STARLING REVIEW

11 β -Hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action

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Abstract

Two isozymes of 11 β -hydroxysteroid dehydrogenase (11 β -HSD1 and 11 β -HSD2) catalyse the interconversion of hormonally active cortisol and inactive cortisone. The enzyme evolved from a metabolic pathway to a novel mechanism underpinning human disease with the elucidation of the role of the type 2 or 'kidney' isozyme and an inherited form of hypertension, 'apparent mineralocorticoid excess'. 'Cushing's disease of the kidney' arises because of a failure of 11 β -HSD2 to inactivate cortisol to cortisone resulting in cortisol-induced mineralocorticoid excess.

Conversely, 11 β -HSD1 has been linked to human obesity and insulin resistance, but also to other diseases in

which glucocorticoids have historically been implicated (osteoporosis, glaucoma). Here, the activation of cortisol from cortisone facilitates glucocorticoid hormone action at an autocrine level. The molecular basis for the putative human 11 β -HSD1 'knockout' – 'cortisone reductase deficiency' – has recently been described, an observation that also answers a long standing conundrum relating to the set-point of 11 β -HSD1 activity. In each case, these clinical studies have been underpinned by studies *in vitro* and the manipulation of enzyme expression *in vivo* using recombinant mouse models.

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Background

In retrospect, the first appreciation of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) activity came through the discovery of cortisone. Hench, Kendall and Reichstein were awarded the Nobel Prize in the 1950s for their discovery of the glucocorticoid, cortisone (Kendall's compound E) (Kendall 1971), and they elucidated its therapeutic use in the treatment of rheumatoid arthritis (Kendall & Allen 1968). Cortisone has also been used in the treatment of adrenal insufficiency since the 1950s (Bush 1969).

However, studies by Cope and Black showed that the biological activity of any glucocorticoid relates, in part, to the presence of a hydroxyl group at position C11 of the steroid structure, and the inactivation of this group to a C-11 oxo group inactivates the steroid (Cope & Black 1958). Cortisol (Kendall's compound F), and the principal glucocorticoid in rodents, corticosterone (compound B), are active steroids whereas cortisone and 11-dehydrocorticosterone (compound A), possessing a C11 keto group, are inactive. Therefore, unbeknown to Hench, Kendall and Reichstein, the bioactivity of

cortisone was dependent upon conversion to cortisol in the liver. This was supported by studies that showed orally administered cortisone was, in part, excreted as cortisol (Sprague *et al.* 1950, Axelrod 1953, Burton *et al.* 1953).

Subsequently, cortisol was characterized as the active ligand and shortly thereafter the first description of tissues converting cortisol to cortisone was published. Amelung and colleagues (1953) discovered that the enzymatic inter-conversion of cortisol in man, and corticosterone in the rodent was performed by the 11 β -HSD enzyme, shown diagrammatically in Fig. 1. 11 β -HSD was designated the number EC1.1.1.146 by the Nomenclature Committee of the International Union of Biochemistry. 11 β -Dehydrogenase activity was subsequently observed in other tissues such as placenta (Osinski 1960, Bernal *et al.* 1980) and kidney (Jenkins 1966). This was surprising, given that Bush *et al.* (1968) described a liver enzyme *in vivo* which was capable of reductase activity, converting cortisone to cortisol. Isotopic studies (Hellman *et al.* 1971) and clinical studies measuring cortisol/cortisone (F/E) levels in patients with renal disease (Srivastava *et al.* 1973, Whitworth *et al.* 1989) confirmed that the kidney was an important site for cortisol to cortisone conversion.

Figure 1 The enzymatic actions of 11B-hydroxysteroid dehydrogenase on its substrates - interconverting inactive and active glucocorticoid.

Selective venous catheterisation studies indicated significantly lower circulating F/E ratios in renal venous blood compared with peripheral values. In contrast, circulating F/E ratios were much higher in hepatic venous blood, confirming that the liver predominantly converts E to F (Walker *et al.* 1993). These data suggested the existence of either a single bi-directional enzyme or two distinct isoforms, one catalysing oxidation the other responsible for reduction (Monder & Lakshmi 1989).

Subsequently, two distinct isoforms of 11β-HSD have been characterised and cloned in human tissues, 11β-HSD1 and 11β-HSD2 (Stewart 1996, White *et al.* 1997). The tissue-specific expression of the isoforms plays a crucial role in corticosteroid physiology by regulating glucocorticoid and mineralocorticoid receptor activation. Although both 11β-HSD isoforms control the inter-

conversion of biologically active glucocorticoids to their inactive 11-keto forms, there are important functional differences such as cofactor specificity, substrate affinity and direction of the reaction, which are summarised in Table 1.

The first 11β-HSD isozyme to be characterised was 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (Agarwal *et al.* 1989) and it was shown to act as a low affinity NADP(H)-dependant bi-directional enzyme, capable of carrying out both 11-oxo-reductase and dehydrogenase reactions. It is highly expressed in glucocorticoid target tissues where it facilitates glucocorticoid exposure to the glucocorticoid receptor (GR). However, the 11β-HSD1 isozyme could not explain the clinical or molecular basis of an inherited form of hypertension thought to be due to defective cortisol metabolism,

Table 1 Direct comparison between the characteristics of 11β-HSD1 and 11β-HSD2 isozymes

	11β-HSD1	11β-HSD2
Chromosomal location	1q32.2	16q22
Size	Gene: 30 kb, 6 exons Protein: 292 aa, 34 kDa	6.2 kb, 5 exons 405 aa, 44 kDa
Enzyme family	SDR superfamily	SDR superfamily
Tissue expression	Liver, lung, gonads, pituitary, brain, adipose	Kidney, colon, salivary glands, placenta
Enzyme kinetics	<i>In vitro</i> bidirectional <i>In vivo</i> mainly reductase, low affinity (K_m - μM) Cofactor NADP(H)	Only dehydrogenase High affinity (K_m - nM) Cofactor NAD
Function	Supplies cortisol to GR	Protects MR from cortisol

aa, amino acids.

'apparent mineralocorticoid excess', and there was great impetus to search for a second isozyme. The second isozyme, 11 β -HSD2, was discovered through the analysis of corticosteroid metabolism in isolated renal collecting duct cells and placental trophoblast. In these tissues, 11 β -HSD activity utilised NAD⁺ solely as a cofactor. Almost no reduction of 11-dehydrocorticosterone to corticosterone occurred, suggesting that the enzyme was a unidirectional dehydrogenase, and must therefore be a separate isozyme from 11 β -HSD1.

Although both isozymes are members of the short-chain dehydrogenase superfamily, they share only 21% homology, which is largely at the sites of conserved regions observed in short chain dehydrogenase/reductase (SDR) members. The isozymes are derived from separate gene products (Tannin *et al.* 1991, Albiston *et al.* 1994).

11 β -Hydroxysteroid dehydrogenase type 2 (11 β -HSD2)

11 β -HSD2 is a unidirectional, NAD-dependant dehydrogenase converting cortisol to cortisone. The isozyme has a high affinity for F with a K_m of 50 nM (100 times that of 11 β -HSD1) and 5 nM for corticosterone (Brown *et al.* 1993, Albiston *et al.* 1994, Stewart *et al.* 1994). 11 β -HSD type 2 cDNA has been isolated and cloned from human (Albiston *et al.* 1994), sheep (Agarwal *et al.* 1994), rat (Zhou *et al.* 1995), rabbit (Naray-Fejes-Toth & Fejes-Toth 1995) and mouse (Condon *et al.* 1997) kidney.

11 β -HSD2 localisation

The tissue distribution of 11 β -HSD2 has been ascertained by RNA blot hybridisation in human tissues, and the enzyme is expressed in high amounts in adult mineralocorticoid target tissues (Edwards *et al.* 1988), such as kidney (Agarwal *et al.* 1994, Albiston *et al.* 1994, Stewart *et al.* 1994) and colon (Whorwood *et al.* 1994). Immunohistochemistry (Krozowski *et al.* 1995) and *in situ* hybridisation studies (Whorwood *et al.* 1995) localised 11 β -HSD2 in the kidney to the renal collecting ducts in the cortex, most of the medulla, the connecting tubule and the distal convoluted tubule. Expression is also seen in surface epithelial cells in the colon (Whorwood *et al.* 1994), in salivary epithelial cells (Roland & Funder 1996) and in human skin (Kenouch *et al.* 1994). The expression of both 11 β -HSD isozymes, 11 β -HSD1 and 11 β -HSD2, in the kidney has been reported and shown to be species-specific, as high levels of 11 β -HSD1 mRNA have been found in the rat kidney, in proximal tubules (Agarwal *et al.* 1989), while in the human and sheep kidney, 11 β -HSD1 mRNA is barely detectable (Tannin *et al.* 1991, Yang *et al.* 1992).

