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11β-Hydroxysteroid Dehydrogenase and the Syndrome of Apparent Mineralocorticoid Excess*

PERRIN C. WHITE, TOMOATSU MUNE†, AND ANIL K. AGARWAL

Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas Texas 75235-9063

Address reprint requests to: Perrin C. White, M.D., University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9063.
*This work is supported by NIH Grant DK-42169.
†Present address: Third Department of Internal Medicine, Gifu University School of Medicine, Gifu 500, Japan.

I. Introduction

STEROID hormones are commonly thought to act by occupying specific receptors in the cytoplasm or nucleus that, once liganded, increase transcription of specific genes by binding to hormone response elements. According to this model, the biological effects of a steroid may be predicted from its ability to bind and activate particular receptors. However, a number of situations have been identified in which steroid hormone action is modified by tissue-specific metabolism. For example, testosterone is converted by steroid 5α-reductase in male genital tissues to dihydrotestosterone, which is a much stronger androgen. This considerably increases the tissue specificity of testosterone's actions (1).

This article reviews a mechanism by which the actions of glucocorticoids are modulated. The studies summarized herein extend more than almost 50 yr and across such fields as pharmacology, clinical endocrinology, and molecular genetics. A theme common to many of these studies is biological effects of glucocorticoids that seem to be paradoxical.

The first of several such paradoxes was noted early in the therapeutic use of corticosteroids. Cortisone first became available for therapeutic use in 1949 and, when administered systemically, was found to effectively treat inflammatory conditions such as rheumatoid arthritis (2, 3). However, it was poorly effective when injected directly into the joint (4). In contrast, when hydrocortisone, a closely related steroid, became widely available 2 yr later, it was found to be effective both systemically and intraarticularly (4).

At around the same time, studies of patients with Cushing's disease and those recovering from surgery demonstrated that hydrocortisone (*i.e.* cortisol) was the principal secretory product of the human adrenal cortex and was presumably the most important glucocorticoid hormone (5). Moreover, systemically administered cortisone was partially excreted as cortisol. These findings suggested that systemically administered cortisone might be biologically active only when converted to cortisol. Thus, cortisone would not be active when injected intraarticularly because the enzyme that converted cortisone to cortisol would not be present in the joint.

An enzymatic activity catalyzing this conversion was identified in rat liver (6, 7) and eventually termed 11β-hydroxysteroid dehydrogenase (11-HSD, EC 1.1.1.140). Interconversion of cortisol and cortisone was subsequently found in many tissues. The physiological consequences of this interconversion are reviewed below, after a description of the main pathways of cortisol metabolism.

II. Biochemistry of Cortisol Metabolism

Plasma cortisol is bound with high affinity to corticosteroid-binding globulin, which protects it from degradation (8). The normal plasma half-life of cortisol is between 60 and 80 min, in contrast to other steroids such as aldosterone that have half-lives of less than 20 min due, in large part, to lower affinity for plasma proteins.

The liver and the kidney are the principal organs involved in metabolizing glucocorticoids and clearing them from the circulation. Metabolism decreases the biological activity of these hormones and increases their water solubility by converting them to hydrophilic compounds that can be excreted in urine. Free cortisol is also present in urine but normally comprises only about 0.1% of the total cortisol metabolites (reviewed in Ref. 9).

Cortisol and corticosterone are metabolized similarly (Fig. 1). The C-4,5 double bond is reduced; if the hydrogen at the 5 position is added in the β-orientation, the product is 5β-dihydrocortisol, whereas 5α-reduction yields 5α-dihydrocortisol. Under normal circumstances, 5β-reduction predominates. The 3-oxo group may also be reduced; 3α-reduction is strongly favored over 3β-reduction. The products of these "A ring" reductions are tetrahydrocortisol (if 5β-reduced) and allo-tetrahydrocortisol (if 5α-reduced). Cortisol or its reduced metabolites may be oxidized at the 11-hydroxy position to cortisone, dihydrocortisone, or tetrahydrocortisone. Tetrahydrocortisol and tetrahydrocortisone also undergo reduction at the C-20 position to yield cortol and cortolone, respectively. Hydroxylation at the 6β position occurs predominantly in fetal life and infancy.

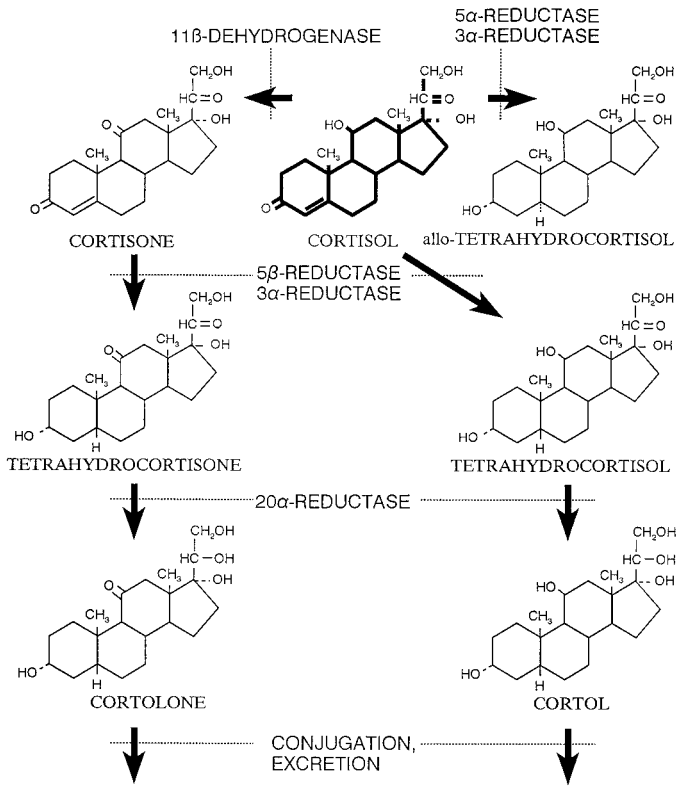

FIG. 1. Pathways of cortisol metabolism. Enzymes mediating each conversion are listed adjacent to each *arrow*. Although only cortol and cortolone are depicted as being conjugated and excreted, any 3α-reduced steroids can be conjugated, and all listed steroids can be excreted in the urine.

