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THE CORTISOL–CORTISONE SHUTTLE AND HYPERTENSION

PAUL M. STEWART[1] and CHRISTOPHER R. W. EDWARDS[2]*

[1]Department of Medicine, Queen Elizabeth Medical Centre, Edgbaston, Birmingham B15 2TH and [2]Department of Medicine, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K.

Summary—11β-OHSD is an enzyme complex consisting of 11β-DH, converting cortisol to cortisone in man and an 11-keto-reductase performing the reverse reaction. Congenital deficiency of 11β-DH should be considered in any child presenting with mineralocorticoid hypertension and suppression of the renin–angiotensin–aldosterone axis. The keystone to diagnosis is the demonstration of a reduced daily production rate of cortisol and an increase in its plasma half-life. In the majority of cases diagnosis can be made from a urinary steroid metabolite profile indicating a high excretion of cortisol relative to cortisone metabolites. Cortisol is the responsible mineralocorticoid, and as such treatment with the pure glucocorticoid dexamethasone will prevent life-threatening hypokalaemia, although additional antihypertensive drugs are usually required to control blood pressure.

Liquorice and carbenoxolone, for years thought to be direct "agonists" of the mineralocorticoid receptor, in fact cause sodium retention through inhibition of 11β-DH.

The demonstration of 11β-DH activity in the vasculature raises the possibility that it locally modules access of glucocorticoids to mineralocorticoid and possibly glucocorticoid receptors in the vessel wall.

It remains possible that subtle alterations of this cortisol–cortisone shuttle are responsible for other forms of hypertension which are currently classified under the umbrella diagnosis of essential hypertension.

INTRODUCTION

In diseases related to steroid excess, much greater emphasis has been placed on steroid secretion rather than metabolism. Thus, even in conditions where steroids may have been implicated in their pathogenesis, normal plasma steroid levels have been interpreted as negative results. In this review we shall show how cortisol (Kendall's compound F), the principal glucocorticoid secreted in man, can produce severe hypertension even when plasma concentrations are normal.

The main site of cortisol metabolism has been thought to be the liver [1] and conversion of cortisol to the inactive steroid cortisone (E) by an enzyme complex 11β-hydroxysteroid dehydrogenase (11β-OHSD) occurs early in its metabolic transformation. Although the existence of 11β-OHSD has been known of since the 1960s our understanding of the physiological significance of this enzyme has come only recently from investigation of the enzyme-deficient state.

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*To whom correspondence should be addressed.

Cortisol metabolism and 11β-hydroxysteroid dehydrogenase

Figure 1 depicts the principal pathways of cortisol metabolism in man. Briefly this involves reduction of the 4–5 double bond (5α and 5β-hydrogenation) and the C_{20} group, hydroxylation at C_6 and the interconversion of the hydroxyl and keto groups at C_{11} carried out by 11β-OHSD. This step is important in that steroids possessing a hydroxyl group at C_{11} are active whilst those with a keto group are inactive. Thus cortisone needs to be converted to cortisol in the liver for activity, and is therefore inactive if given parenterally.

11β-OHSD activity was first shown in placental tissue in the late 1950s [2] and since then its activity has been shown in numerous tissues; kidney [3, 4, 5], liver [6, 7], lung [8, 9], muscle [4], adrenal [10], thyroid [4], colon [11] and gonad [12] (see Ref. [13] for a review). It is known that this enzyme complex exists as two distinct dehydrogenase and reductase components [14, 15], with activity dependent on NADP/NAD [16], and regulated at least in part by progesterone and its hydroxylated derivatives [17]; thyroid [18, 19] and sex hormones [20]. More recently Carl Monder's group has fully