A number of studies demonstrated NAD⁺-dependent dehydrogenase enzyme activity in human placenta (Brown

et al. 1993, Lakshmi *et al.* 1993) with oxo-reductase predominating in the decidua (Lopez & Craft 1981, Baggia *et al.* 1990). Studies have shown that 11 β -HSD2 is expressed in the placental trophoblast (Brown *et al.* 1993, Krozowski *et al.* 1995, Stewart *et al.* 1995, Sun *et al.* 1997). The capacity for 11 β -HSD2 to inactivate glucocorticoids in the placenta is very powerful, and this is supported by the placenta being the most abundant source of the enzyme, per mg of wet weight tissue (Shams *et al.* 1998). 11 β -HSD2 expression increases to term in the human, baboon and rat placenta (Burton & Waddell 1994, Shams *et al.* 1998, Pepe *et al.* 1999). In contrast, in the mouse placenta expression is reduced from gestation day 13 (term = 19.5 days) (Brown *et al.* 1996, Condon *et al.* 1997).

11 β -HSD2 is also documented in some malignant tissues; as expression is not seen in their non-malignant counterparts this has been implicated as part of the oncogenic process (see later).

The functional role of 11 β -HSD2 in mineralocorticoid receptor (MR)-expressing epithelia is to protect the MR from cortisol. The ligand for the MR *in vivo* is aldosterone but *in vitro* studies investigating MR binding (Krozowski & Funder 1983, Arriza *et al.* 1987) indicated that cortisol, corticosterone, and aldosterone all have equal binding affinities for the MR, with an affinity of around 0.5–1 nM (Arriza *et al.* 1988). The realisation of the importance of 11 β -HSD2 in this regard came through our understanding of an inherited form of hypertension – 'apparent mineralocorticoid excess'.

Apparent mineralocorticoid excess (AME)

Defective peripheral conversion of cortisol to cortisone (F-E) in patients with AME, reflecting impaired activity of 11 β -HSD2, was first suggested by Ulick and co-workers in the late 1970s (Ulick *et al.* 1979).

AME is a monogenic cause of hypertension, with an autosomal recessive inheritance pattern. Approximately 100 cases have been reported worldwide, including several cases of affected siblings (Ulick *et al.* 1979, Shackleton *et al.* 1985, Monder *et al.* 1986, Shackleton & Stewart 1990). AME is characterised by low renin, low aldosterone hypertension, and hypokalaemia. Patients with AME present as children or young adults with severe hypertension and hypokalaemia, suppressed plasma aldosterone concentrations, low plasma renin activity, and extended half-life of plasma cortisol (Ulick *et al.* 1979, White *et al.* 1997). The profound hypokalaemia can cause rhabdomyolysis and nephrogenic diabetes insipidus. Other renal abnormalities including renal cysts and nephrocalcinosis have been observed (Milford 1995, Moudgil 2000). Other clinical features include intra-uterine growth restriction (IUGR), short stature, thirst, polyuria and post-natal failure to thrive. The hypertension can be treated with the blockade of mineralocorticoid receptors with the potassium sparing diuretic, Spironolactone, or by a low salt

diet, thus it was initially thought that mineralocorticoid excess was causing the disorder. However, all known mineralocorticoids were found to be normal or reduced.

In the 1970s Ulick *et al.* (1979) suggested that the features of AME could be attributed to defective peripheral conversion of cortisol (F) to cortisone (E) due to impaired activity of 11 β -HSD. Urinary steroid metabolites from AME patients supported this hypothesis as AME was associated with abnormalities in the peripheral metabolism of cortisol, specifically an increase in the urinary excretion of the A-ring reduced cortisol metabolites (tetrahydrocortisol (THF) and allo-THF) versus those of cortisone (tetrahydrocortisone (THE)), suggesting defective 11 β -HSD2 activity (Mune *et al.* 1995). The excretion of 5 α -cortisol metabolites exceeds that of 5 β -cortisol metabolites, resulting in a high urinary allo-THF/THF ratio and suggesting an additional defect in 5 β -reductase activity (Shackleton *et al.* 1985, Monder *et al.* 1986). Typical THF+allo-THF:THE ratios range from 8 to greater than 70 in AME (reference range 0.7–1.1). However, the *in vivo* assessment of 11 β -HSD2 activity is complicated. The THF+alloTHF:THE measurement provides an index of overall 11 β -HSD activity with the principal contributors being 11 β -HSD1 in the liver and 11 β -HSD2 in the kidney. Therefore, unless the THF+alloTHF:THE is grossly abnormal it is unclear whether 11 β -HSD1 or 11 β -HSD2 is the main contributor to the ratio. The urinary free cortisol/urinary free cortisone ratio (UFF:UFE) may more accurately reflect renal 11 β -HSD2 activity. Normal individuals excrete $\sim$ twofold more UFE than UFF (reference range 0.5–0.8), reflecting 11 β -HSD2 activity in the kidney. In AME, UFE excretion is virtually undetectable (Palermo *et al.* 1996). However, others dispute the suggestion that the UFF:UFE ratio is more sensitive than the THF+alloTHF:THE ratio for detecting decreased 11 β -HSD2 activity (Ferrari *et al.* 2001).

Lack of 11 β -HSD2 in AME leads to a failure of the 'protective' mechanism preventing illicit MR activation and results in cortisol acting as a potent mineralocorticoid (Stewart *et al.* 1988). However, AME patients do not present with Cushingoid features. In the face of reduced metabolism, the presence of a normal negative feedback system causes a decrease in cortisol secretion rates, thus normal circulating cortisol levels are maintained (Stewart *et al.* 1988).

Therapeutic treatment of AME

The main aim of treating patients with AME should be to correct life-threatening hypokalaemia and to control blood pressure. Therapeutically, patients have been treated using a wide range of drugs, including potassium supplementation and the MR antagonist Spironolactone. Spironolactone has been of variable benefit, due to high doses being required to block the MR from cortisol activation. Triamterene and Amiloride, potassium sparing diuretics

which act by blocking the tubular sodium channels, have also had some success in the treatment of AME. Dexamethasone has had variable success, and works by suppression of cortisol secretion (dexamethasone itself is not metabolised to any great degree by 11 β -HSD2). Often, additional conventional anti-hypertension treatment is also required, such as angiotensin converting enzyme (ACE) inhibition in order to completely control blood pressure. Interestingly, Captopril (an ACE inhibitor) has been shown to be capable of enhancing renal 11 β -HSD2 activity and it has been theorised that it could be useful in treating AME patients with partial enzyme activity (Riddle & McDaniel 1994). Finally, a 'cure' for AME has been reported in a patient following kidney transplantation presumably because of normal 11 β -HSD2 activity in the transplanted kidney (Palermo *et al.* 1998).

Molecular basis for AME

At the time of its description, it was thought that mutations in the gene encoding 11 β -HSD1 might be responsible for AME. However, no mutations were identified in the four unrelated AME patients whose gene was first sequenced (White *et al.* 1994). With the discovery of the 11 β -HSD2 isozyme and the elucidation of its gene structure, the focus for determining the causative mutation for AME moved to 11 β -HSD2. The *HSD11B2* gene is 6.2 kb in length containing 5 exons and is located on chromosome 16q22 (Agarwal *et al.* 1995b). The *HSD11B2* gene from AME patients was screened for mutations by sequence analysis, and mutations were initially found to be present in exons 3, 4 and 5 in all the patients studied (Mune *et al.* 1995). Subsequently, mutations have been identified throughout the gene, although exons 3–4 remain a hotspot for mutations. Mutations on both alleles were found in all cases and thus AME is an autosomal recessive disorder. At present, over 33 different mutations have been defined within *HSD11B2* in approximately 60 kindreds; a comprehensive list of the mutations responsible for causing AME is shown in Table 2.