At least 90% of cortisol and cortisone metabolites are normally excreted as sulfate or glucuronide conjugates. Conjugation of cortisol and cortisone can occur at the 21 position, and 3α-reduced steroids can also be conjugated at the 3α position (10).

III. Mineralocorticoid Receptor Function

A. Actions of aldosterone

Aldosterone regulates electrolyte excretion and intravascular volume mainly through its effects on the renal distal convoluted tubule and cortical collecting duct, where it acts to increase resorption of sodium from the urine through at least two mechanisms. The first step in sodium resorption is by passive diffusion through sodium-permeable channels in the apical membranes of the epithelial cells lining the distal convoluted tubule and collecting duct. Aldosterone increases the apparent number of such channels. This may reflect an increase in the percentage of time that each channel stays open, possibly mediated by methylation of the channel (11), and/or an increase in the actual number of channels (12). Aldosterone also increases synthesis of a sodium/potassium ATPase located in the basolateral cell membrane, which generates the electrochemical gradient that drives diffusion through the sodium channels (13).

Although membrane receptors for aldosterone may exist (14), most effects of aldosterone are mediated by a specific nuclear receptor referred to as the mineralocorticoid or "type 1 steroid" receptor.

These receptors are expressed at high levels in renal distal convoluted tubules and cortical collecting ducts but also in other mineralocorticoid target tissues, including salivary glands and the colon. They are also found at multiple sites in the brain and at low levels in the myocardium and in the peripheral vasculature (see below).

B. Structure and function of the mineralocorticoid receptor

The mineralocorticoid receptor has a high degree of sequence identity with the glucocorticoid or "type 2" receptor (15). These receptors are least similar (<15% identical) in their amino-terminal domains. In the glucocorticoid receptor, this region is known to interact with other nuclear transcription factors, and this region presumably has a similar function in the mineralocorticoid receptor (16). The center of the molecule contains a DNA-binding domain consisting of two "zinc fingers"; this region is also involved in dimerization of liganded receptors. The amino acid sequences of the mineralocorticoid and glucocorticoid receptors are 94% identical in this region. The carboxyl terminus is a ligand-binding domain that is 57–60% identical in amino acid sequence in the two receptors.

As is the case with the glucocorticoid receptor, the unliganded mineralocorticoid receptor is located mainly in the cytoplasm (17). Once liganded, the receptor is translocated to the nucleus where it dimerizes and binds to hormone re-

sponse elements in the 5′-flanking regions of specific genes, thus increasing their transcription.

When cDNA encoding the mineralocorticoid receptor was cloned and expressed (15), it became apparent that this receptor had very similar binding affinities for aldosterone and for glucocorticoids such as corticosterone and cortisol. This was consistent with observations that this receptor in the rat hippocampus had identical affinities for aldosterone and corticosterone (18, 19), but it was difficult to reconcile with the fact that corticosterone and cortisol are relatively weak mineralocorticoids *in vivo*.

It was initially proposed that the discrepancies between the ligand specificities of the mineralocorticoid receptor in hippocampus and kidney resulted from the presence of extravascular corticosteroid-binding globulin ("tissue transcortin") in renal but not in hippocampal cytosol. Corticosteroid binding globulin, which also circulates in the blood, can sequester cortisol and corticosterone but not aldosterone (18, 20). However, this hypothesis was rendered untenable by the finding that the renal mineralocorticoid receptor in neonatal rats has the same ligand specificity as adult kidney despite very low levels of corticosteroid-binding globulin (21).

A recent study suggested that aldosterone and glucocorticoids in fact do not behave identically at the mineralocorticoid receptor; aldosterone apparently dissociates much more slowly from the receptor than corticosterone does, and thus receptor liganded with aldosterone transactivates reporter genes much more strongly than receptor liganded with corticosterone (22). However, this study detected agonist effects of aldosterone at concentrations well below those that are required for effects *in vivo*, casting some doubt on its validity. At this time, it awaits independent confirmation.

C. Hypothesis: 11-HSD protects the mineralocorticoid receptor

A mechanism conferring ligand specificity on the mineralocorticoid receptor was deduced from studies of two conditions in which the normal specificity of the receptor was lost, the syndrome of apparent mineralocorticoid excess (AME) and licorice intoxication. These conditions have three features in common: cortisol acts as a much stronger mineralocorticoid agonist than is normally the case, the plasma half-life of cortisol is prolonged, and urinary excretion of cortisone metabolites is decreased relative to excretion of cortisol metabolites, implying that 11-HSD, the enzyme catalyzing the conversion of cortisol to cortisone, is decreased in activity.