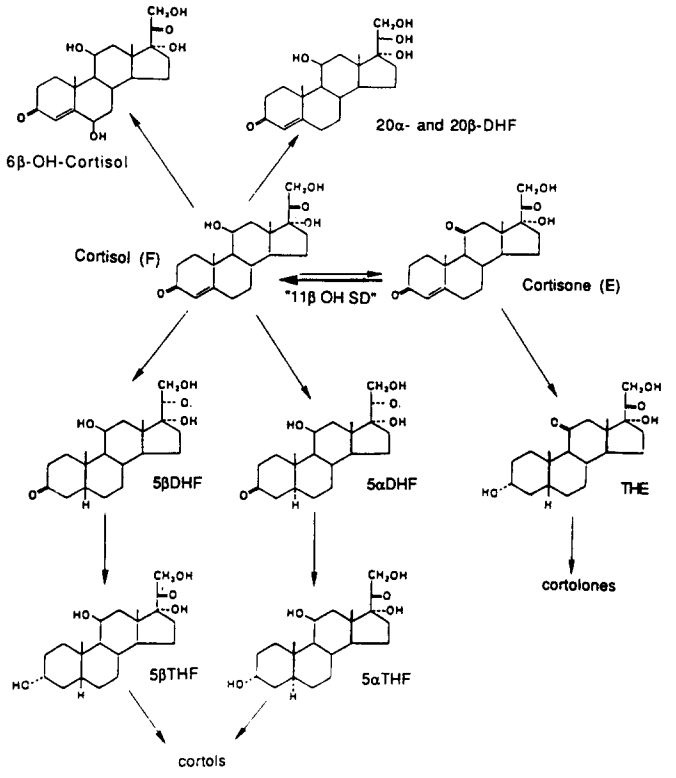

Fig. 1. Major pathways of cortisol metabolism. The broad arrow indicates a shift of metabolism towards F in AME.

characterized 11β-dehydrogenase (11β-DH) from rat liver microsomes [15], and, using a series of detergents has purified and cloned 11β-DH [21, 22]. The 11β-DH cDNA is 1.2 kb in length encoding 287 amino acids to produce a glycoprotein of mol. wt 34 kDa, and has marked similarity to other dehydrogenases. When 11β-DH cDNA was transfected into Chinese hamster ovary cells, these cells were able to convert F to E but also E to F, and thus suggested that a single gene may be encoding one enzyme with bidirectional activity rather than two separate enzymes. This issue is still not resolved and we shall refer 11β-DH and 11-keto reductase converting F to E and E to F respectively.

Congenital deficiency of 11β-dehydrogenase: the syndrome of apparent mineralocorticoid excess (AME)

Congenital deficiency of 11β-dehydrogenase presents as a rare but often fatal cause of mineralocorticoid hypertension, i.e. severe hypertension with suppression of the renin–angiotensin–aldosterone axis and hypokalaemia. Worldwide only 19 children and 1 adult have been reported. Children classically present with failure to thrive, short stature and thirst, polyuria and polydipsia secondary to nephrogenic diabetes insipidus induced by hypokalaemia. Presentation with rickets is also documented. Table 1 shows in more detail blood pressure and biochemical data on these patients [23–33]. It was in the late 1970s that Stanley Ulick investigating two children with this disorder first documented a defect in the peripheral metabolism of cortisol to cortisone [23]. This results in a prolonged plasma cortisol half-life, but plasma cortisol concentrations remain normal due to a concomitant reduced daily cortisol secretion rate brought about through the ACTH negative feedback mechanism. Thus other ACTH-dependent steroids such as corticosterone and deoxycorticosterone are low.

A defect in 11-keto-reductase has also been reported in three women (two were siblings) presenting with hirsutism and menstrual irregularity. Cortisol production is very high but is rapidly subjected to "one-way" conversion to cortisone. A high ACTH-mediated androgen level results and is thought to be responsible for the clinical picture [34, 35].

A characteristic urinary steroid metabolic profile (measured by gas chromatography/mass spectrometry) is produced in 11β-DH deficiency

Table 1. Reported cases of congenital 11β-dehydrogenase deficiency (AME)