The mutations cause premature stop codons or amino acid substitutions which cause critical changes to protein structure. Expression of 11 β -HSD2 mutant cDNAs results in proteins that either have severely attenuated enzyme activity or else enzyme activity is absent. Therefore, there appears to be a close correlation between genotype and biochemical phenotype ranging from severe early-onset disease to a milder variety that may mimic adult-onset low-renin hypertension (Wilson *et al.* 1995a, Ferrari *et al.* 1996, Mune & White 1996). Genotypically, severe AME correlates with homozygous mutations that generate null enzymes conferring no protection on the MR, while mild AME due to mutations that generate enzymes capable of partial 11 β -HSD2 activity confer some MR protection.

Table 2 A comprehensive list of mutations identified in the gene encoding 11 β -HSD2 known to cause AME, and the number of cases identified

	Gene location	No. of patients	Reference
HSD11B2 mutation			
R74G	Ex 1	1	Quinkler <i>et al.</i> 2004
P75, Δ 1nt	Ex 1	1	Quinkler <i>et al.</i> 2004
L114, Δ 6nt	Ex 2	2	Odermatt <i>et al.</i> 2001
L179R	Ex 3	1	Nunez <i>et al.</i> 1999
S180F	Ex 3	1	Nunez <i>et al.</i> 1999
R186C	Ex 3	2	Ferrari <i>et al.</i> 1996; Dave-Sharma <i>et al.</i> 1998
R208C	Ex 3	3	Dave-Sharma <i>et al.</i> 1998; Mune <i>et al.</i> 1995
R208H	Ex 3	1, 1	Kitanaka <i>et al.</i> 1997; Nunez <i>et al.</i> 1999
R213C	Ex 3	4	Morineau <i>et al.</i> 1999; Mune <i>et al.</i> 1995; Rogoff <i>et al.</i> 1998
A221V	Ex 3	1	Quinkler <i>et al.</i> 2004
A–G nt 1	Int 1	1	Lavery GG <i>et al.</i> 2003
C–T nt 14	Int 3	1, 1	Mune <i>et al.</i> 1995; Nunez <i>et al.</i> 1999
D224N	Ex 4	1	Dave-Sharma <i>et al.</i> 1998
Y226N	Ex 4	1	Lavery GG <i>et al.</i> 2003
P227L	Ex 4	1	Wilson <i>et al.</i> 1998; Ugrasbul <i>et al.</i> 1999
Y232 Δ 9nt	Ex 4	1	Mune <i>et al.</i> 1995
Y232C	Ex 4	1	Lavery GG <i>et al.</i> 2003
A237V	Ex 4	1	Nunez <i>et al.</i> 1999
F246+1 frameshift	Ex 4	1	Nunez <i>et al.</i> 1999
L250P, L251S	Ex 4	3	Ferrari <i>et al.</i> 1996; Dave-Sharma <i>et al.</i> 1998; Mune <i>et al.</i> 1995
L250R	Ex 4	1	Dave-Sharma <i>et al.</i> 1998
C771G	Ex 4	1	Lavery GG <i>et al.</i> 2003
R279C	Ex 5	4	Li <i>et al.</i> 1998
N286 – 1 frameshift	Ex 5	1	Dave-Sharma <i>et al.</i> 1998
Δ 299	Ex 5	1	Lin-Su <i>et al.</i> 2004
G305 Δ 11nt	Ex 5	1	Mune <i>et al.</i> 1995
V322ins9nt	Ex 5	1	Quinkler <i>et al.</i> 2004
A328V	Ex 5	3, 1	Li <i>et al.</i> 1997; Morineau <i>et al.</i> 1999; Nunez <i>et al.</i> 1999
R337H, Δ Y338	Ex 5	3, 1	Dave-Sharma <i>et al.</i> 1998; Kitanaka <i>et al.</i> 1997; Mune <i>et al.</i> 1995
E356-1 frameshift	Ex 5	1	Ferrari <i>et al.</i> 1996; Dave-Sharma <i>et al.</i> 1998
R359W	Ex 5	1	Lavery GG <i>et al.</i> 2003
R374X	Ex 5	5	Stewart <i>et al.</i> 1996; Whorwood & Stewart 1996
L376P	Ex 5	1	Lavery GG <i>et al.</i> 2003

Ex, exon.

Recently, nine patients with AME from four families from Oman have been reported. Five mutations, four novel and the previously reported L114 Δ 6nt (Odermatt *et al.* 2001) were identified and, for the first time, mutations were identified within exon 1 of HSD11B2 (Quinkler *et al.* 2004). With the addition of this study, mutations have been identified in all exons and two introns of the HSD11B2 gene.

AME is most commonly found in consanguineous families (Mune *et al.* 1995, Wilson *et al.* 1995a,b, Stewart *et al.* 1996, White *et al.* 1997). Homozygosity in AME is thought to result from endogamy or a founder effect in Native American families with the R208C and E356 Δ 1nt mutations, and the L250S, L251P mutation (Mune *et al.* 1995, Wilson *et al.* 1995b). The fact that six kindreds are of Native American origin has prompted speculation as to a possible selective advantage of heterozygotes. Such

individuals may have an increased ability to conserve salt under conditions of extreme sodium deprivation (White *et al.* 1997). The Oman population comprises 2.2 million people with a majority (73%) of Omanis with tribal customs consisting of a strong preference for inter-family marriages. In 1995, Wilson *et al.* (1995a) described a family from Oman with two boys having AME syndrome, the parents and three other siblings being heterozygous for a R208C mutation. The more recent Oman cases derive from four different tribes; however the parents of one kindred and the grandparents of another kindred were second degree relatives. This suggests that the homozygosity of those mutations may be explained by consanguinity in the tribal society. Therefore, the diagnosis of AME should be suspected in patients from tribal societies who present with the features of low birth weight, failure to thrive, polyuria and polydipsia, and hypokalaemia and

hypertension (Quinkler *et al.* 2004). These data suggest that AME may be a common cause of hypertension in certain ethnic groups, such as the Oman population.

AME has generated interest in *HSD11B2* as a possible candidate gene for analysis in populations with essential hypertension, more specifically those with low renin, low aldosterone hypertension. Abnormalities in 11 β -HSD2 activity have been shown in patients with essential hypertension either by increases in the plasma [11- 3 H]cortisol half life or the THF+alloTHF:THE ratio (Walker *et al.* 1993, Soro *et al.* 1995). Genetic association studies using polymorphic markers within *HSD11B2* have been carried out to determine linkage between 11 β -HSD2 and hypertension. An Italian population was genotyped for a CA repeat polymorphism (11 alleles) in the first intron of *HSD11B2*, and an association between salt sensitivity (observed in AME hypertension) and shorter CA repeat length was observed, suggesting decreased *HSD11B2* expression with shorter CA repeat length. However, functional analysis has failed to support these data (Agarwal *et al.* 2000). A further study by our group has shown weak associations between the short alleles of the microsatellite within the *HSD11B2* gene, and type 1 diabetes mellitus and nephropathy – salt sensitivity and hypertension being phenotypes characteristic of diabetic nephropathy (Lavery *et al.* 2002). However, linkage and/or association studies to date have mainly been negative, probably because of underpowered studies; a direct genetic link remains to be defined (Brand *et al.* 1998, Smolenicka *et al.* 1998).

11 β -HSD2 knockout (KO) mouse

To further assess the role of 11 β -HSD2 *in vivo*, mice have been generated with targeted disruption of the 11 β -HSD2 gene. The homozygous mutation was generated by removing the genomic fragment encompassing exons 2–5 through specific recombination in mouse embryonic stem cells (Kotelevtsev *et al.* 1999). The mice appear normal at birth, but around 50% show motor weakness and die within 48 hours of birth. Female and male survivors were fertile and exhibited severe hypertension, hypokalaemia and polyuria, thus exhibiting a typical human AME syndrome phenotype. The epithelium of the distal tubule of the nephron displayed hypertrophy and hyperplasia. Interestingly, the heterozygote mice appeared to be like the wild-type littermates for all parameters studied, and to date no phenotype has been described. The 11 β -HSD2 KO mice will be a useful model to evaluate the role of cortisol in hypertension.