It was proposed (23) that whereas AME represented a congenital deficiency of this enzyme, licorice intoxication resulted from pharmacological inhibition of 11-HSD. In either case, intrarenal concentrations of cortisol would be abnormally high as a consequence of deficient metabolism by 11-HSD and would thus saturate mineralocorticoid receptors. This model was refined by studies of tissue distribution of 11-HSD activity (24, 25). Activity was high in tissues such as the kidney and parotid gland, in which mineralocorticoid receptors were specific for aldosterone, and low in the hippocampus and heart, tissues in which glucocorticoids are able to bind this receptor. Moreover, inhibition of 11-HSD in various tissues by active components of licorice resulted in loss of ligand specificity as evidenced by increased ability of tissues or their cytosols to bind glucocorticoids.

Thus, it was hypothesized (24, 25) that oxidation by 11-HSD of cortisol or corticosterone to cortisone or 11-dehydrocorticosterone, respectively, represented the physiological mechanism conferring specificity for aldosterone upon the mineralocorticoid receptor (Fig. 2). Although cortisol and corticosterone bind the receptor well *in vitro,* cortisone and 11-dehydrocorticosterone are poor agonists for this receptor. Aldosterone is a poor substrate for 11-HSD because, in solution, its 11-hydroxyl group is normally in a hemiacetal conformation with the 18-aldehyde group.

Evidence supporting this hypothesis is discussed in detail in the following sections of the review.

IV. Loss of Function of 11-HSD

A. Syndrome of AME

1. Clinical features. A hypertensive syndrome was first described more than 20 yr ago in which children presented with hypertension, hypokalemia, and reduced PRA, all signs of mineralocorticoid excess. A low salt diet or blockade of mineralocorticoid receptors with spironolactone ameliorated the blood pressure. ACTH exacerbated the hypertension. Therefore, it was initially suspected that these patients had elevated levels of an unknown ACTH-inducible mineralocorticoid, but levels of all known mineralocorticoids were low (26–28). For this reason, this was termed a syndrome of "apparent mineralocorticoid excess" (AME) (29). This disorder was distinguished clinically from a similar disorder, Liddle's syndrome, by the therapeutic response to spironolactone (30). Patients with Liddle's syndrome do not respond to blockade of the mineralocorticoid receptor but can be treated by direct blockade of the renal tubular sodium channel with triamterine. It is now known that Liddle's syndrome is caused by mutations in the β- or γ-subunits of the sodium channel, leading to constitutive activation of this channel (31, 32).

Other clinical features reported in patients with AME include moderate intrauterine growth retardation and postnatal failure to thrive (26, 27, 29, 33–48) (Table 1). Consequences of the often severe hypokalemia include nephrocalcinosis and nephrogenic diabetes insipidus, which leads to polyuria and polydipsia. Deleterious effects on muscle range from elevations in serum creatine phosphokinase to frank rhabdomyolysis.

In some patients, the hypertension tends to be labile or paroxysmal with severe emotional stress as a precipitating factor. Complications of hypertension have included cerebrovascular accidents. Several patients have died during infancy or adolescence. The reasons for their demise are not known but might include either electrolyte imbalances leading to cardiac arrhythmias or vascular sequelae of hypertension.

Several affected sibling pairs have been reported, but parents have usually been asymptomatic, suggesting that AME is a genetic disorder with an autosomal recessive mode of inheritance (34, 40, 44, 45). However, occasional abnormalities, including hypertension and hypokalemic alkalosis, have been observed in parents (43).