Patient	Age at investigation (yr)	BP (mmHg)	Na (mmol/l)	K (mmol/l)	HCO_3 (mmol/l)	PRA (ng/ml/h)	Aldosterone (ng/100 ml)	THF + 5αTHF/THE	Growth	Ref. No.
1	3+	180/140	—	3.7	—	1.000	—	16.2	<3rd percentile	[23]
2	9	250/180	—	2.0	—	0.7900	<1.00	10.0	Growth retarded	[23]
3	2	140/90	154	2.2	36.0	0.013	<1.33	15.9	<3rd percentile	[24]
4	3	175/118	160	2.8	—	ND	1.90	10.2	Growth retarded	[25]
5	$5\frac{9}{12}$	190/120	150	2.5	32.0	ND	ND	19.8	—	[26]
6	$2\frac{9}{12}$+	180/120	148	2.6	31.7	0.200	ND	9.8	—	[27]
7	$3\frac{3}{12}$	125/85	—	—	—	—	—	40.0	—	Unpublished
8	$1\frac{9}{12}$	150/110	140	2.6	25.0	0.400	3.00	32.5	—	[28]
9	19	120/80	143	3.2	30.0	0.400	2.40	15.5	—	[29]
10	$\frac{5}{12}$+	200/100	—	1.8	—	0.020	1.04	68.8	—	[30]
11	11	190/120	143	2.7	—	—	1.20	31.2	<3rd percentile	[27]
12	$3\frac{9}{12}$	200/129	—	2.3	—	—	—	13.4	<5th percentile	[27]
13	6+									[27]
14	$9\frac{4}{12}$	130/90	138	3.8	26.0	0.210	ND	8.9	<3rd percentile	[31]
15	$4\frac{4}{12}$	142/98	144	2.8	—	0.070	ND	20.1	—	[31]
16	8	130/90	140	2.4	36.0	0.070	ND	14.9	<3rd percentile	[31]
17	21	200/145	148	1.7	34.0	0.050	<3.40	13.5	Normal	[32]
18	7	160/120	145	1.8	28.0	—	—	29.8	<2.5 percentile	[33]
19	3	200/110	—	2.2	—	—	—	7.5	—	Unpublished
20	9	170/100	145	2.5	28.8	ND	1.80	17.4	Weight <5th percentile	Unpublished

AME, Apparent mineralocorticoid excess; BP, blood pressure; PRA, plasma renin activity; THF, tetrahydrocortisol; THE, tetrahydrocortisone; ND, not detectable; +, deceased.

with an increase in the ratio of cortisol metabolites (principally tetrahydrocortisol (5β-THF) and its isomer allo-tetrahydrocortisol (5α-THF) to cortisone metabolites (tetrahydrocortisone (THE)). In addition, the ratio of 5α-THF/5β-THF is raised suggesting an associated defect in 5β-reductase. Urinary free cortisol is usually high [27, 31].

11β-DH deficiency is familial. All three siblings in one family (patients 11, 12 13 in Table 1) had the condition and patients 14 and 15 were sisters. The mother of patient 17 appears to heterozygous for 11β-DH deficiency having mild low-renin hypertension, hypokalaemia and a prolonged plasma cortisol half-life. The genetics of AME have yet to be elucidated.

For several years the mineralocorticoid responsible for this condition was unknown. Numerous bioassays on plasma and urine from such patients failed to detect increased mineralocorticoid activity. From Dr Ulick's original case a high urinary excretion of 5α-dihydrocortisol had been observed, and studies suggested that this might be a potent mineralocorticoid [36]. However when infused into subjects with AME the syndrome was not reproduced and hence the term syndrome of "*apparent* mineralocorticoid excess" (AME) was coined. In 1983 Oberfield *et al.* documented sodium retention and hypertension following ACTH and cortisol infusions into patient 1 in Table 1 [37]. They postulated an abnormality in the mineralocorticoid receptor, recognizing cortisol as a mineralocorticoid.

In 1985 we described the first adult case of congenital 11β-DH deficiency a 21-yr-old male presenting with blurred vision and polydipsia/polyuria [38]. Blood pressure was elevated at 240/140 mmHg, plasma potassium was low at 1.7 mmol l^{-1}, bicarbonate high at 34 mmol l^{-1}. Shortly after admission to hospital he sustained a cardiac arrest secondary to hypokalaemia from which he was successfully resuscitated. Investigations showed suppressed plasma renin activity and aldosterone (0.1 ng ml^{-1} h^{-1} (normal range 0.5–1.5) and less than 100 pmol l^{-1} (reference range 150–500) respectively). Plasma cortisol circadian rhythm was normal but plasma cortisone measured by an in-house RIA was low at 8.4 nmol l^{-1} (reference range 40–70 nmol l^{-1}). The ratio of urinary 5β-THF + 5α-THF/THE was grossly elevated at 13.6 (reference range 0.7–1.3). Deficiency of 11β-DH was confirmed by studying the metabolism of 11α-tritiated cortisol (11α-[^{3}H]F) (which when acted on by 11β-DH forms cortisone and tritiated water). The half-life for this isotope in our patient was prolonged at 131.8 min (reference range 31.3–48.5 min, $n = 16$). As previously reported in AME conversion of cortisone to cortisol was unimpaired, indicating normal 11-keto-reductase activity.