Malignancy and tumorigenesis

It has been well documented that glucocorticoids have an antiproliferative action by inducing cell cycle arrest at the

G1 phase (Sanchez *et al.* 1993, Rogatsky *et al.* 1997). 11 β -HSD2 expression has now been described in a number of tumours including adrenal adenomas and carcinomas (Coulter *et al.* 1999), lung adenocarcinomas (Suzuki *et al.* 2000) and adrenocorticotrophin (ACTH)-producing small cell lung cancer (Parks *et al.* 1998). Recent studies of bone and the pituitary have shown that both 11 β -HSD isozymes may be expressed at these sites. Specifically, our group has described a shift from predominant 11 β -HSD1 expression in normal tissue to 11 β -HSD2 in tumours (Bland *et al.* 1999, Cooper *et al.* 2000, Eyre *et al.* 2001, Korbonits *et al.* 2001). In each case the presence of 11 β -HSD2 in neoplastic cells was associated with GR rather than MR expression, suggesting an alternative function for the isozyme that is distinct from its classical role in MR-rich tissues, such as the colon or kidney. In view of the fundamental transregulatory role of the GR and its potential impact on cell proliferation and differentiation, we have hypothesised that abnormal expression of 11 β -HSD2 in GR-rich tissues will confer growth advantage and may be an important component of tumour initiation and/or progression.

11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1)

From the pioneering studies of Monder and White (1993), 11 β -hydroxysteroid dehydrogenase type 1 was purified from rat liver, which by sequence comparison was recognised to be an SDR family member. An antiserum was raised against the protein used to clone a rat cDNA that was shown to encode a protein of 287 amino acids (Lakshmi & Monder 1988, Agarwal *et al.* 1989, Monder & Lakshmi 1989), although the cDNA sequence was subsequently updated in 2002 (Nobel *et al.* 2002). The protein was subsequently termed 11 β -HSD1 and is a glycoprotein of 34 kDa. It has been shown to be expressed in a variety of tissues, including liver (Ricketts *et al.* 1998), lung (Hirasawa *et al.* 1999), testis (Monder *et al.* 1994) and decidua (Stewart *et al.* 1995, Sun *et al.* 1997).

11 β -HSD1 is a low affinity NADP(H)-dependent bi-directional enzyme, capable of carrying out both 11-oxo-reductase and dehydrogenase reactions, interconverting inactive cortisone and active cortisol (Fig. 2). The enzyme is microsomal (Ozols 1995) and NADP dependent; in a cell-free system it behaves mainly as a dehydrogenase and no reductase activity is detected in the purified preparation, as described by Ozols (1995). Homogeneous enzyme gave rectilinear Eadie plots and K_m constants of $1.83 \pm 0.06 \mu\text{M}$ for corticosterone and $17.3 \pm 2.24 \mu\text{M}$ for cortisol. First order rate constants were an order of magnitude higher for corticosterone than cortisol, but maximal velocities were similar (Lakshmi & Monder 1988).

Subsequently, 11 β -HSD1 cDNAs and proteins have been published for the human (Tannin *et al.* 1991),

Figure 2 The enzymatic reactions of 11 β -HSD1. 11 β -HSD1 activity levels can be determined by the urinary ratio THF+alloTHF:THE.

mouse (Rajan *et al.* 1995), squirrel monkey (Moore *et al.* 1993), sheep (Yang *et al.* 1992), rabbit (Ozols 1995), pig (Klemcke *et al.* 2003), cow (Tetsuka *et al.* 2003) and guinea-pig (Pu & Yang 2000, Shafqat *et al.* 2003). Human liver 11 β -HSD1 was purified in an active form and was postulated to exist as a dimer (Maser *et al.* 2002). The value of the K_m determined for 11 β -HSD1 dehydrogenase activity is puzzling given that it is more than two orders of magnitude higher than the circulating level of free cortisol (1–100 nM). Maser *et al.* (2002, 2003) discovered an unusual kinetic mechanism of action of the human liver 11 β -HSD1. They determined that this isoform exhibits Michaelis-Menton kinetics with respect to cortisol but cooperative kinetics with cortisone. In this way, 11 β -HSD1 could operate at both nanomolar and micromolar substrate concentrations.

Set-point of 11 β -HSD1 activity

In original purification studies, liver 11 β -HSD1 was shown to be bi-directional although, in contrast with its dehydrogenase activity, the reductase activity was unstable *in vitro* (Lakshmi & Monder 1988). More recently, a series of studies suggests that the enzyme prefers the reductase direction unless cells are disrupted. This applies in primary cultures of cells from liver (Jamieson *et al.* 1995), adipose tissue (Bujalska *et al.* 1997), lung (Hundertmark *et al.* 1995), hippocampus (Rajan *et al.* 1996), and vascular smooth muscle (Brem *et al.* 1995). In a few studies, for example in Leydig cells, 11 β -dehydrogenase activity has been reported in apparently intact cell preparations (Phillips *et al.* 1989), but others have found predominant 11 β -reduction (Leckie *et al.* 1998) and argued that

Figure 3 (A) Mutations found in HSD11B1 in CRD patients. (B) Mutations found in H6 PDH in CRD patients. CRD is a tri-allelic digenic disease requiring 3 of 4 mutations in both HSD11B1 and H6 PDH genes.

some 11 β -HSD1 must be liberated from damaged cells to detect 11 β -dehydrogenase activity. This striking change in directionality between intact cells and homogenates has never been satisfactorily explained, but is likely to reflect the specific intracellular localization of 11 β -HSD1 within the lumen of the endoplasmic reticulum (ER), where neighbouring enzymes may be powerful generators of the reduced co-substrate NADPH. Hexose-6-phosphate dehydrogenase (H6 PDH) is the first enzyme in the microsomal version of the cytosolic based pentose phosphate pathway, and is related to glucose-6-phosphate dehydrogenase (G6 PDH). It generates NADPH through the oxidation of various hexose-6-phosphates including glucose-6-phosphate and appears to be responsible for NADPH provision for 11 β -HSD1 thus regulating its set-point of activity (see later).

The human gene encoding 11 β -HSD1 (HSD11B1)

The human *HSD11B1* gene was first cloned and isolated by Tannin *et al.* in 1991. A rat 11 β -HSD cDNA was used to probe a human cDNA library (testis) and the corresponding human 11 β -HSD cDNA was used to determine chromosomal localisation. *HSD11B1* was localised to chromosome 1 by human/hamster cell hybrid panels, and clones containing the *HSD11B1* gene were isolated from a chromosome 1 genomic library. The human cDNA predicted a protein of 292 amino acids and was 77% identical at the amino acid level to the rat enzyme (Tannin *et al.* 1991). The human *HSD11B1* gene is localised to chromosome 1 (1q32–41) and consists of 6 exons (Fig. 3). When first cloned, the gene was thought to be approximately 9 kb in size; however, subsequent studies revealed a much larger than previously recognised intron 4 of approximately 25 kb, expanding the size of the 11 β -HSD type 1 gene to approximately 30 kb.

Very few polymorphisms have been identified in the *HSD11B1* gene, to date 28 polymorphisms are documented in the GenBank single nucleotide polymorphism (SNP) database (dbSNP at <http://www.ncbi.nlm.nih.gov/SNP/>). These polymorphisms were identified from sequencing studies as part of the human genome project. All but one polymorphism is located in non-coding regions of the gene; 20 SNPs within intron 4, 1 SNP in the 3' untranslated region (UTR), and 7 SNPs within 2 kb of the mRNA transcript (3 in 5' regions of the gene and 4 in 3' gene regions). The coding mutation represents a T to C single base change leading to a silent synonymous amino acid change, a serine to a serine at codon 204 in the mRNA sequence. By gene modelling, this residue does not appear to be part of any of the important conserved regions in the 11 β -HSD1 protein. More recently, our group has identified two polymorphisms within intron 3 of *HSD11B1* that are in complete linkage disequilibrium; these polymorphisms will be discussed in more detail with

regard to their role in causing the 11 β -HSD1 deficient state – 'cortisone reductase deficiency'.