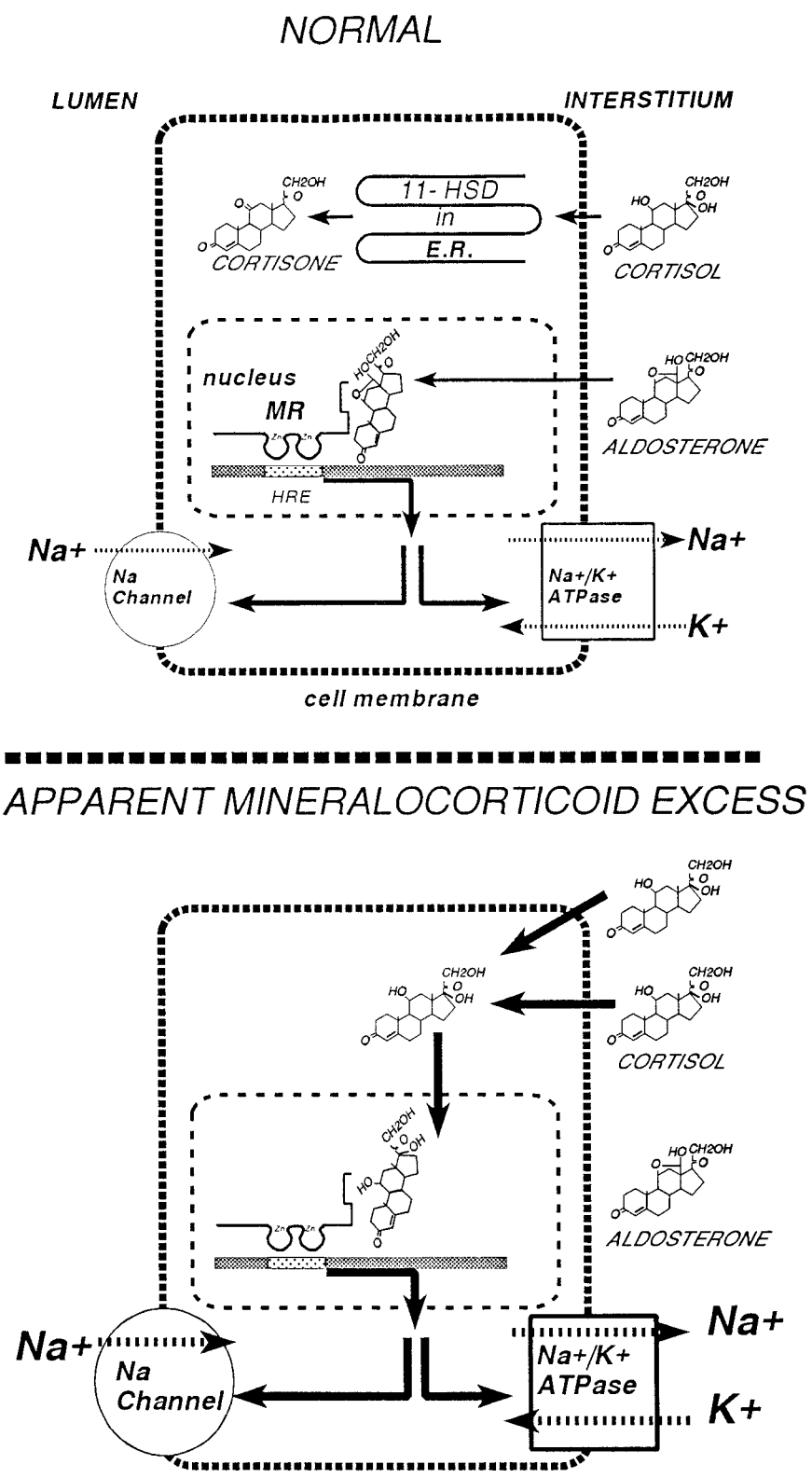

FIG. 2. Schematic of mineralocorticoid action. *Top*, A normal mineralocorticoid target cell in a renal cortical collecting duct. Aldosterone occupies nuclear receptors (MR) that bind to hormone response elements, increasing transcription of genes and directly or indirectly increasing activities of apical sodium (Na) channels and the basolateral sodium-potassium (Na/K) ATPase. This increases resorption of sodium from and excretion of potassium into the tubular lumen. Cortisol, which circulates at higher levels than aldosterone, cannot occupy the receptor because it is oxidized to cortisone by 11-HSD. *Bottom*, A cell from a patient with the syndrome of AME. Because 11-HSD is absent, cortisol inappropriately occupies mineralocorticoid receptors, leading to increased gene transcription, increased activity of sodium channels and the Na/K ATPase, increased resorption of sodium and excretion of potassium, and hypertension.

2. Biochemical findings. It was quickly realized that patients with AME had abnormal cortisol metabolism. It was first noted that there was an increase in the ratio of 5α to 5β-reduced cortisol metabolites (28). For this reason, it was proposed that 5α-dihydrocortisol might be the culprit mineralocorticoid, but absolute levels of this steroid were not elevated in these patients. It was subsequently found that there was a marked prolongation in plasma cortisol half-life from the normal of approximately 80 min to 120–190 min (29, 39, 40). Moreover, there was a marked deficiency in 11β-

