Tissue-specific Cushing's syndrome, 11β-hydroxysteroid dehydrogenases and the redefinition of corticosteroid hormone action

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Abstract

Two isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD) interconvert the active glucocorticoid, cortisol, and inactive cortisone. 11β-HSD1 acts predominantly as an oxo-reductase *in vivo* using NADP(H) as a cofactor to generate cortisol. In contrast, 11β-HSD2 is a NAD-dependent dehydrogenase inactivating cortisol to cortisone, thereby protecting the mineralocorticoid receptor from occupation by cortisol. In peripheral tissues, both enzymes serve to control the availability of cortisol to bind to corticosteroid receptors. 11β-HSD2 protects the mineralocorticoid receptor from cortisol excess; mutations in the *HSD11B2* gene explain an inherited form of hypertension, the syndrome of 'apparent mineralocorticoid excess', in which 'Cushing's disease of the kidney' results in cortisol-mediated mineralocorticoid excess. Inhibition of 11β-HSD2 explains the mineralocorticoid excess state seen following liquorice ingestion and more subtle defects in enzyme expression might be involved in the pathogenesis of 'essential' hypertension.

11β-HSD1 by generating cortisol in an autocrine fashion facilitates glucocorticoid receptor-mediated action in key peripheral tissues including liver, adipose tissue, bone and the eye. 'Cushing's disease of the omentum' has been proposed as an underlying mechanism in the pathogenesis of central obesity and raises the exciting possibility of selective 11β-HSD1 inhibition as a novel therapy for patients with the metabolic syndrome.

'Pre-receptor' metabolism of cortisol via 11β-HSD isozymes is an important facet of corticosteroid hormone action. Aberrant expression of these isozymes is involved in the pathogenesis of diverse human diseases including hypertension, insulin resistance and obesity. Modulation of enzyme activity may offer a future therapeutic approach to treating these diseases whilst circumventing the endocrine consequences of glucocorticoid excess or deficiency.

European Journal of Endocrinology **149** 163–168

Introduction

Harvey Cushing's elegant description of bilateral adrenal hyperplasia in association with pituitary basophilia did far more than define a new disease entity, 'Cushing's disease'. His meticulous depiction of the clinical features of the disease that bears his name informed us of the diverse and deleterious effects of cortisol excess. We now appreciate that Cushing's syndrome results in hypertension in >90% of cases, visceral obesity in 80% of cases and osteoporosis in 50% of cases. In many cases these features are reversible on correcting the cause – proof beyond doubt of the consequences of circulating cortisol excess. In subsequent years investigators have attempted to apply these findings to broader populations – subjects with 'essential' hypertension, 'simple' obesity and idiopathic osteoporosis but have drawn a blank. Endogenous Cushing's syndrome is excessively rare (incidence approximately one per million) and circulating cortisol concentrations are invariably normal (if not slightly reduced) in the above disease processes. Our emphasis has been to identify factors that might regulate corticosteroid hormone action at a tissue-specific level independent of these normal circulating concentrations. Here the metabolism of cortisol by isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) is of great relevance.

Cortisol metabolism and 11β-HSDs

The metabolism of cortisol is both complex and tissue dependent, but a major pathway is the interconversion

of active cortisol to inactive cortisone by 11β-HSD. To date, two isoforms of 11β-HSD have been extensively characterised. 11β-HSD1 was originally isolated from liver (1) but is also expressed in adipose tissue, gonad, brain, bone, ocular tissues and muscle (2). The *HSD11B1* gene is located on chromosome 1q32.2, is over 30 kb in length, principally due to the length of intron 4 (25 kb), comprises 6 exons and encodes a 34 kDa protein. The enzyme resides within the endoplasmic reticulum and activity is NADP(H) dependent. Activity is bi-directional possessing both dehydrogenase (cortisol to cortisone) and reductase (cortisone to cortisol) activity but *in vivo* in intact cells the enzyme appears to function almost exclusively as a reductase (3, 4). As a consequence the enzyme facilitates glucocorticoid receptor (GR)-mediated hormone action in tissues where it is expressed.

By contrast, 11β-HSD2 utilises NAD to inactivate cortisol to cortisone. The *HSD11B2* gene is found on chromosome 16q22, is ~6.2 kb in length, comprising 5 exons, and encoding a 44 kDa protein. The enzyme is located to the endoplasmic reticulum although perinuclear localisation has been demonstrated. 11β-HSD1 and 11β-HSD2 share only 14% sequence homology. In normal adult tissues, 11β-HSD2 is expressed in mineralocorticoid receptor (MR)-rich tissues, kidney, colon and salivary gland (5) where it serves to protect the MR from cortisol excess. *In vitro* the MR has similar affinity for cortisol and its cognate ligand, aldosterone; aldosterone occupies the MR *in vivo* only when cortisol is inactivated to cortisone in an autocrine fashion by 11β-HSD2.

11β-HSD2 is also expressed in many fetal tissues including the placenta (6). Recent data also suggest high levels of expression in some malignant tissues. At these sites 11β-HSD2 appears to protect the GR rather than the MR.

Cortisol secretion and metabolism are intricately linked and tightly controlled in order to maintain circulating cortisol levels. With increasing metabolic clearance of cortisol there is an associated increase in adrenocorticotrophin (ACTH) secretion and cortisol production in order to maintain circulating levels. The metabolism of cortisol therefore plays a critical role in determining the activity of the hypothalamo−pituitary−adrenal (HPA) axis. Following interconversion of cortisol and cortisone, A ring reduction by 5α/5β-reductases and 3α-hydroxysteroid dehydrogenase yields tetrahydrocortisone (THE), 5β-tetrahydrocortisol (THF) and 5α-tetrahydrocortisol (allo-THF). The ratio of urinary free cortisol (UFF) to urinary free cortisone (UFE) is an accurate measure of renal 11β-HSD2 activity (7). Abnormalities in this ratio can then be reflected in the urinary THF + allo-THF:THE ratio but if the UFF:UFE ratio is normal any change in the THF + allo-THF:THE ratio indicates an alteration in 11β-HSD1 activity (7) (Fig. 1).
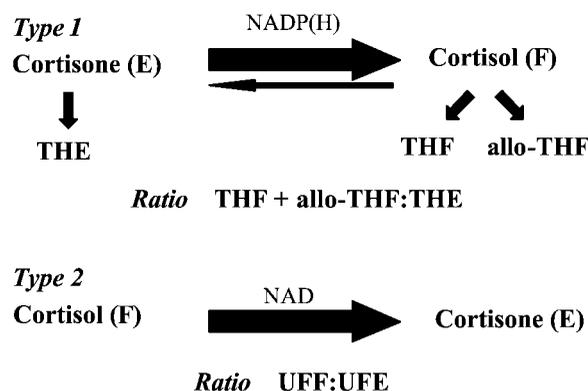

Figure 1 Two isoforms of 11β-hydroxysteroid dehydrogenase catalyse the interconversion of cortisol (F) to cortisone (E). Type 1, *in vivo*, functions almost exclusively as a reductase generating cortisol from inactive cortisone. Type 2 functions as a dehydrogenase inactivating cortisol and thus protecting the mineralocorticoid receptor.

11β-HSDs and human disease

Apparent mineralocorticoid excess and hypertension

Apparent mineralocorticoid excess (AME) is an inherited form of hypertension. The prevalence of the condition is unknown but is likely to be rare as less than 100 cases have been reported (8, 9). Presentation is usually in neonatal life or childhood with low birth weight, failure to thrive, hypertension and hypokalaemia. Hypokalaemia may result in arrhythmias, nephrogenic diabetes insipidus and rhabdomyolysis. Both plasma renin activity and aldosterone levels are suppressed in all cases of AME and the diagnosis is made by a urinary cortisol metabolite analysis with an increase in the UFF:UFE and THF + allo-THF:THE ratios. The half-life of cortisol is prolonged in AME as a consequence of the inhibition of cortisol-to-cortisone conversion. As a compensatory mechanism, endogenous cortisol secretion rate is reduced so as to maintain normal circulating corticosteroid levels. A milder variant of AME may present in later life. Again, patients are hypertensive with hypokalaemia but the THF + allo-THF:THE ratio is only mildly abnormal. In all cases cortisol is the offending mineralocorticoid because of failure to inactivate cortisol to cortisone at the site of the MR and the ensuing 'Cushing's disease of the kidney'.

AME is inherited as an autosomal recessive trait and is explained on the basis of mutations in the *HSD11B2* gene. There is a close correlation between genotype and phenotype − inactivating mutations result in severe, often fatal hypertension with presentation in early neonatal life, whilst mutations that encode cDNAs with residual enzyme activity present later in life with a milder phenotype (10). A summary of
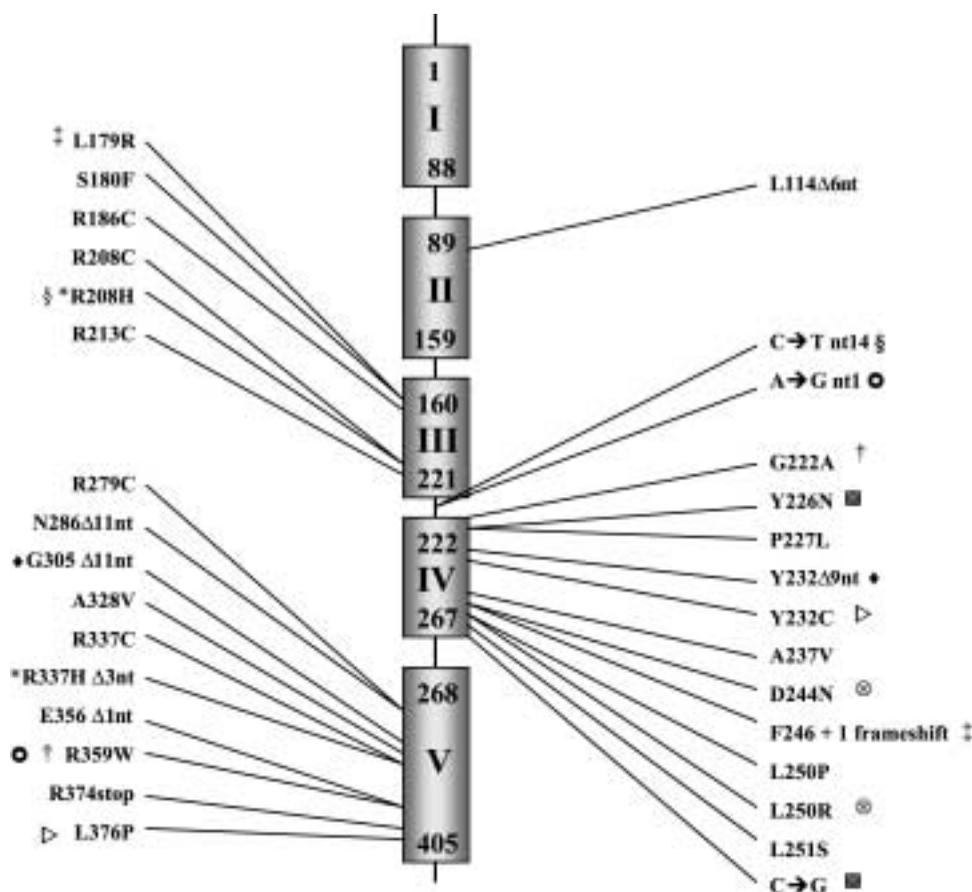
Figure 2 Mutations within the *HSD11B2* gene giving rise to the syndrome of 'apparent mineralocorticoid excess' (paired symbols represent compound heterozygotes).

their location within the *HSD11B2* gene is shown in Fig. 2.

Dexamethasone is not metabolised by 11β-HSD2 and, by suppressing cortisol secretion rate, has been used therapeutically in affected cases. Control of hypertension can be difficult and multiple anti-hypertensive agents are often needed.

These studies have raised the important question as to the role of 11β-HSD2 in patients with 'essential hypertension'. The prevalence of AME is unknown but milder cases are reported together with heterozygous HSD11B2 mutations presenting as 'essential hypertension' (11).

Association and linkage studies using CA nucleotide repeats and single nucleotide polymorphisms close to or within the *HSD11B2* gene have failed to demonstrate any association between HSD11B2 and essential hypertension *per se*, but the studies were not powered to exclude a minor effect. Association with hypertensive intermediate or sub-phenotypes including nephropathy and salt sensitivity has been demonstrated however and linked functionally to reduced renal 11β-HSD2 expression (12, 13).

Liquorice ingestion

The active component of liquorice is glycyrrhetinic acid (GE) and the use of liquorice as an anti-indigestion remedy ultimately led to the use of a derivative of GE, 18β-glycyrrhetinic acid, in the development of the anti-ulcer drug, carbenoxolone. The mineralocorticoid side effects of both liquorice and carbenoxolone are well documented and include oedema, dyspnoea and hypertension with hypokalaemia. It was initially thought that GE and carbenoxolone were direct agonists upon the MR, but our studies, endorsed by others, established that these compounds inhibit 11β-HSD2 to account for their mineralocorticoid effects. Treatment with spironolactone ameliorates these side effects. The clinical picture is one of an acquired form of AME as a result of cortisol-induced mineralocorticoid excess (14).

Ectopic ACTH syndrome

Patients with Cushing's syndrome secondary to ectopic ACTH production invariably develop hypertension with

hypokalaemic alkalosis due to a state of mineralocorticoid excess. This is explained on the basis of exceedingly high cortisol concentrations that saturate 11β-HSD2. The very high levels of urinary free cortisone indicate that the enzyme is performing at its maximal level, but nevertheless is insufficient to metabolise the excessive quantities of cortisol. Cortisol spills over to act on the MR to cause an AME phenotype (7, 15).

Cortisone reductase deficiency and polycystic ovary syndrome

Cortisone reductase deficiency (CRD) is in many ways the exact opposite of AME. Patients display a defect in the conversion of cortisone to cortisol, suggesting inhibition of 11 oxo-reductase activity and therefore, by implication, inhibition of 11β-HSD1. Less than 10 cases have been described, with one exception all are female (16–18). Aberrant cortisone metabolism increases the metabolic clearance rate of cortisol with activation of the hypothalamo–pituitary–adrenal axis and increased ACTH secretion to maintain normal circulating cortisol concentrations but at the expense of adrenal androgen excess. Therefore, patients have presented with a polycystic ovary syndrome (PCOS)-like phenotype with hirsutism, oligoamenorrhoea and infertility. Urinary tetrahydro-metabolites show almost exclusively THE with little or no detectable THF or allo-THF (THF + allo-THF:THE ratio < 0.05). Further studies have also shown impaired cortisol generation following an oral dose of cortisone acetate (16), data that implicate a defect in 11β-HSD1 as being causative in the syndrome of CRD. Recent in-house data suggest that CRD might represent a digenic disease, caused by mutations in HSD11B1 (that reduce but do not abolish 11β-HSD1 expression) and mutations in a novel endoplasmic reticulum enzyme, hexose-6-phosphate dehydrogenase that generates NADPH thereby conferring reductase activity upon 11β-HSD1 (19).

The prevalence of CRD in patients with PCOS is unknown but milder abnormalities in the activity of 11β-HSD1 have been reported in some PCOS cohorts (20).

Obesity, insulin resistance and the metabolic syndrome

The link between central or visceral adiposity and insulin resistance and premature mortality from cardiovascular disease has focussed attention on identifying factors that regulate fat distribution in addition to absolute fat mass. Patients with Cushing's syndrome develop florid, but reversible insulin resistance and central obesity in the setting of circulating glucocorticoid excess, but circulating cortisol concentrations are invariably normal in obesity (21). 11β-HSD1 is expressed in abundance in adipose tissue, specifically omental fat, where it acts as a reductase generating cortisol locally and facilitating glucocorticoid-induced adipocyte differentiation (4, 22). 'Cushing's disease of the omentum', mediated via the autocrine expression of 11β-HSD1, may explain the propensity of an individual to develop a central obese phenotype, an hypothesis that is supported through the phenotype of transgenic mice with targeted overexpression of 11β-HSD1 in adipose tissue (23). In the liver 11β-HSD1 also serves an important autocrine role by regulating glucocorticoid-induced hepatic gluconeogenesis. Thus mice lacking 11β-HSD1 resist hyperglycaemia following stress and over-feeding and show reduced levels of expression of gluconeogenic enzymes (e.g. phosphoenolpyruvate carboxykinase) (24). By promoting adipocyte differentiation and hepatic glucose output it is easy to envisage how 11β-HSD1 has become a novel therapeutic target in the treatment of metabolic syndrome. To support this concept selective inhibitors of 11β-HSD1 have been developed, that unlike the liquorice derivatives (glycyrrhetinic acid, carbenoxolone) have no inhibitory action upon 11β-HSD2. Preliminary and as yet short-term animal experiments indicate a beneficial effect of these arylsulphonamides upon glucose tolerance (25).

It seems unlikely, however, that a primary overexpression of 11β-HSD1 is a cause of central obesity. The data are conflicting but clinical studies suggest global inhibition, not stimulation, of 11β-HSD1 activity in obesity. THF + allo-THF:THE ratios are slightly reduced in obese subjects as are circulating cortisol concentrations following an oral bolus of cortisone. Furthermore, expression analyses conducted on both subcutaneous and omental fat biopsies show a trend (at least in our studies) for reduced 11β-HSD1 mRNA and activity with increasing body mass index (BMI) (26), findings that are reversible with weight loss. It is important to recognise the opposing effects of cortisol upon pre-adipocyte proliferation (inhibitory) and differentiation (stimulatory) and at an autocrine level 11β-HSD1 plays a regulatory role in both of these processes (Fig. 3). It should perhaps be regarded as an important protective rather than pathogenetic mechanism with inhibition of activity with increasing BMI (and thus reduced adipocyte differentiation and hepatic glucose output) serving to attenuate the deleterious consequences of obesity upon glucose tolerance. Human translational studies are now required utilising the selective 11β-HSD1 inhibitors to fully define the role of this enzyme in human obesity.

Other diseases

The recent identification of 11β-HSD1 within osteoblasts has implications for the development of both age-related and glucocorticoid-induced osteoporosis (27). 11β-HSD1 expression in bone increases with advancing age and is also positively regulated by pro-inflammatory cytokines including tumour necrosis factor-α and interleukin-1 (28, 29). Recent studies
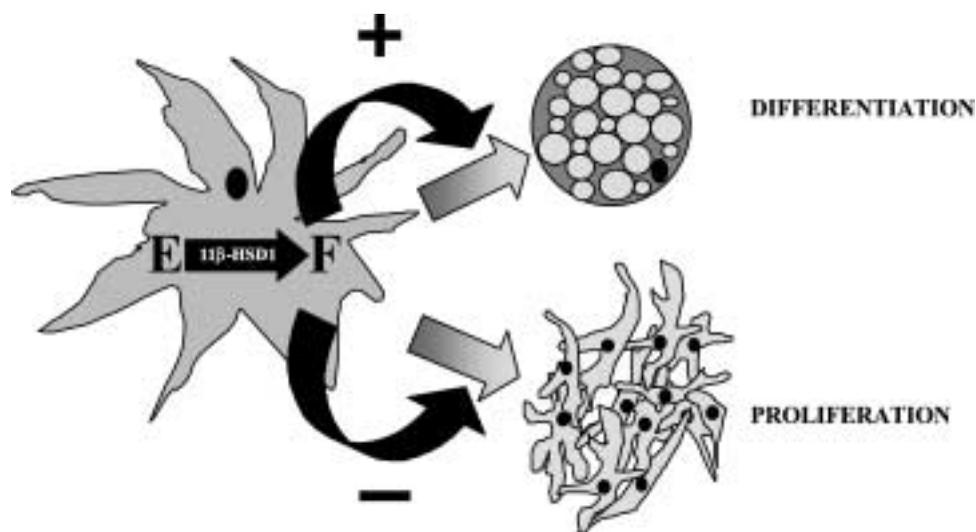
Figure 3 Proposed model for the role of 11β-HSD1 in regulating adipose stromal cell proliferation and adipocyte differentiation in human adipose tissue. The net effect of increased expression in whole adipose tissue would be to reduce preadipocyte proliferation whilst at the same time stimulating adipocyte differentiation.

also highlight a predictive role for 11β-HSD1 in the development of glucocorticoid-induced osteoporosis – individuals with the highest levels of 11β-HSD1 had the greatest deleterious effects of glucocorticoids upon the skeleton as measured by markers of bone formation.

Finally, and perhaps of some surprise for a sodium transporting epithelial tissue, the ciliary body of the eye expresses an abundant amount of 11β-HSD1 but no 11β-HSD2 (30). Here clinical data support the concept of local generation of cortisol within this intraocular tissue that serves to stimulate epithelial sodium transport and the production of intraocular fluid. Systemic inhibition of 11β-HSD1 is associated with a reduction in intraocular pressure, raising the intriguing possibility that topical inhibition of 11β-HSD1 within the eye might represent a novel approach to treat patients with glaucoma.

Summary

11β-HSDs play a pivotal role in the metabolism of cortisol, tightly controlling the exact concentration of cortisol that is available to bind to the GR (11β-HSD1) yet simultaneously protecting the MR from illicit occupation by cortisol (11β-HSD2). The two isoforms identified to date interconvert cortisol and cortisone, type 1 acting principally as a reductase *in vivo* to generate cortisol and type 2 acting exclusively as a dehydrogenase to inactivate cortisol to cortisone. Inhibition of 11β-HSD2 underpins the mineralocorticoid hypertension induced by liquorice consumption and carbenoxolone therapy. Defects in the *HSD11B2* gene are responsible for an inherited form of hypertension, AME, and the close relationship between genotype and phenotype in this condition raises the possibility that milder forms of AME or polymorphisms within the *HSD11B2* gene may be implicated in the pathogenesis of essential hypertension. Conversely, CRD is an inherited form of PCOS explained by mutations in the *HSD11B1* gene and a novel endoplasmic reticulum-specific re-dox enzyme, H6PDH. 11β-HSD1 appears to be intricately involved in insulin resistant states such as central obesity, and may also be implicated in the pathogenesis of osteoporosis and glaucoma. Importantly, 'tissue-specific Cushing's syndrome' mediated through aberrant expression of the 11β-HSD isozymes has uncovered novel therapeutic targets in the future treatment of common medical diseases.

Acknowledgements

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