Structural analysis of 11 β -HSD1

Little is known about the 11 β -HSD1 protein as its crystal structure has yet to be determined. The structure has been modelled on other SDR family members where the crystal structure has been identified.

The amino acid sequence of 11 β -HSD1 was determined by Edman degradation of 11 β -HSD1 purified from rabbit liver microsomes. The protein was determined to have 291 amino acid residues. This sequence was later shown to have 80% homology with human liver 11 β -HSD1. Residues 17–49 were shown to be highly conserved, whereas the C-terminus shows most variability across species (Ozols 1995). The topology of 11 β -HSD1 was elucidated by utilising 11 β -HSD1 constructs with attached FLAG epitopes at the N- and C-terminal regions. 11 β -HSD1 is intrinsic to the ER membrane, the N-terminus region of 11 β -HSD1 traverses the ER membrane and the bulk of 11 β -HSD1 is orientated in the lumen, whereas the N-terminus is cytoplasmic (Odermatt *et al.* 1999).

Co-factor binding sites for pyridine nucleotides, NADP/NAD were localised to residues 41–47 within the 11 β -HSD1 sequence (GXXXGXG). A putative active site region identified by Tyr-X-X-X-Lys sequence was identified at residue 182 of 11 β -HSD1 (Ozols 1995). This sequence has been implicated as part of the enzyme active site in some members of the short-chain dehydrogenase family (Persson *et al.* 1991). Mutagenesis studies of Tyr-179 and Lys-183 have shown these residues to be essential for enzymatic activity of 11 β -HSD1 (White *et al.* 1994). Mutation of the serine residues within the YSASK region of rat 11 β -HSD1 has indicated that these residues play an important role in determining the rate of the catalytic reaction (Obeyesekere *et al.* 1998).

Within the primary structure of 11 β -HSD1, the protein was found to be associated with oligosaccharides, Asn-X-Ser glycosylation motifs, at three specific sites. The Asn-X-Ser sites are at positions 123–125, 162–164, and 207–209 of the protein. The importance of N-linked glycosylation to 11 β -HSD1 activity has been controversially discussed in the literature. Ozols reported that de-glycosylation of rabbit 11 β -HSD1 resulted in no alteration in oxo-reducing ability (Ozols 1995). However, Agarwal *et al.* (1990) showed that glycosylation of rat 11 β -HSD1 was essential for dehydrogenase activity (<50%), but did not alter reductase activity. Also mutagenesis of the three potential N-glycosylation sites resulted in either reduced or complete abolition of enzyme activity (Agarwal *et al.* 1995a). This study was one of the first to show that HSDs could be regulated by post-translational modification. However, the same group also reported that the activity of de-glycosylated 11 β -HSD1 purified from

human liver showed no difference to native enzyme activity in both dehydrogenase and reductase directions (Odermatt *et al.* 1999, Blum *et al.* 2000a). In support of this finding, recent data conclusively show fully active non-glycosylated 11 β -HSD1 enzyme activity generated in *E. coli*, with kinetic properties for both dehydrogenase and reductase activities similar to those reported in mammalian systems (Walker *et al.* 2001). These data suggest glycosylation is not required for correct protein folding or enzyme activity (Blum *et al.* 2000b). Glycosylation of 11 β -HSD1 may still play a role in preventing protein aggregation, in addition to stabilising the overall structure within the ER (Ozols 1995).

Studies of purified protein have helped to elucidate some sites of post-translational modification, and the enzyme's orientation within the ER. 11 β -HSD1 has been difficult to purify as the enzyme has remained largely insoluble due to its glycosylation state and N-terminal region. Expression of 11 β -HSD1 constructs in *E. coli*, containing an N-terminal 6 xHis tag, has yielded a purified (157-fold) protein exhibiting full enzyme activity, with K_m s equivalent to those seen in mammalian systems (Walker *et al.* 2001).

11 β -HSD1 knockout mouse

An important advance in understanding the role of 11 β -HSD1 in glucocorticoid action was the creation of the 11 β -HSD1 knockout mouse ($-/-$) (Kotelevtsev *et al.* 1997). The *HSD11B1* homozygous mutant was generated by removing the genomic fragment encompassing exons 3 to 4 through specific recombination in mouse embryonic stem cells.

The $-/-$ mouse is incapable of converting 11-dehydrocorticosterone to active corticosterone – this confirmed the fact that the only enzyme capable of 11 β -reduction *in vivo*, at least in mice, is 11 β -HSD1, refuting suggestions of a third isozyme. The mice showed no visible abnormalities, and retained a fully active 11 β -HSD2 isozyme. The mice were fertile, producing normal litters. The adrenal glands of male 11 β -HSD1 $-/-$ mice are enlarged, suggesting adrenocortical hyperplasia. This could be explained by stimulation of the hypothalamic–pituitary–adrenal (HPA) axis to restore corticosterone levels (Holmes *et al.* 2001). The overcompensation of the HPA axis, leading to greater corticosterone levels than wild-type mice, may be explained by the absence of 11 β -HSD1 in the CNS, removing a level of regulation (Holmes *et al.* 2001).

The mouse studies highlighted the importance of hepatic 11 β -HSD1 in activating gluconeogenic enzymes and regulating hepatic glucose output. A high fat diet led to 11 β -HSD1 $-/-$ mice that had significantly lower fasting plasma glucose levels than weight-matched litter mates. Glucocorticoids stimulate gluconeogenesis,

and phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6 Pase) catalyse key steps of gluconeogenesis. Upon starvation these enzymes are induced by glucocorticoids; however, the 11 β -HSD1 $-/-$ mice failed to show normal induction of G6 Pase and PEPCK. Fed mice accumulated liver glycogen, reflecting lack of glucocorticoid-induced glycogenolysis. Thus 11 β -HSD1 $-/-$ mice are able to resist the hyperglycaemia seen in obese wild-type mice (Kotelevtsev *et al.* 1997).

11 β -HSD1, human obesity, insulin resistance and metabolic syndrome

Obesity is a major risk factor for cardiovascular disease, diabetes and premature mortality. However, it is the amount of omental fat that has been shown to correlate strongly with glucose intolerance and insulin resistance independently from total adiposity and subcutaneous abdominal adipose tissue (Fujioka *et al.* 1987, Despres *et al.* 1989). These data highlight the importance of investigating factors which control adipose tissue distribution in addition to absolute mass. The metabolic syndrome refers to a cluster of cardiovascular risk factors which include insulin resistance, alterations in glucose and lipid metabolism, increased blood pressure and visceral obesity. Patients with circulating cortisol excess, Cushing's syndrome, demonstrate many features of the so-called metabolic syndrome. Based on the precedent of AME and 'Cushing's disease of the kidney', several groups have now evaluated cortisol metabolism in patients with obesity-metabolic syndrome with exciting results.

Studies have demonstrated that primary cultures of adipose stromal cells (ASCs) isolated from patients undergoing elective abdominal surgery, express 11 β -HSD1 but not 11 β -HSD2. Activity studies show conversion of inactive cortisone to active cortisol through the expression of 11 β -HSD1 which is significantly higher in omental fat than in subcutaneous fat (Bujalska *et al.* 1997). Cortisol and insulin treatment were shown to increase differentiation of preadipocytes to adipocytes (Bujalska *et al.* 1999). Exposure to cortisol also increased expression and activity of 11 β -HSD1, providing a fast-forward feedback system for the local generation of active glucocorticoid within omental adipose tissue (Bujalska *et al.* 1997). *In vivo*, such a mechanism would ensure a constant exposure of active glucocorticoid specifically to omental adipose tissue, suggesting that central obesity may reflect 'Cushing's disease of the omentum' (Bujalska *et al.* 1997). However, *in vivo* studies are less clear-cut with increased (Paulmyer-Lacroix *et al.* 2002, Lindsay *et al.* 2003), unchanged or even reduced 11 β -HSD1 activity reported in obesity. Our own data are consistent with a reduction in hepatic 11 β -HSD1 reductase activity with increasing body mass index (BMI) (Stewart *et al.* 1999, Rask *et al.* 2001). Expression in

adipose tissue in obesity is more contentious with increased 11 β -HSD1 expression and/or activity again reported in subcutaneous sites. Our data in omental fat suggests no change or even a slight reduction similar to that seen in the liver (Tomlinson *et al.* 2002). Conversely, enzyme expression increased in obese subjects on a strict calorie-restricted diet (Tomlinson *et al.* 2004).

Obesity is thought to result from an expansion of adipose tissue mass by hypertrophy of existing adipocytes by lipid accumulation, differentiation of preadipocytes (adipose stromal cells (ASC)) through to adipocytes, or proliferation of preadipocytes (Yau *et al.* 1995, Tomlinson & Stewart 2001). Cortisol has been shown to cause differentiation of preadipocytes to adipocytes (Hauner *et al.* 1987), and the expression of 11 β -HSD1 *in vitro* is sufficient to allow differentiation of omental preadipocytes by cortisone alone (Bujalska *et al.* 1999). This is supported by inhibition of 11 β -HSD1 with glycerhethinic acid which prevented cortisone-induced adipocyte differentiation. However, glucocorticoids generally inhibit cellular proliferation, and 11 β -HSDs can regulate this process (Rabbitt *et al.* 2002). In addition, our group has recently demonstrated inhibition of human omental preadipocyte proliferation with cortisol, modulated by 11 β -HSD1. Thus, in whole adipose tissue, 11 β -HSD1 activity would lead to increased adipocyte differentiation, but simultaneous inhibition of preadipocyte proliferation, and thus the overall impact upon adipose tissue mass needs to be evaluated (Tomlinson & Stewart 2001). Our latest data, supported by data shown in the 11 β -HSD1 KO mouse (Morton *et al.* 2004), suggest that reduced expression of 11 β -HSD1 in liver and fat might represent an important protective mechanism offsetting the deleterious metabolic consequences of increasing BMI (i.e. reduced hepatic glucose output, reduced adipocyte differentiation). Of interest is that a BMI-related reduction in 11 β -HSD1 activity is not observed in type 2 diabetes mellitus (Valsamakis *et al.* 2004). Further prospective studies are required to evaluate the consequences of this upon the development of diabetes in obese subjects.

An adipose-specific 11 β -HSD1 transgene has been used to further *in vivo* studies of the role of 11 β -HSD1 in visceral obesity. 11 β -HSD1 cDNA was linked to the adipocyte fatty-acid-binding protein (aP2) promoter (Masuzaki *et al.* 2001), which caused a sevenfold amplification of 11 β -HSD1, leading to a viscerally obese phenotype (transgene mice 16% heavier than wild-type mice after 15 weeks). Significantly, this caused a 15–30% elevation in local adipose tissue corticosterone levels but circulating levels remained normal. Adipocyte number remained the same between transgenic and wild-type mice, although exposure of adipocytes to increased glucocorticoid levels resulted in lipid accumulation and an increased adipocyte size in transgenic mice. The transgenic mice were also markedly insulin resistant and glucose intolerant (Masuzaki *et al.* 2001).

Understandably, these *in vitro*, clinical and recombinant mouse studies have been the impetus for several pharmaceutical companies to develop selective 11 β -HSD1 inhibitors (selective in that they do not inhibit the related 11 β -HSD2). Many pharmaceutical companies have already published encouraging results using such inhibitors, which are discussed below, and many more are currently investigating newly developed compounds.

Biovitrum-Amgen has shown that arylsulphonamido-thiazole compounds inhibit 11 β -HSD1 both *in vivo* and *in vitro* (Alberts *et al.* 2002, Barf *et al.* 2002) and have shown encouraging results in animal studies. The diethylamide derivative was shown to inhibit human 11 β -HSD1 with an IC₅₀ of 52 nM, and an N-methylpiperazinamide form (BVT.2733) was shown to be specific for the mouse enzyme (IC₅₀ of 96 nM). Both compounds showed >200-fold selectivity over human and murine 11 β -HSD2 (Barf *et al.* 2002). In the hyperglycaemic mouse strain KKA(y), this compound lowered hepatic PEPCK and glucose-6-phosphatase mRNA, blood glucose and serum insulin concentrations, supporting data that 11 β -HSD1 is a key regulator of gluconeogenesis. This raises the possibility that inhibition of 11 β -HSD1 might be used therapeutically to treat patients with insulin resistance.

Merck has shown that insulin sensitising thiazolidinediones peroxisome proliferator-activated receptor (PPAR)- γ agonists may mediate their action, in part, through inhibition of adipose 11 β -HSD1, an effect demonstrated in cultured adipocytes (Berger *et al.* 2001). It was shown that thiazolidinedione and nonthiazolidinedione agonists of PPAR γ markedly inhibit expression of 11 β -HSD1 in 3T3-L1 adipocytes. This decrease in expression correlated with a significant decrease in the cellular conversion of cortisone to active cortisol. The half-maximal inhibitory effect of the thiazolidinedione, rosiglitazone, occurred at a concentration that supported a PPAR γ -mediated mechanism of action. It was also demonstrated that the inhibitory action of PPAR γ agonists on 11 β -HSD1 mRNA expression appears to take place at the level of transcription. In addition, treatment of diabetic db/db mice with rosiglitazone inhibited expression of 11 β -HSD1 in adipose tissue. This decrease in enzyme expression correlated with a significant decline in plasma corticosterone levels (Berger *et al.* 2001). Furthermore, the lipid lowering agent fenofibrate (a PPAR- α agonist) inhibits 11 β -HSD1 in hepatocytes (Hermanowski-Vosatka *et al.* 2000).

More recently, Merck has identified a new 11 β -HSD1 specific inhibitor – adamantly triazole, and have shown that chronic oral administration in a murine model system lowered triglycerides, insulin, fasting glucose and reduced body weight (Thieringer *et al.* 2004). This suggests pharmacological inhibition of 11 β -HSD1 improves several key features of the metabolic syndrome.

Glaucoma

11 β -HSD isozyme expression has recently been described in the human and rodent eye (Stokes *et al.* 2000, Rauz *et al.* 2001, Suzuki *et al.* 2001), notably 11 β -HSD1 in ciliary epithelial cells, raising the possibility for a role in aqueous humour production and the regulation of intraocular pressure. Within the aqueous humour, cortisol concentrations exceed cortisone by approximately 14-fold (Rauz *et al.* 2001). This is highly suggestive of predominantly 11 β -HSD1 reductase activity. Treatment with carbenoxolone in a randomised, double-blind placebo-controlled study of glaucoma sufferers significantly reduced intra-ocular pressure (Rauz *et al.* 2001) and topical 11 β -HSD1 inhibitors may represent a future novel therapeutic strategy in glaucoma.

Glucocorticoid-induced osteoporosis

Glucocorticoid-induced osteoporosis (GIOP) was first described as a recognised feature of Cushing's syndrome (Cushing 1932). Since then the increasing use of pharmacological doses of corticosteroids has brought attention to GIOP (Canalis 1996). Studies *in vivo* clearly show that administration of pharmacological doses of glucocorticoids stimulate bone resorption and inhibit bone formation, therefore decreasing bone mass and increasing the risk of fractures, and causing profound effects on bone cell replication, differentiation and function (Canalis & Delany 2002).

Several studies have demonstrated GR expression in osteoclasts and osteoblastic cells (Chen *et al.* 1977); GR α and MR are present in osteoclasts and osteoblasts (Beavan *et al.* 2001) and it is thought that many of the effects of glucocorticoids on bone are GR mediated. Studies by our group have demonstrated the expression of 11 β -HSD isozymes in bone. Predominant 11 β -HSD1 expression was demonstrated by enzyme activity studies, RT-PCR and immunohistochemistry, in osteoblast primary cultures (Bland *et al.* 1999), cell lines and normal adult bone (osteoblasts and osteoclasts) (Cooper *et al.* 2000). It is proposed that the expression of 11 β -HSD1 in osteoblasts facilitates the local synthesis of active glucocorticoids with consequent effects on osteoblastic proliferation and differentiation. In contrast to liver and adipose tissue, where reductase activity predominates, both reductase and dehydrogenase activities of 11 β -HSD1 are evident in bone chips and primary cultures of human osteoblasts (Cooper *et al.* 2000).

Tissue damage by pro-inflammatory cytokines is attenuated at both systemic and cellular levels by anti-inflammatory factors such as glucocorticoids. Within the human osteosarcoma cell-line (MG-63) pro-inflammatory cytokines, interleukin (IL)-1 β and tumour necrosis factor (TNF)- α potentially inhibited 11 β -HSD2 activity, and

reciprocally raised 11 β -HSD1 activity and expression. In addition, a rise in 11 β -HSD1 reductase activity was observed in primary cultures of osteoblasts treated with TNF- α (Cooper *et al.* 2001). These data suggest that local tissue metabolism of glucocorticoids is likely to be important in determining the sensitivity of both osteoblasts and osteoclasts to glucocorticoids in normal bone homeostasis. In addition, variation in 11 β -HSD isozyme expression and activity may explain individual differences in susceptibility to glucocorticoid-induced osteoporosis.

Importantly, 11 β -HSD1 reductase activity in osteoblast cultures has been shown to correlate positively with age. Glucocorticoid treatment caused a time- and dose-dependent increase in 11 β -HSD1 activity with similar increases in 11 β -HSD1 mRNA expression (Cooper *et al.* 2002). These studies indicate that activation of glucocorticoids at an autocrine level within bone is likely to play an important role in the age-related decrease in bone formation and increased risk of glucocorticoid-induced osteoporosis (Cooper *et al.* 2002).

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, affecting approximately 5–10% of all women. PCOS is an important cause of menstrual irregularity, infertility and androgen excess in women (Scarpitta & Sinagra 2000).

Over 10 years ago, our group postulated that defective 11 β -HSD1 activity may be an important mechanism in patients with PCOS (Stewart *et al.* 1990). The resulting increase in cortisol metabolic clearance rate would lead to stimulation of the HPA axis in an attempt to normalise serum cortisol levels, and might account for the ACTH-mediated hyperandrogenism in such patients. Alterations of peripheral metabolism of adrenal steroids, specifically increased 5 α -reductase and 11 β -HSD isozyme activities, have been identified in women with PCOS (Stewart *et al.* 1990, Rodin *et al.* 1994, Chin *et al.* 2000, Moghetti *et al.* 2002, Fassnacht *et al.* 2003, Tomlinson *et al.* 2003). This is perhaps best exemplified through patients with the opposite of AME – 'cortisone reductase deficiency'.

Cortisone reductase deficiency

The existence of a deficiency in the peripheral enzymatic conversion of E to F (the opposite of AME) was first described in 1984 by Taylor *et al.*, and has since been described in eleven individuals, all but one being female (Taylor *et al.* 1984, Savage 1991, Nikkila *et al.* 1993, Phillipov *et al.* 1996, Jamieson *et al.* 1999, Nordenstrom *et al.* 1999). The clinical characteristics of these patients are

Table 3 Clinical characteristics of patients with cortisone reductase deficiency

Age (years)	Sex	Clinical features	Serum androgens	THF+allo THF:THE ratio	Comments	Reference
28	F	Hirsutism	↑ Test. ↑ DHEAS ↑ Androst	—	Fall in androgens with dexamethasone treatment	Taylor <i>et al.</i> 1984
17	F	oligo-amennorrhoea, hirsutism, acne, obesity	↑ Test. ↑ DHEAS	0.039	Fall in androgens with dexamethasone treatment although developed Cushingoid side effects.	Phillipov <i>et al.</i> 1996
18	F	oligo-amennorrhoea, hirsutism, acne.	↑ DHEAS	0.045	Sibling of above patient. Fall in androgens with dexamethasone treatment.	Phillipov <i>et al.</i> 1996
30	F	oligo-amennorrhoea, hirsutism, infertility.	↑ Test.	—	Fall in testosterone with dexamethasone treatment.	Jamieson <i>et al.</i> 1999; Savage 1991
27	F	oligo-amennorrhoea, hirsutism	↑ Test. ↑ DHEAS ↑ Androst	—	Normal menstrual cycle and suppression of elevated androgens with dexamethasone treatment	Taylor <i>et al.</i> 2003
	M	Excess body hair (sibling of above patient)		—	Sibling of above patient.	Taylor <i>et al.</i> 2003; Nikkila <i>et al.</i> 1993
37 (32)	F	obesity, oligo-amennorrhoea, hirsutism	↑ Test. ↑ DHEAS ↑ Androst	0.03	—	Jamieson <i>et al.</i> 1999
4	F	CAH diagnosed shortly after birth (21 hydroxylase deficient). 17 OHP levels unresponsive to cortisone acetate.	—	↓	17 OHP levels suppressed completely with prednisolone indicative of an inability to activate cortisone acetate.	Nordenstrom <i>et al.</i> 1999
55	F	Androgenetic alopecia, mild hirsutism	↑ Test ↑ DHEAS ↑ Androst	0.05	—	Biason-Lamber <i>et al.</i> 2000
44	F	Hirsutism	↑ Test. ↑ Androst	0.04	Fall in androgens with dexamethasone treatment	Laing <i>et al.</i> 2002
7	M	Precocious puberty	↑ Test ↑ DHEAS ↑ Androst	0.04	Pubertal development halted with dexamethasone treatment.	Malunowicz <i>et al.</i> 2003

Test., testosterone; DHEAS, dehydroepiandrosterone sulphate; Androst, androstenedione; CAH, congenital adrenal hyperplasia; 17OHP, 17- α hydroxyprogesterone.

summarised in Table 3. This rare condition named 'cortisone reductase deficiency' (CRD) is a form of PCOS in which the underlying defect appears to be deficient activity of the 11 β -HSD1 enzyme. Patients with CRD share many clinical characteristics associated with PCOS and have invariably presented in adolescence or early adulthood with features of hyperandrogenism (acne, hirsutism, oligo-amennorrhoea, infertility). Obesity has been a feature of some cases. Serum androgens (dehydroandrosterone sulphate (DHEAS), androstenedione and testosterone) have been elevated in each case, but are readily suppressed following dexamethasone administration.

Studies indicate an increased excretion of total cortisol metabolites indicative of enhanced cortisol secretion rates, often to values reported in patients with Cushing's syndrome. However, virtually all the urinary metabolites are excreted as 11-oxo-metabolites (THE) with very low/undetectable levels of THF and allo-THF appearing in the urine. Typical THF+allo-THF:THE ratios of <0.05 (normal adult range 0.7–1.3) have been reported (Phillipou & Higgins 1985). (It is of interest that similar patterns of cortisol metabolism are observed in the normal neonatal period.) These data, together with an attenuated plasma cortisol response following oral cortisone acetate, suggest defective 11-oxo-reductase activity (and thus 11 β -HSD1

Figure 4 Biochemical scenario in 11 β -HSD1 deficiency. Aberrant metabolism drives steroid secretion in CRD. Lack of 11 β -HSD1 leads to an increase in cortisol to cortisone conversion; the decrease in cortisol feeds back onto the HPA axis to increase cortisol levels, and therefore also increases androgen production by the adrenal glands. Circulating F levels remain normal due to a balance between increased F metabolism compensated for by an increase in F secretion.

activity). The defect in E to F conversion results in an increased metabolic clearance rate for cortisol; through the negative feedback mechanism the HPA axis is activated and ACTH secretion is increased to maintain normal circulating cortisol concentrations, but at the expense of ACTH-mediated androgen excess. This leads to a polycystic ovarian syndrome-like phenotype in females (Biaison-Laubier *et al.* 2000), as summarised in Fig. 4. The phenotypic consequences of CRD in males are likely to be milder, with a report of premature adrenarche/puberty in a boy aged 7 years (Biaison-Laubier *et al.* 2000). Dexamethasone, by suppressing endogenous ACTH drive has been used therapeutically to control the hyperandrogenism.

It has been proposed that this condition could be due to mutations within the 11 β -HSD1 gene (*HSD11B1*). The mode of inheritance of CRD is thought to be autosomally recessive, as two sets of sib-pairs have been identified without affected parents. Analysis of genomic DNA has been undertaken in a number of patients to try and determine a mutation within *HSD11B1*. Southern blot analysis of *HSD11B1* from one CRD patient showed no gross deletions or re-arrangements (Nikkila *et al.* 1993). In addition, further sequencing of the coding region of *HSD11B1* within four CRD patients revealed no mutations (Nikkila *et al.* 1993, Jamieson *et al.* 1999, Nordenstrom *et al.* 1999, Biaison-Laubier *et al.* 2000). Nevertheless, a functional abnormality of this enzyme remains the most likely cause of CRD.

Recently, we have reported an extensive genetic investigation of *HSD11B1* in three CRD kindreds, each with a single affected case. The CRD cases, one of which is male, all exhibited characteristically low urinary THF+allo-THF:THE ratios of between 0.03 and 0.04 (reference range 0.7–1.1), and have been described previously in the literature (Jamieson *et al.* 1999, Laing *et al.* 2002, Malunowicz *et al.* 2003). In all three CRD cases, two polymorphisms in complete linkage disequilibrium within intron 3 of *HSD11B1* were identified; an A insertion (83557), and 40 base pairs downstream a T to G substitution (83597) (Fig. 3A). The allele frequency for the 83557A/83597T-G haplotype was 14% in control populations.

Although the 11 β -HSD1 cDNA sequence was normal in one case, adipose tissue mRNA levels were 28-fold lower when compared with an unaffected sister, and the corresponding oxo-reductase activity was absent (0% conversion of E to F versus 14% in the unaffected sibling). In luciferase reporter assays, there was a 2.5-fold reduction in transcriptional activity in *HSD11B1* constructs containing the intron 3 83557A/83597T-G mutation compared with wild-type, suggesting that this region of the gene acts as an intronic enhancer of *HSD11B1* expression. These data are in keeping with published precedents suggesting a silencer/enhancer role for intronic sequences in many genes including *HSD11B2* (Agarwal 2001). The impact of heterozygosity for the 83557A/83597T-G mutation upon

Figure 5 H6 PDH generates NADPH by the conversion of glucose-6-phosphate (G6P) transported via the G6P translocase (GT), to 6-phosphogluconate (6 PG) within the endoplasmic reticulum (ER). 11 β -HSD1 uses NADPH as cofactor allowing conversion of cortisone to cortisol.

cortisol metabolism is unknown, but the CRD phenotype cannot be explained by heterozygosity or homozygosity at this single locus because this was present in 25% and 3% of normals respectively.

As discussed previously, 11 β -HSD1 oxo-reductase activity requires NADPH. The purified enzyme behaves as an NADP-dependent dehydrogenase and the switch to oxo-reductase activity upon tissue homogenization (Agarwal *et al.* 1990) suggests a close association between 11 β -HSD1 and a NADPH generation system. The G6 PDH enzyme of the pentose phosphate pathway has been considered to be the major source of intracellular NADPH (Kletzien *et al.* 1994), but this is a cytosolic enzyme and the membrane-binding domain of 11 β -HSD1 directs the active site of the enzyme towards the ER lumen, away from the cytosol (Ozols 1995). Here H6 PDH, an enzyme of previously uncertain significance but related to G6 PDH, can catalyze the first two steps of the pentose phosphate pathway and generate NADPH (Kimura *et al.* 1979, Stegeman & Klotz 1979). H6 PDH is present in most tissues but is highly expressed in liver and adipose tissue, sites of 11 β -HSD1 oxo-reductase activity.

Sequencing of the *H6PD* gene, localized to chromosome 1p36.2 (see Mason *et al.* 1999), revealed mutations within exon 5 in all three CRD cases. Case 1 was heterozygous for 620 ins29 bp621, an insert of 29 bp between residues 620 and 621 that results in the inclusion of 3 new amino acids and a stop codon, truncating the

protein by 171 amino acids. Cases 2 and 3 were homozygous for R453Q, a non-conservative missense amino acid change. The position of the mutations within H6 PDH are shown in Fig. 3B. When *H6PD* mutant cDNAs were synthesized and expressed in hepatic WRL68 cells, the 620 ins29 bp621 mutant was devoid of H6 PDH activity and the R453Q mutant demonstrated residual activity that was consistently less than 50% of wild-type. The impact of the R453Q mutation in the context of a normal *HSD11B1* genotype is unknown. The allele frequency of R453Q in both Scottish and Indo-Asian controls is 21–22%, so that ~4% of the normal population is homozygous for this mutation. The combination of homozygosity for the *H6PD* R453Q and heterozygosity for the *HSD11B1* intron 3 83557A/83597T-G mutations was not observed in our control subjects, and its presence in 2/3 CRD patients is unlikely to be due to chance ($P=0.0008$, Fisher's exact test). Based on our normative allelic frequency data we can predict a prevalence rate for CRD of approximately 0.1% for the mutations *HSD11B1*^{+/–} and R453Q *H6PD*^{–/–}. Alternatively, interaction with an unidentified third locus, modifying penetrance, might be implicated.

Thus, a combination of mutations in the *HSD11B1* and *H6PD* genes interact to cause CRD manifesting in a reduction in 11 β -HSD1 expression and impaired provision of NADPH to an enzyme that is critically dependent on reduced co-factor for oxo-reductase activity. This is

summarised in diagrammatic form in Fig. 5. A digenic triallelic mode of inheritance is proposed, in which three distinct alleles, from two (or more) loci (*HSD11B1* and *H6PD*), are necessary for trait manifestation (Fig. 3A and B).

CRD defines H6 PDH as the regulator of a novel redox potential within the ER. The oligogenic basis for CRD has identified susceptibility loci for the complex, heterogeneous disease, PCOS.

Conclusions

This review celebrates the 100th anniversary of the term 'hormone'. Some 50 years after this discovery, Hench, Kendall and Rubenstein won the Nobel Prize for their discovery of cortisone and the description of its anti-inflammatory properties in patients with rheumatoid arthritis. These findings established the endocrine importance of 11 β -HSD isozymes, due to their pre-receptor action, playing a critical role in regulating cortisol metabolism and clearance.

11 β -HSD2, being a unidirectional dehydrogenase, controls renal cortisol concentrations and, via its pre-receptor role, protects the MR from illicit activation by cortisol. Mutations in the gene encoding 11 β -HSD2 lead to the monogenic form of hypertension – AME. It has been suggested that mild mutations in *HSD11B2* may account for some cases of idiopathic hypertension.

More recently, attention has focused on the 11 β -HSD1 isoform and its role in human disease states, in particular features of the metabolic syndrome such as obesity and insulin resistance, and also glucocorticoid-related diseases such as osteoporosis and glaucoma. Thus far, studies have predominantly concentrated upon the liver and adipose tissue and the role of 11 β -HSD1 in regulating hepatic gluconeogenesis and fat mass. Obviously, this makes selective inhibitors of 11 β -HSD1 an attractive proposition for the pharmaceutical industry, who are currently rigorously searching for, and developing, 11 β -HSD1 inhibitors with the aim of finding a therapeutic target for the metabolic syndrome. In addition to the clear clinical impact of these inhibitors, they will also be crucial for increasing our understanding of the role of 11 β -HSD1 *in vivo*. Some caution should be considered here, as these inhibitors are likely to be systemic, and not tissue specific and it will therefore be of importance to tease out the effect of inhibition within individual tissues that show a wide range of 11 β -HSD1 expression. In addition, as many inhibitors are derived from high throughput assays in intact cell systems, it is exciting to speculate that they might be inhibitors of H6 PDH rather than acting directly upon the 11 β -HSD1 enzyme.

The 'human 11 β -HSD1 knockout', termed cortisone reductase deficiency has enabled greater understanding of the set-point of activity of 11 β -HSD1. Mutations were

found in both *HSD11B1* and the gene encoding hexose-6-phosphate dehydrogenase. It is now thought that H6 PDH is responsible for conferring reductase activity upon 11 β -HSD1 by the production of cofactor (NADPH). Further studies are clearly needed to assess the broader impact of regulation of H6 PDH expression and activity as a determinant of 11 β -HSD1 reductase activity and hence autocrine cortisol generation. The next generation of experiments may be to place the last twenty years of 11 β -HSD1 research in the context of H6 PDH activity and expression.

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