TABLE 1. Clinical data on patients with AME

Patient[a]	Ethnic Group	Sex	Age[b]	Birth Wt (g)	BP (mm Hg)	FTT	DI	Other Sx	K+ mM	HCO_3 mM	$\frac{THF + aTHF}{THE}$	$\frac{aTHF}{THE}$	$t_{1/2}$ min	Low salt	Spiro	Dex	Other Rx	Ref
1	White	F	3 y	2500	175/118	+			2.8		10.2				300		Amiloride	(26)
2*	Native N. American	F	9 m; 3 y	1870	180/140	+	+		2.7		16.2		187	+	100	NE		(27)(29)
3	Native N. American	F	3 w; 2 y, 9 m		180/120			Died 12 y, CVA	2.5	31.7	9.8	3.4			300	NE	Triamterine	(33)(34)
4*	Native N. American	M	3 m; 1 y, 7 m		140/100		+		3.1	26.6	19.8	5.4		+	300	NE	Triamterine	(33)(34)
5*	Indian	M	7 y; 9 y	2000	250/180	+	+	CVA, 7 y	2.6		10.0		122	+	+	NE		(29)
6	Japanese	F	3 y	2330	144/88		+	Paralysis	1.4	35.2	11	11			NE	+	Adrenalectomy	(35)
7	White	M	2 y		140/90	+	+		2.2	36	15.9	6.2		+	100	NE	Triamterine	(36)
8	White	M	1 m; 1 y, 7 m	2170	150/110	+	+	Retinopathy	2.6	28	45	2.3		NE	NE	NE	Triamterine Furosemide	(37)
9	White	M	1 m; 5 m	2360	200/100			Died 5 m	1.8	29	68.8	13.6						(38)
10	White	F	9 m, 19 y		150/100	+			3.0	32	15.5		109	+	200	NE	Amiloride	(39)
11a*	Native S. American	F	3 y; 11 y		170/110	+	+		2.7		31.3	4.0					Triamterine Nifedipine	(34)
11b*	Native S. American	F	3 y 9 m; birth		200/129	+	+		2.3		13.4	4.0			200		Triamterine	(34)
12a*	African American	F	2 y; 9 y, 4 m	2100	130/90	+		Weakness; nephrocalcinosis	1.4		8.9	2.0	113		100	NE		(40)(41)
12b*	African American	F	2 y, 6 m; 4 y, 4 m	2600	142/98			Nephrocalcinosis	2.8		20.1	2.2	128		100			(40)(41)
13*	Indian	M	9 m; 8 y	2000	145/palp	+	+	Nephrocalcinosis	1.9		14.9	2.2	137		100		Triamterine	(40)(41)
14	White	F	2 y		160/120	+	+	Rickets	1.8	28	29.8			+	400		Triamterine	(42)
15	White	M	21 y		200/145		+	Nephrocalcinosis; heart arrhythmia	1.7	32	13.6	1.9	131			2		(43)
16a*	Asian	M	6 m; 3 y, 6 m		141/117	+	+	Nephrocalcinosis	2.1	29	20.0	1.7	152		30		Amiloride	(44)
16b*	Asian	M	1 y, 5 m		144/91	+	+	Nephrocalcinosis	3.0		43.4	1.8						(44)
17a*	Iranian	F	14 y, 2 m		220/160				2.8	24	8.9	2.3			+		Furosemide Captopril	(45)
17b	Iranian	M	11 y, 6 m		170/110			Paralysis	2	24	6.8	2.2			+			(45)
17c*	Iranian	F	4 y, 2 m		160/100				3.1	22	6.9	2.1			+			(45)
18*	Native N. American	M	9 y	1940	170/100	+	+	CVA	2.6	29	14.4	1.6			200			(46)
19*	Native N. American	F	1 y	1710 33 wk	142/92	+		Weakness	2.8	33	73.8	5.7		NE	NE		Triamterine RCl	(46)
20*	Native N. American	M	3 y, 4 m	1960 36 wk	205/130	+		Calciuria, delayed development	0.9	31	25.6	4.8		+	75	0.5	Nifedipine	(46)
21*	Mexican American	F	26 y		180/120				4.1	26	7.9	2.9			200	NE	Furosemide	(46)
22*	White	M	2 y, 3 m	1990 37 wk	149/83	+		Rhabdomyolysis; nephrocalcinosis	2.4	34	134	10.8			150			(46)
23*	Indian	M	1 y, 6 m; 2 y, 6 m		150/100	0			1.8	32	5.9							(47)
24a*	Arab	M	<1 y; 10 y		160/118	+		Nephrocalcinosis; facial palsy	2.9		59						KCl	(47)
24b*	Arab	M	<1 y, 9 y		170/110	+		Nephrocalcinosis; facial palsy	1.7		9.2						KCl	(47)
25	Japanese	M	2 y	2532	190/110	+	+	Rhabdomyolysis	2.1		43.7/17.4	2.6		+	100	NE	Nifedipine	(48)

BP, Blood pressure; FTT, failure to thrive; DI, nephrogenic diabetes insipidus; Sx, symptoms; CVA, cerebrovascular accident; K+, serum potassium; HCO_3, serum bicarbonate, $t_{1/2}$, plasma cortisol half-life, low salt, therapeutic effectiveness of low sodium diet (+, effective; NE, not effective); spiro, effective dose in mg/d of spironolactone, dex, effective dose in mg/d of dexamethasone.

[a] Patients marked by an *asterisk* have had their mutations identified (Table 2).

[b] Where more than one age is listed for a patient, the first is the age at presentation and second is the age at the time of diagnostic studies.

[c] Normal values for (THF + aTHF)/THF are 1.07 ± 0.30 (4–8 yr) and 1.30 ± 0.30 (adult). Normal values for aTHF/THF are 1.26 ± 0.54 (4–8 yr).

dehydrogenation; very low levels of cortisone metabolites were excreted when labeled cortisol was administered. However, 11-reduction was unimpaired; labeled cortisone was excreted entirely as cortisol and other 11β-reduced metabolites (29).

Total urinary excretion of cortisol metabolites was markedly decreased, but patients did not have any signs or symptoms of Addison's disease, and serum cortisol levels were normal. Presumably, these findings were a consequence of the prolonged serum half-life of cortisol, which led to suppression of ACTH secretion. Consistent with this idea, cortisol excretion was markedly increased by ACTH administration (27, 36).

These findings suggested that cortisol itself might be acting as a strong mineralocorticoid in these patients. This was confirmed by administering hydrocortisone (*i.e.* cortisol), which aggravated the hypertension and hypokalemia in a dose-dependent manner (50). In contrast, administering aldosterone had no effect on blood pressure; this was assumed to reflect saturation of mineralocorticoid receptors by cortisol.

In subsequent studies, the biochemical parameter most frequently used to diagnose this disorder has been a precursor-product ratio reflecting 11β-dehydrogenase activity—the sum of the urinary concentrations of tetrahydrocortisol and allo-tetrahydrocortisol, divided by the concentration of tetrahydrocortisone, abbreviated (THF+aTHF)/THE (34). The relative activity of 5β-reductase is measured by the ratio of the 5α-reduced to 5β-reduced metabolites, aTHF/THF. Although both ratios are abnormal in patients with AME (34, 40), the two ratios are linearly related, and the (THF+aTHF)/THE is usually much higher than the aTHF/THF ratio (Fig. 3), implying that the primary defect in this disorder is indeed one of 11β-dehydrogenation. This has been assayed directly by administering 11α[^{3}H]-cortisol to subjects and measuring the appearance of [^{3}H]water (29, 39, 41, 43).

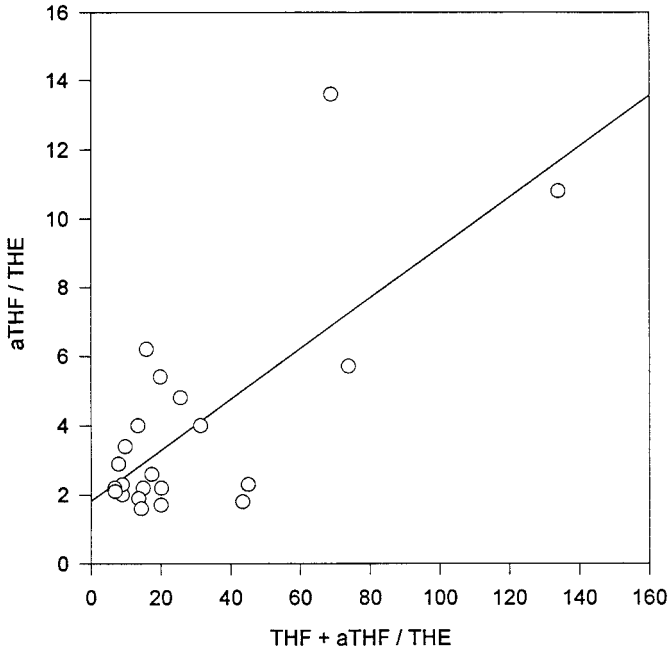

FIG. 3. Correlations between the precursor-product ratios for 5β-reduction (aTHF/THF) and 11β-dehydrogenation ([aTHF+THF]/THE) in patients with AME.

3. Treatment. A low-salt diet, potassium supplementation, and spironolactone have been the most often used therapies for AME (Table 1). Triamterine, another potassium-sparing diuretic, has also been successfully used, particularly because it is difficult to distinguish AME from Liddle's syndrome without specialized steroid assays. Triamterine inhibits the tubular sodium channel. Amiloride has similar effects and is also effective therapy for AME. The hypertension of AME has also been treated with nifedipine. Although ACTH exacerbates the signs of this disorder, suppression of ACTH secretion with dexamethasone has rarely been effective in ameliorating hypokalemia and hypertension. However, dexamethasone was effective in a patient in whom bilateral subtotal adrenalectomy had previously been performed, suggesting that the lack of efficacy of this agent is related to incomplete suppression of cortisol secretion (35). Angiotensin-converting enzyme inhibitors such as captopril enhance renal 11-HSD activity and might theoretically be useful in patients with partially active enzymes (51).

B. Licorice intoxication

Licorice is an extract of the roots of *Glycyrrhiza glabra*. It has been used as a flavoring extract and herbal remedy for millenia (52). It promotes healing of peptic ulcers. The active component of licorice is glycyrrhetinic acid, and its hemisuccinate derivative, carbenoxolone, has enjoyed wide use in Europe and Japan for treatment of ulcers.

It has been known for more than 40 yr that licorice has undesirable mineralocorticoid-like side effects including hypertension, edema, heart failure, and hypokalemia (53). Hypokalemia may in turn cause muscle weakness and, if severe, rhabdomyolysis. These side effects are shared by glycyrrhetinic acid and carbenoxolone. Although therapeutic use of carbenoxolone has decreased, licorice flavoring is still used in candy and in chewing tobacco, and sufficient quantities may be ingested to lead to significant medical problems (54–56).

Although patients suffering from licorice intoxication have signs of mineralocorticoid excess, they have low circulating levels of aldosterone. The most obvious explanation for this is that licorice and its active components themselves have mineralocorticoid activity. This idea seems particularly reasonable considering that glycyrrhetinic acid has a structure closely resembling steroids. However, glycyrrhetinic acid and carbenoxolone bind mineralocorticoid receptors very weakly. Moreover, they have no mineralocorticoid effects in patients or animals without functioning adrenal glands (57), suggesting that they must act by amplifying mineralocorticoid actions of some endogenous steroid(s), particularly glucocorticoids. In rats treated with carbenoxolone, exogenously administered glucocorticoids have mineralocorticoid effects that are blocked by mineralocorticoid antagonists (58).

Humans who ingest large quantities (200 g/day) of licorice excrete urinary steroids in a manner similar to patients with AME although the abnormalities are much milder; they have

decreased excretion of cortisone metabolites and increased urinary excretion of free cortisol (23, 59). Similar effects have been noted in rats (60). These findings suggest that licorice inhibits 11-HSD. This has been confirmed by studies of isolated rat kidney microsomes (60, 61). Thus, it appears that licorice intoxication is a reversible pharmacological counterpart to the inherited syndrome of AME.

However, glycyrrhetinic acid inhibits other enzymes including 5β-reductase, 3β-dehydrogenase (62), and 17β-dehydrogenase. Therefore, it cannot be assumed that all biological effects of licorice result from its ability to inhibit 11-HSD. Indeed, synergism of carbenoxolone with 11-deoxycorticosterone (which is not a substrate for 11-HSD) has been reported (63). Moreover, the biological effects of glycyrrhetinic acid and carbenoxolone are not identical; carbenoxolone apparently inhibits both 11β-dehydrogenase and 11-reductase activities, whereas glycyrrhetinic acid inhibits only 11β-dehydrogenase (64). The reasons for this have not been elucidated in detail but may involve differences in the ease in which these compounds enter cells and inhibit the different isozymes of 11-HSD.

The important physiological effects of active compounds in licorice have prompted a search for other substances that have similar effects, with particular attention being paid to compounds that are known to increase urinary potassium losses. Furosemide is a weak competitive inhibitor (K_i of approximately 20 μm) of both liver and kidney 11-HSD (65, 66). Gossypol, a constituent of cotton seeds, has been tested as a potential male contraceptive, but its use has been complicated by hypokalemia. It, too, inhibits renal 11-HSD (67). Flavenoids in grapefruit juice, such as naringenin, inhibit this enzyme *in vitro* (66), and adults drinking a quart of grapefruit juice a day have changes in urinary cortisone-cortisol ratios, consistent with inhibition of 11-HSD (68).

C. Ectopic ACTH syndrome

Certain tumors, particularly small cell lung and medullary thyroid carcinomas, secrete ACTH at levels far in excess of normal and well above those seen in Cushing's disease of the pituitary. Consequently, patients with this "ectopic ACTH" syndrome have markedly elevated serum cortisol levels (69, 70). They also display signs of mineralocorticoid excess, such as hypertension and hypokalemic alkalosis, although levels of aldosterone are low. The severity of these signs exceeds that seen in pituitary Cushing's disease. These patients have high (approximately 3 times normal) ratios of cortisol to cortisone in plasma, suggesting that 11β-dehydrogenase is insufficiently active to handle the high circulating levels of cortisol (69). Other cortisol-metabolizing enzymes, particularly 5α-reductase, are apparently also affected as evidenced by abnormal urinary metabolite ratios (70); very high levels of free cortisol are excreted in the urine in this disorder.

The simplest explanation for these findings is that cortisol-metabolizing enzymes are saturated by high circulating levels of cortisol, thus permitting cortisol to occupy renal mineralocorticoid receptors. However, it is not clear whether decreased activity of 11-HSD is the most physiologically significant change seen in this disorder, considering that ratios of cortisol to cortisone are only moderately elevated and correlate poorly with the degree of hypokalemia. It is also unclear from the available data whether the observed effects on 11-HSD are most consistent with saturation or inhibition of the enzyme. ACTH infusions in normal humans raise plasma cortisol but not cortisone levels, whereas hydrocortisone infusions do raise cortisone levels. This suggests that ACTH inhibits 11-HSD, possibly due to secretion of other adrenal steroids, such as corticosterone, that competitively inhibit the enzyme (69, 71).

D. Essential hypertension

Because apparent 11-HSD deficiency causes severe hypertension, it was reasonable to hypothesize that milder decreases in enzymatic activity might be associated with common "essential" hypertension. Several lines of evidence have been developed in support of this hypothesis.

1. Birth weight, placental weight, and essential hypertension. In addition to childhood hypertension, patients with AME are often born with a mild to moderate degree of intrauterine growth retardation (Table 1). Although the reason for this is not known, it seems likely that deficiency of 11-HSD in the placenta (see below) permits excessive quantities of maternal glucocorticoids to cross the placenta and thus inhibit fetal growth (72). A hypothetical mild form of 11-HSD deficiency might also present with low birth weight and subsequent hypertension (73). In rats, placental 11-HSD activity is inversely correlated with placental weight and directly correlated with term fetal weight (74). In human population studies, most of which are retrospective, low birth weight and increased placental weight are indeed risk factors for subsequent development of adult hypertension (75–80). Although variations in 11-HSD might in principle be responsible for this correlation, a recent study in humans (81) did not find such a correlation between placental 11-HSD activity and placental weight. A weak but significant positive correlation was observed between 11-HSD activity and fetal birth weight, but a subsequent larger study of the identical population was unable to confirm this (F. M. Rogerson, K. M. Kayes, and P. C. White, unpublished observations). Thus, the currently available data do not support the idea that low 11-HSD activity is a risk factor for low birth weight in humans who do not suffer from AME.

2. Endogenous inhibitors of 11-HSD. Endogenous inhibitors of *in vitro* hepatic 11-HSD activity are excreted in human urine and in increased amounts during pregnancy. Because glycyrrhetinic acid, a component of licorice (see below), is a widely used inhibitor of 11-HSD, the endogenous inhibitors have been termed "glycyrrhetinic acid-like factors" or GALFs (82).

It was speculated that increased circulating levels of GALFs might inhibit 11-HSD activity and thus cause hypertension. Studies aimed at testing this hypothesis have obtained somewhat contradictory results. One study quantitated GALF activity using rat liver microsomes (83) and found that GALFs had no diurnal rhythm of excretion and were no different after dexamethasone treatment or in patients with hypopituitarism, pituitary Cushing's disease, and the ectopic ACTH syndrome. Thus, GALFs are presumably

not metabolites of adrenal steroids. GALF levels were not increased in hypertensive individuals and were not correlated with plasma cortisol half-life or with the precursor-product ratio, (THF+aTHF)/THE. A second study (84) quantitated GALF levels using human kidney microsomes; as discussed below, human kidney and rat liver express distinct 11-HSD isozymes. In contrast to the previous study, individuals with low-renin essential hypertension excreted significantly larger amounts of GALFs than normals. This difference might be a result of the different assay conditions as well as the examination in the second study of a specific subgroup of hypertensive individuals, those with low PRA. Nevertheless, there was again no correlation between GALF levels and (THF+aTHF)/THE ratios.

The physiological significance of GALFs remains unclear at present. GALF levels in normotensives are affected by varying the amount of sodium in the diet, and inhibitor levels are correlated with sodium excretion (84). This suggests that the increased GALF levels seen in low-renin hypertension may be a consequence rather than a cause of the volume expansion that is associated with this type of hypertension. If GALFs inhibited 11-HSD *in vivo*, this would allow renal mineralocorticoid receptors to be occupied by cortisol, causing sodium retention and decreased rather than increased sodium excretion.

The identities of GALFs have not yet been determined. The inhibitors can be extracted by reversed phase chromatography under conditions that suggest that they could be steroids. One candidate is 11β-hydroxyprogesterone, which is a potent inhibitor of 11-HSD (85) that could potentially be synthesized *in vivo* from progesterone. It might plausibly be excreted in larger amounts during pregnancy. However, 11β-hydroxyprogesterone is eluted separately from the peak of the inhibitory activity during HPLC (84).

3. Variations in 11-HSD activity in essential hypertension. Precursor-product ratios and plasma cortisol half-life have been compared in normal and hypertensive individuals with inconsistent results. One study found higher (THF+aTHF)/THE ratios in untreated hypertensive individuals (86), but other investigators found no such differences (83, 84, 87, 88) regardless of whether a low renin subgroup was selected. Moreover, hypertensive patients who developed hypokalemia while receiving diuretic therapy (AME patients usually have hypokalemia) had similar precursor-product ratios to those individuals who did not develop hypokalemia (89). In contrast, two small studies have demonstrated prolonged plasma cortisol half-life in hypertensive individuals as compared with matched controls (87, 90). It is difficult to draw conclusions from these studies. Prolonged cortisol half-life could be due to decreased activity of some other enzyme such as 5β-reductase (88). Most of the study groups have been relatively small, and the results have generally been at levels of statistical significance in the $0.01 < P < 0.05$ range. Additionally, the study groups have not been identical in ethnic group, age, and selection criteria. The data do suggest that precursor-product ratios are not a sensitive way of identifying mild individual differences in 11-HSD activity, should they exist. Unfortunately measurements of cortisol half-life generally require administration of radioactively labeled steroids and are thus not easily used in large studies.

E. Related conditions

1. 11-Reductase deficiency. Two pairs of sisters who presented with signs of androgen excess including hirsutism, acne (one patient), oligomenorrhea (two patients), and infertility (one patient) have been reported (91, 92, 92a). There were no signs of Cushing's syndrome, and blood pressure was normal. Plasma cortisol and androgens were elevated in the two patients tested. All four patients had abnormal urinary excretion of steroids with virtually absent 11β-hydroxy cortisol metabolites (*i.e.* tetrahydrocortisol and allo-tetrahydrocortisol) as compared with tetrahydrocortisone. A rapid metabolic clearance rate was detected in one patient after injection of radioactively labeled cortisol. Administration of high doses (100 mg) of hydrocortisone (*i.e.* cortisol) or cortisone acetate did not suppress adrenal androgen production in one patient, and these steroids were excreted in the urine almost entirely as tetrahydrocortisone. However, 2 mg of dexamethasone (equivalent to ~50 mg of cortisol in glucocorticoid potency) completely suppressed the adrenal (91). Parents of the affected individuals were asymptomatic, suggesting an autosomal recessive mode of inheritance.

Apparently this disorder represents the reverse of the 11β-dehydrogenase defect seen in AME. These patients apparently are able to convert cortisol to cortisone but cannot carry out the reverse reaction. As a result, cortisol is cleared from the circulation and excreted more rapidly than normal. This increases secretion of ACTH and adrenal steroidogenesis, which, in turn, causes excessive secretion of adrenal androgens. Circulating cortisol levels remain normal, explaining the lack of signs of Cushing's syndrome. Exogenous hydrocortisone is rapidly oxidized by 11-HSD and thus cannot suppress ACTH secretion by the pituitary, but dexamethasone is a poor substrate for 11-HSD and is thus effective.

The low number of reported cases of this disorder probably results from a problem of ascertainment. These patients are clinically indistinguishable from the very large number of women with polycystic ovary syndrome unless detailed urinary steroid profiles are obtained, and this test is not routinely available.

The presence of apparently distinct 11-reductase and 11β-dehydrogenase deficiencies suggested that these activities were mediated *in vivo* by distinct gene products, although allelic variation in a single gene could also account for these observations.

2. Type 2 AME. Five patients in three kindreds were identified who had signs and symptoms of AME including severe childhood hypertension, hypokalemia, polyuria and polydipsia secondary to nephrogenic diabetes insipidus, and failure to thrive (in three patients). PRA and aldosterone secretion were suppressed. These patients responded to blockade of the mineralocorticoid receptor with spironolactone or to suppression of cortisol secretion with dexamethasone (93–96).

One kindred had three affected members including two siblings (a brother and a sister) and a cousin. The parents of

the affected siblings were first cousins and were asymptomatic. They had five other children who were also asymptomatic. This pattern is most consistent with an autosomal recessive mode of inheritance.

Unlike other patients with AME, these affected individuals excreted normal ratios of cortisol to cortisone metabolites in the urine, and were, therefore, proposed to have a distinct "type 2" form of AME [we have assigned one of the first reported AME patients (93) to this group retrospectively based on her urinary steroid profile]. Although the clinical presentation raised the possibility that these patients had Liddle's syndrome, the mode of inheritance and the therapeutic response to spironolactone are not consistent with this diagnosis. Moreover, these patients did have an abnormal pattern of steroid secretion in the urine; total secretion of corticosteroid metabolites was subnormal, as was the ratio of tetrahydro metabolites (tetrahydrocortisol, allotetrahydrocortisol, and tetrahydrocortisone) to free cortisol.

These findings suggested that there might be a defect in reduction of the "A-ring" of cortisol and cortisone; *i.e.* the 3-keto group and the C4-5 double bond. A-ring reduction (in particular, 5β-reduction) is also abnormal in "type 1" AME. