, which is associated with improvements in neurogenesis and markers of CNS plasticity. Encouragingly, the effects of 11β-HSD1 deletion on cognitive function are recapitulated with enzyme inhibitors. Short-term studies in aged mice demonstrate improvements in tests of cognitive function after only two weeks of treatment with a CNS-active 11β-HSD1 inhibitor.[11] Clinical studies show evidence of improvement in cognitive function with the use of non-selective 11β-HSD1 inhibitors: in two small, randomised, placebo-controlled trials, treatment with carbenoxolone resulted in improvements in verbal fluency in healthy elderly men and verbal memory in patients with type 2 diabetes.[12] The key next step is to establish whether these beneficial effects extend to patients with dementia, including those with Alzheimer's disease.

Summary

The development of sophisticated techniques to assess glucocorticoid activity – from a whole-body level to a subcellular level – has enabled us to begin to explain the similarity between Cushing's syndrome and a number of human degenerative conditions. Tissue-specific modulation of glucocorticoid action seems to be a common feature in the pathophysiology of cardiovascular disease and cognitive decline and may also be important in other conditions such as chronic inflammatory disease, osteoporosis and sarcopenia. Importantly, this process is amenable to pharmacological intervention. The long-term nature of these conditions means that clinical trials to assess such potential therapies will be challenging. However, as the role of endogenous glucocorticoids in an increasing number of human diseases becomes clearer, it is likely that efforts to translate these therapies into clinical practice will intensify.

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