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Cortisol as a mineralocorticoid in human disease ☆

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Abstract

The type 2 isozyme of 11β-hydroxysteroid dehydrogenase inactivates cortisol to cortisone and enables aldosterone to bind to the MR. Congenital deficiency of the enzyme results in cortisol-mediated mineralocorticoid excess and arises because of inactivating mutations in the HSD11B2 gene. Inhibition of the enzyme following licorice or carbenoxolone ingestion results in a similar, though milder phenotype and the enzyme is overwhelmed in ectopic ACTH syndrome. Loss of 11β-HSD2 expression may be important in sodium balance and blood pressure control in some patients with renal disease. Finally, while some studies demonstrate impaired 11β-HSD activity in broader populations of patients with hypertension, further studies are required to clarify the role of 11β-HSD2 in 'essential' hypertension.

1. Introduction

Conventionally we have considered the human adrenal gland to secrete two principal classes of corticosteroid hormone, glucocorticoids and mineralocorticoids. Glucocorticoids (cortisol, corticosterone) are secreted in relatively large amounts and have a diverse array of physiological roles, whilst mineralocorticoids (aldosterone) are produced in low amounts and have a more defined role to stimulate epithelial sodium transport. With the elucidation of the mechanisms underlying corticosteroid hormone metabolism, however, there are numerous disease processes in which cortisol has been shown to act as a mineralocorticoid. Most of these examples arise in the setting of reduced 11β-hydroxysteroid dehydrogenase expression. Two isozymes of 11β-HSD catalyse the interconversion of hormonally active cortisol (F) to inactive cortisone (E); these have been cloned and characterised and are discussed in more detail in Dr Krozowski's article in this special issue. It is the type 2 11β-HSD isoform (11β-HSD2) which plays a crucial role in normal physiology in dictating specificity upon the mineralocorticoid receptor (MR). In vitro, this receptor has similar affinity for aldosterone and cortisol [1], aldosterone gains access to the MR in vivo only if cortisol is inactivated to cortisone at the site of the receptor through the activity of 11β-HSD2 [2,3].

The purpose of this review is to detail the clinical scenarios in which cortisol acts as a mineralocorticoid.

2. The syndrome of apparent mineralocorticoid excess (AME)

AME is a cause of low-renin, low-aldosterone hypertension and hypokalaemia found predominantly in children, worldwide approximately 30–50 cases have been reported [4–14]. Children present with failure to thrive (low birth weight is a recognised feature), short stature, and have severe and often fatal hypertension and hypokalaemia. The profound hypokalaemia may cause rhabdomyolysis and nephrogenic diabetes insipidus manifesting as thirst and polyuria. Other renal abnormalities include renal cysts and nephrocalcinosis. Several cases with affected siblings have been reported and the condition is inherited as an autosomal recessive condition.

Defective peripheral conversion of cortisol to cortisone reflecting impaired activity of 11β-HSD was first suggested by Ulick, New and co-workers in patients

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with AME in the late 1970's [4] and has been further investigated by other groups. Urinary steroid metabolite profiles on such patients indicate that the majority of cortisol metabolites are excreted as A-ring reduced metabolites of cortisol itself (THF and allo-THF) with very low or absent levels of THE in the urine. The excretion of 5α-cortisol metabolites exceeds that of 5β-cortisol metabolites resulting in a high urinary allo-THF/THF ratio suggesting an additional defect in 5β-reductase activity [6,7]. The incremental increase in the THF + allo − THF/THE compared to the allo − THF/THF ratio, however, is much larger, with typical THF + allo − THF/THE ratios ranging from 8 to >70 in AME. The plasma half-life of [11α-3$_H$]-cortisol (which when metabolised by 11β-HSD yields tritiated water and cortisone), may more accurately reflect renal 11β-HSD2 activity [4,10] as may the ratio of urinary free cortisol/urinary free cortisone (UFF/UFE) [15]. Normal subjects excrete 2–3-fold more UFE than UFF, reflecting the significant activity of renal 11β-HSD2. In AME, however, UFE excretion is virtually undetectable. The conversion of cortisone to cortisol is normal in AME [4,10], all of which results in a marked increase in the plasma cortisol half-life. Despite this defect in the conversion of F to E, patients with AME are not Cushingoid, due to a normal intact negative feedback mechanism, cortisol secretion rate falls often to very low levels which maintain normal circulating concentrations in the face of impaired cortisol metabolism.

Initial studies indicated that a low salt diet, together with mineralocorticoid receptor blockade with spironolactone lowered blood pressure, and ACTH exacerbated the condition. However, despite an extensive search for an 'ACTH-dependent mineralocorticoid', none was identified and patients were labelled as suffering from the syndrome of 'apparent' mineralocorticoid excess.

Subsequently, despite normal circulating cortisol concentrations, cortisol was shown to have profound effects in the kidney and colon in AME patients by acting as a potent mineralocorticoid [9,10]. Thus an infusion of only 10 mg/day hydrocortisone was shown to lower the urinary Na/K ratio, to suppress plasma renin activity and to increase measurements of subtraction potential difference (a marker of mineralocorticoid activity) across the rectal colon. Dexamethasone, by suppressing endogenous cortisol secretion, resulted in a natriuresis, potassium retention and lowered blood pressure with restoration of a normal renin-angiotensin-aldosterone system.

Therapeutically, patients have been successfully treated with triamterene and/or amiloride. Spironolactone has been of variable benefit, presumably because very high doses would be required to block the mineralocorticoid effects of cortisol on the MR.

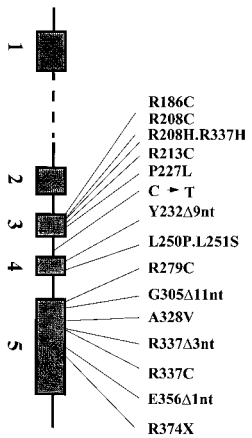

Fig. 1. Diagram illustrating the mutations reported to date in the HDS11B2 gene in patients with apparent mineralocorticoid excess.

Dexamethasone has been very effective in some cases, but not in others and this may relate to inadequate suppression of cortisol secretion. As is the case with all secondary forms of hypertension, however, removal of the source only restores blood pressure to normal in approximately 60% of cases and additional antihypertensive medication with calcium channel antagonists or angiotensin converting enzyme inhibitors may be required. The main aim of treating patients with AME should be to correct life-threatening hypokalaemia. Amiloride and/or triamterene or dexamethasone should achieve this in the majority of cases though conventional anti-hypertensive therapy may be required in addition to control blood pressure. The poor growth rate seen in many children with AME usually responds to correction of the profound hypokalaemia. However, 11β-HSD2 is expressed in high amounts in many fetal tissues including placenta [16,17] and it is possible that 'glucocorticoid excess' in AME patients in utero consequent upon absent or impaired 11β-HSD2 activity may impair fetal growth [13].

A second 'variant' of AME, so-called 'type II AME' has been documented. Type II AME has been described in 3 Sardinian patients and a further two cases from mainland Italy [18,19]. This variant is characterised by a milder phenotype, with onset in late adolescence or early adulthood and by a relatively normal urinary THF + allo − THF/THE ratio. A generalised defect in cortisol A-ring metabolism was proposed as the underlying defect. However, the UFF/UFE excretion is high in the type II variant, and the metabolism of 11α-triated cortisol (directly reflecting 11β-HSD activity) is grossly deranged, suggesting deficiency of 11β-HSD2 [20]. Evidence suggests that cortisol is also the offending mineralocorticoid in this 'type II' variant [21].

Information on the structure and sequence of the

HSD11B2 gene has enabled the identification of mutations in AME patients. HSD11B2 is 6.2 kb in length, is composed of five exons and is located on chromsome 16q22. To date 15 mutations have been reported in the HSD11B2 gene in 26 patients with AME type 1 (Fig. 1) [14,22–29]. Only two patients are compound heterozygotes with each allele coding for an enzyme devoid of activity. All other type I AME patients are homozygous for mutations causing full, or partial loss of activity. In one case (mutation R374Stop), affected placental tissue was obtained from an AME kindred and absent conversion of cortisol to cortisone confirmed in vitro [25]. AME is an autosomal recessive disease and is most commonly found in consanguineous families. A founder effect is evident in three families homozygous for the R337HΔ3 nt mutation which results from the deletion of three nucleotides across the two codons coding for R337 and Y338. One of these families are Zoroastrians from Iran, and the others come from the Bombay area to which the religious group emigrated in the seventh century. This mutation also appears to have arisen independently in a compound heterozygote from Japan [26]. Homozygosity in AME is thought to result from endogamy or a founder effect in the Native American families with the R208C and R356Δ1 nt mutations, and the L250 S,L251P mutation. The fact that six kindred are of native American origin has prompted speculation as to a possible selective advantage of heterozygotes. It has been suggested that such individuals may have an increased ability to conserve salt under conditions of extreme sodium deprivation [14].

Heterozygotes are not normally clinically affected, though in one report both parents were found to be mildly hypertensive and had evidence of mineralocorticoid based hypertension [10], while in another family the father of an affected case, who in turn was heterozygous for the A328 V mutation, developed hypertension at age 38 and displayed a moderately elevated THF + allo − THF/THE ratio of 2.47 [27]. Because AME is usually diagnosed in childhood, prolonged follow-up of the relatively young parents into late adulthood is required to analyse the full functional significance of the heterozygote state.

Recent data suggest that Type II AME can also be explained on the basis of homozygous mutations in the HSD11B2 gene. In an extensive Sardinian kindred, a novel homozygous mutation (R279C) was found in all 4 affected cases. In keeping with the mild phenotype the mutation resulted in a mutant enzyme with only minor disturbances in activity [28]. Classification of AME into distinct variants is therefore inappropriate.

The correlation between phenotype and genotype has received some attention. In AME the biochemical phenotype is best characterized by the urinary THF + allo − THF/THE ratio, a value which can vary considerably within patients, and the most informative patients are those with partially active 11β-HSD2. Given the low numbers of such patients caution must be exercised in drawing correlations. Nevertheless, one study has suggested a correlation between metabolite ratios and genotype [29], and the emerging data from the AME type II variant would support these conclusions.

3. Licorice and carbenoxolone ingestion

Licorice has been used medically for at least 5000 years, but its mineralocorticoid activity was first documented in the 1940's in Holland. A preparation of the licorice root, succus liquiritiae, was successfully used to treat patients with peptic ulceration. Such observations were 'the basis for the development of the effective anti-ulcer drug', carbenoxolone which is a hemisuccinate derivative of 18β-glycyrrhetinic acid. However, both licorice and carbenoxolone induced mineralocorticoid side effects (oedema, shortness of breath on exertion and increased blood pressure) in up to 50% of patients consuming these compounds.

In Europe, licorice is mainly ingested as a confectionery sweet (the botanical name of the licorice plant, glycyrrhiza glabra, literally means sweet root) with as little as 50 g/day required to induce mineralocorticoid hypertension [30]. In North America and Europe, glycyrrhizin is found in some confectioneries, but is also a sweetener in chewing gums and chewing tobacco [31,32].

Patients consuming excessive quantities of licorice present with hypertension and hypokalaemia which may be severe enough to cause myopathy and cardiac arrhythmias. Both plasma renin activity and aldosterone levels are suppressed and exchangeable Na levels are increased. The condition responds to spironolactone and is reversible upon stopping licorice ingestion. The 'active' mineralocorticoids in licorice are glycyrrhizic acid (GI) and its hydrolytic product glycyrrhetinic acid (GE), specifically the 3-monoglucuronyl metabolite of GE. Although GI and GE bind to the MR, both compounds have a very low affinity for the MR (approximately 1/15,000th that of aldosterone). Furthermore, their activity was shown to be dependent upon the presence of functional adrenal tissue, activity was absent in patients with Addison's disease and in rodents subjected to bilateral adrenalectomy, arguing against a direct effect of GI and GE on the MR. It was subsequently shown that GI, GE and carbenoxolone inhibited both 11β-HSD isozymes, and that their mineralocorticoid activity is mediated through cortisol via inhibition of 11β-HSD2 [33–35]. GI and GE were shown to inhibit renal 11β-HSD activity both in vitro

and in vivo in rodents prior to the characterisation of two distinct isozymes [36] and more recently to be competitive inhibitors of 11β-HSD2 with a Ki of approximately 5–10 nM [37,38]. Thus it is now established that licorice induces an acquired and milder form of AME, causing its mineralocorticoid effects through inhibition of 11β-HSD2.

Carbenoxolone also inhibits 11β-HSD2 activity, potentiating the mineralocorticoid activity of glucocorticoids [39] and causing cortisol-mediated mineralocorticoid excess in man [40]. However, there is little if any change in the THF + allo − THF/THE ratio in volunteers given carbenoxolone, which may reflect co-existing inhibition of 11β-HSD1.

4. Ectopic ACTH syndrome

Eighty percent of patients with Cushing's syndrome have hypertension and this increases to over 95% in the subgroup of patients with ectopic ACTH syndrome. The severity of hypertension is a key factor in predicting morbidity and mortality from the disease yet its pathogenesis remains largely unknown. A further factor which characterises the ectopic ACTH syndrome is mineralocorticoid excess, with hypokalaemic alkalosis found in 95–100% of cases, in contrast to $<10\%$ in other forms of Cushing's syndrome. Although hyperdeoxycorticosteronism has been postulated to play a role, it is the level of cortisol secretion which correlates best with the degree of mineralocorticoid excess [41].

Several studies now suggest that saturation of 11β-HSD2 by the very high cortisol concentrations seen in the ectopic ACTH syndrome explains the mineralocorticoid excess state. Both the urinary ratio of THF + allo − THF/THE and UFF/UFE are elevated, not because of impaired 11β-HSD2 activity, but simply because of substrate saturation. At high cortisol secretion rates the enzyme is overwhelmed by substrate, cortisol cannot be inactivated to cortisone at the site of the MR and 'spills over' onto the MR to cause mineralocorticoid hypertension [42–44].

5. Renal disease

The role of the human kidney in metabolising cortisol to cortisone in vivo was established in the early 1970's [45]. Patients with uraemia have a prolonged plasma cortisol half-life and plasma cortisone concentrations are reduced in patients with renal disease [46,47], with an inverse correlation observed between cortisone values and plasma creatinine. It remains to be seen whether this impairment of metabolism of cortisol to cortisone results in enhanced mineralocorticoid activity within the kidney. If so then alterations in 11β-HSD2 activity may account for the sodium retention seen in some forms of renal diseases, for example the nephrotic syndrome.

6. 'Essential' hypertension

The recent discovery of the molecular basis for AME has once again focussed attention on the role of steroid hormones in the pathogenesis of hypertension. There is a long literature on the role of adrenocortical hormones in the pathogenesis of hypertension, but such studies have invariably concentrated on circulating steroid concentrations rather than deranged steroid metabolism. Recent studies have demonstrated variations in 11β-HSD activity in hypertensive subjects with either increases in the plasma [11α-^{3}H]-cortisol half life [48] or the THF + allo − THF/THE ratio [49]. The results however, have not been consistent, and even in patients with defective conversion of cortisol to cortisone, mineralocorticoid excess has not been demonstrated. A single study has reported an association between a microsatellite marker close to the HSD11B2 gene (D16S496) and hypertension in 79 blacks with hypertensive end stage renal disease [50] suggesting that region 16q22.1 may harbor a gene conveying susceptibility for hypertension. We have been unable to confirm this in other cohorts of hypertensive Afro-Caribbeans using a microsatellite marker adjacent to the HSD11B2 gene (Stewart and White, personal communication [51]). Future studies are required to further evaluate the prevalence of AME in patients currently labelled as having 'essential' hypertension.

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