During a series of metabolic balance studies we demonstrated that cortisol was acting as a potent mineralocorticoid. Dexamethasone by suppressing plasma cortisol, caused a natriuresis and potassium retention. Thus on a fixed dietary sodium/potassium intake, dexamethasone 2 mg/day for 48 h suppressed urinary free F from

628 nmol/24 h (reference range 80–450 nmol/24 h) to less than 30 nmol/24 h, and urinary Na^+/K^+ ratio rose from 1.2 to 3.3. Whilst taking dexamethasone, cortisol in a dose of only 10 mg/day was infused subcutaneously via a mini-pump for 4 days. This reproduced the syndrome with marked sodium retention and a kaliuresis (urinary Na^+/K^+ ratio falling from 1.2 to 0.15) (Fig. 2). Plasma K^+ fell from 4.5 to 3.3 mmol l^{-1} and blood pressure rose from 161/105 to 177/114 mmHg. On 1.25 mg of dexamethasone/day (0.5 mg at 0900 h, 0.75 mg at 2300 h) we maintained suppression of urinary free cortisol to less than 30 nmol/24 h and restored normokalaemia on a long-term basis where numerous antihypertensive and potassium sparing diuretics had failed.

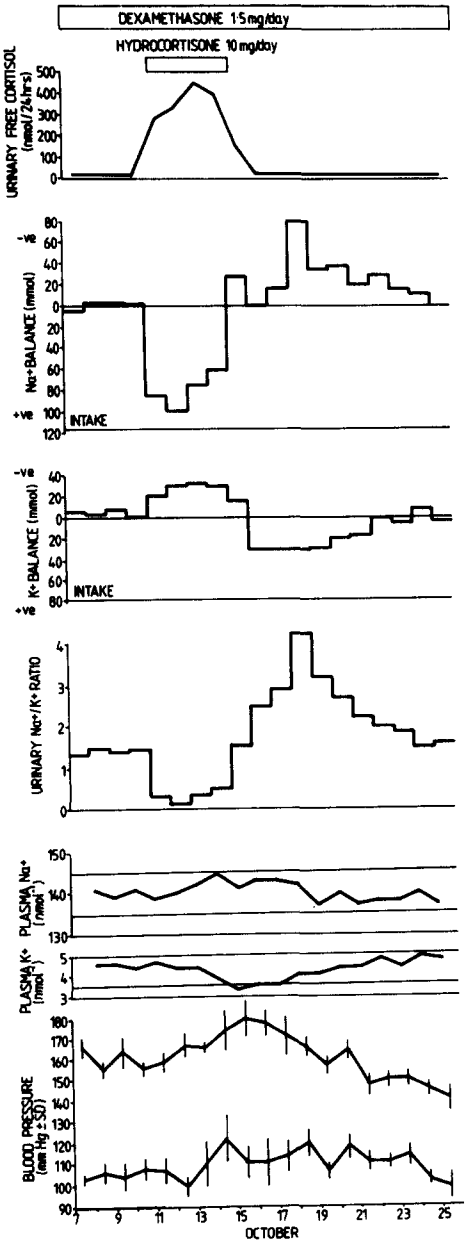

Fig. 2. Metabolic balance data, urinary free cortisol and blood pressure across hydrocortisone administration for 4 days to our index case of congenital 11β-dehydrogenase deficiency. The mean of 10 daily readings for blood pressure is shown.

It seems clear therefore that cortisol itself is the potent mineralocorticoid in congenital 11β-DH deficiency [32], a finding which has subsequently been confirmed by other workers [39]. Treatment should aim to prevent life-threatening hypokalaemia and restore normotension. Dexamethasone carefully titrated to suppress endogenous cortisol production prevents hypokalaemia. However, diuretics and angiotensin converting enzyme inhibitors may be needed in addition to control blood pressure. Table 2 shows the recent status and therapy in 10 of the patients depicted in Table 1.

Clearly cortisol does not have such catastrophic effects in normals and we believed that our index case was telling us something important about the tissue control of steroid activity. We hypothesized that renal 11β-DH was an important physiological mechanism, protecting exposure of the kidney to cortisol by shuttling it to the inactive steroid cortisone. Failure of this mechanism in AME results in the kidney seeing cortisol as a potent mineralocorticoid. Further evidence to support our hypothesis came from the investigation of subjects with liquorice and carbenoxolone induced mineralocorticoid excess.

Acquired inhibition of 11β-DH: effects of liquorice and carbenoxolone administration

Liquorice has been used medically for at least 5000 years but scientific interest was aroused in 1946 in the small Dutch village of Heerenveen. It was here that Reevers studied many patients suffering from peptic ulcer who improved after taking a proprietary liquorice preparation (succus liquoritiae) from the local chemist. However, 2 years later Reevers went on to report that one in five of these patients developed oedema, shortness of breath and hypertension [40]. Since then there have been numerous reports of liquorice-induced hypertension with hypokalaemia, complicated in some cases by myopathy and cardiac arrythmias [41–43]. Patients present as a mineralocorticoid excess state with sodium retention, suppression of the renin-aldosterone system and hypokalaemia [44]. The condition is reversible on stopping liquorice and responds to spironolactone administration [45].

Table 2. Recent status and drug therapy of patients with apparent mineralocorticoid excess (AME)

Patient	Age now (yr)	Age first seen	Age last seen	Blood pressure (mmHg)	K (mmol/l)	Na (mmol/l)	Drugs and diet
4	23	3.0	22.0	125/80	4.40	138	Spironolactone 300 mg/day Amiloride 5 mg/day Chlorthalidone 50 mg/day
5	22	1.6	20.0	128/99	3.40	138	Amiloride 10 mg/day Captopril 25 mg/day Nadolol 160 mg/day Prazosin 5 mg/day
7	13	—	10.0	105/70	3.70	143	Triamterene 150 mg/day Low dietary sodium
8	11	1.5	9.0	115/80	Low normal	138	Triamterene Furosemide Amiloride
9	27	—	9.3	140/100	3.70	142	Amiloride 150 mg/day Low dietary sodium 80 mmol/day
11	19	3.0	17.5	160/100	4.12	—	Nifedipine 10 mg/day Triamterene 100 mg/day
12	9	1.0	7.5	134/82	3.99	—	Spironolactone 75 mg/day Dexamethasone 1.0 mg/day
17	26	21.0	25.0	130/90	4.10	142	Dexamethasone 1.25 mg/day Captopril 25 mg/day Furosemide 40 mg/day
18	12	—	9.3	140/100	3.70	162	Spironolactone 200 mg/day Dietary sodium 80 mEq/day
20	10	8.0	9.0	114/78	6.30		Spironolactone 200 mg/day

The active mineralocorticoids in liquorice are glycyrrhizic acid (GI) and its hydrolytic product glycyrrhetinic acid (GE), and it is widely believed on the basis of two receptor studies that they exert their effect via a direct action on the mineralocorticoid receptor [46, 47]. However it seemed unlikely from previous findings that this could be the explanation. In some elegant balance studies by Dutch workers in the 1950s liquorice was shown to be ineffective in subjects with Addison's disease [48]. Borst in 1953 concluded that the "deoxycortone-like action of liquorice always present in people with intact suprarenal glands, was absent in three patients with Addison's disease". However 10 mg cortisone given daily to these patients fully restored the mineralocorticoid effect [49]. Similarly liquorice had no biological effect in adrenalectomized rats [50, 51]. These studies therefore point strongly to the requirement of functional adrenal tissue and/or the presence of glucocorticoids for liquorice to possess mineralocorticoid activity and argue against affinity for the mineralocorticoid receptor being the predominant mechanism (which would be unaltered in Addison's disease or adrenalectomized patients).

There were other pointers in the literature to suggest that 11β-DH deficiency might be important in this area. Workers in New Zealand had shown an apparent change in the renal handling of cortisol in subjects consuming liquorice, in that 10 of 13 volunteers who consumed 100–200 g liquorice/day had a doubling of their urinary free cortisol excretion with no change in plasma cortisol [52]. Dexamethasone has been shown to have an antimineralocorticoid effect in subjects given glycyrrhetinic acid i.e. causes a natriuresis and potassium retention [53]. Thus there are many similarities between 11β-DH deficiency and liquorice-induced mineralocorticoid excess. It was on this background that we evaluated the effects of liquorice ingestion on 11β-DH activity in man [54]. Seven normal male volunteers (mean age 30.1 ± 1.9 (SEM) yr) were established on a fixed Na^+/K^+ diet (130 mmol sodium, 80 mmol potassium/day). After a 5 day run-in period liquorice 200 g/day (containing 580 mg GI/day) was given in divided doses for 10 days. As shown in Fig. 3 liquorice produced significant sodium retention with suppression of the renin–aldosterone axis. There was a marked kaliuresis significant on each day of liquorice ingestion resulting in a fall of plasma potassium from 4.1 ± 0.2 to 3.7 ± 0.1 mmol l^{-1} ($P < 0.05$). Urinary free cortisol rose across liquorice administration but there was no change in 0900 h plasma cortisol. 0900 h plasma cortisone fell from 42.8 ± 6.4 nmol l^{-1} to 10.2 ± 0.9 on day 10 of liquorice ingestion ($P < 0.001$). As shown in Table 3 the 5β-THF + 5α-THF/THE ratio increased suggesting inhibition of 11β-DH and this was confirmed by demonstrating an increase in the plasma half-life of 11α-[^{3}H]F from 40.7 ± 0.7 min to 84.3 ± 5 min ($n = 3$) after one week of liquorice ingestion. Plasma glycyrrhetinic acid was measured across liquorice administration and was consistently less than

1 μg/ml (i.e. much lower than would be required to displace aldosterone as calculated from the previous mineralocorticoid receptor studies).

Animal experiments performed in parallel with these studies in man have confirmed that GI and GE inhibit renal 11β-DH both *in vitro* and *in vivo*. *In vitro* a K_i of 10^{-9} M for GE was observed, indicating potent inhibition [55].

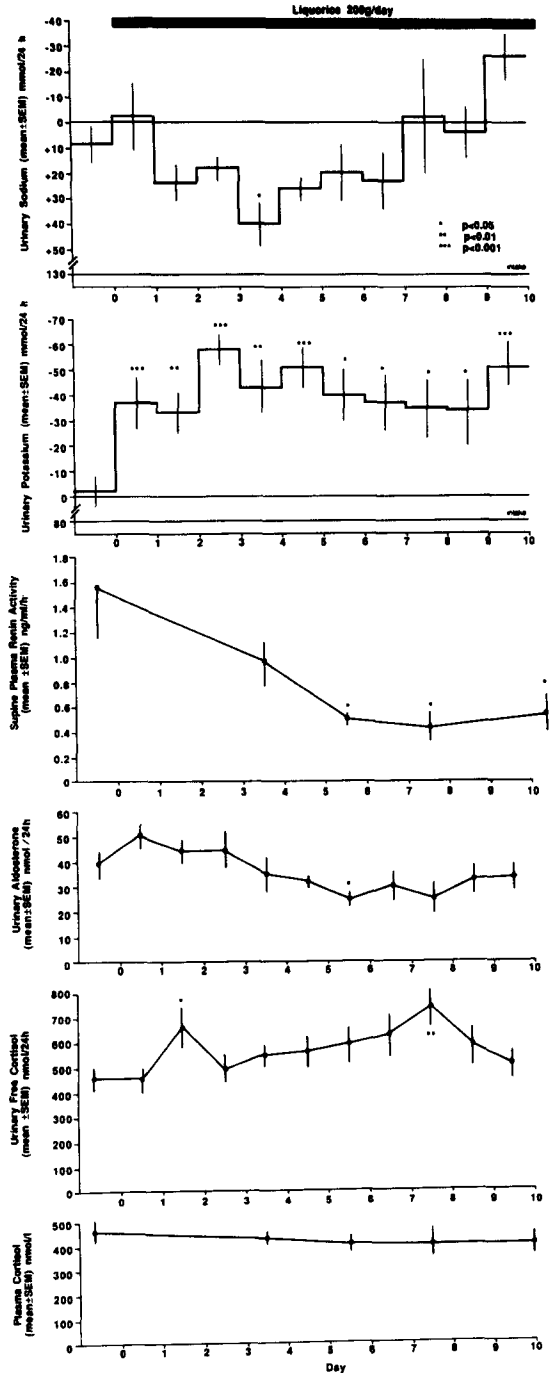

Fig. 3. Mean 24 h urinary sodium, potassium, aldosterone, cortisol, plasma renin activity and plasma cortisol for the seven volunteers on a fixed metabolic dietary intake and receiving liquorice 200 g/day (reproduced with permission of the *Lancet*). *P* values comparing results on day indicated with those on day −1.

Carbenoxolone is a synthetic derivative of GE used in the treatment of peptic ulceration. Its use is now declining, principally because of the effectiveness of H_2 receptor antagonists but also because of mineralocorticoid side-effects seen in up to 50% of patients taking the drug [56]. As with liquorice it was widely thought that carbenoxolone acted directly on the mineralocorticoid receptor [57]. Recently we have shown in 6 volunteers that the sodium retention following carbenoxolone ingestion is associated with inhibition of 11β-DH [58], and again these findings have been confirmed in animal studies [59, 60]. However, we did see striking differences between liquorice and carbenoxolone administration. Although carbenoxolone did inhibit 11β-DH (as judged by a three-fold increase in the plasma half-life of 11α-[^{3}H]F), and reduced daily cortisol production rate, there was no change in the urinary 5β-THF + 5α-THF/THE ratio, nor in plasma cortisone. In addition plasma cortisol following oral cortisone was diminished whilst taking carbenoxolone suggesting inhibition of 11-keto-reductase in addition to 11β-DH. This has similarities to three recently described patients with the so-called "type 2" variant AME, who present as AME patients with reduced cortisol metabolism and production rates, but with normal 5β-THF + 5α-THF/THE ratios [61].

It would appear therefore that the mineralocorticoid activity of liquorice and carbenoxolone is mediated via cortisol through acquired inhibition of 11β-DH and not, as previously thought, via a direct action of GI and GE on the mineralocorticoid receptor. Studies with liquorice and carbenoxolone therefore added further weight to our hypothesis regarding a paracrine role for the cortisol–cortisone shuttle. For the first time we were able to show that

Table 3. Urinary tetrahydrocortisol (THF) + allo-tetrahydrocortisol (allo-THF)/tetrahydrocortisone (THE) ratio and allo-tetrahydrocortisol/tetrahydrocortisol ratio on days −1, +4 and +10 of liquorice ingestion

	THF + allo − THF/THE			allo-THF/THF		
Subject	Day −1	Day 4	Day 10	Day −1	Day 4	Day 10
1	0.96	0.97	1.30	0.51	0.57	0.53
2	0.60	0.71	0.70	0.26	0.32	0.26
3	0.95	1.03	1.08	0.76	0.77	0.98
4	1.17	1.50	1.47	0.89	1.24	1.20
5	0.91	1.05	1.04	0.72	0.80	0.57
6	0.84	0.91	1.05	0.62	0.71	0.86
7	1.04	1.52	1.61	0.97	0.99	1.47
Mean	0.92	1.10[a]	1.18[b]	0.67	0.77	0.84
(SEM)	(0.07)	(0.11)	(0.12)	(0.09)	(0.11)	(0.16)

For comparison with value on day −1: [a]$P < 0.05$; [b]$P < 0.01$.

11β-DH deficiency was relevant in mineralocorticoid excess states other than that occurring in a small number of hypertensive children.

The kidney as a site of cortisol metabolism

Although the liver has been thought to be the major site of cortisol metabolism, for our hypothesis to be correct the kidney must play an important role. Previous studies have shown a prolonged cortisol half-life in patients with chronic renal failure [62], and cortisol isotopic studies suggested that renal conversion of F to E did occur [63].

In vitro we have shown that for a fixed mg protein/g wet weight tissue (and hence enzyme concentration), the kidney was more effective in converting F to E than the liver, the reverse being true for the conversion of E to F [64].

Recently 88 patients with a variety of renal diseases have been compared with 47 normal controls [65]. Renal patients were divided on the basis of their plasma creatinine and a marked reduction in 0900 h plasma cortisone with increasing renal impairment was observed. However 0900 h plasma cortisol levels were similar in all groups. There was a negative correlation between plasma creatinine and cortisone ($r = 0.52$, $P < 0.01$, $n = 88$). In 5 nephrectomized patients plasma cortisone was between 5.1 and 6.3 nmol l^{-1}. (In normal subjects plasma cortisone levels ranged from 40–60 nmol l^{-1}.)

These results would suggest therefore that in man the kidney is the major site for the conversion of cortisol to cortisone.

11β-DH activity in blood vessels

An interesting development is the demonstration of 11β-DH activity and "aldosterone-selective" mineralocorticoid receptors in mesenteric blood vessels [66], raising the possibility that the cortisol–cortisone shuttle in resistance vessels may also be important in blood pressure control.

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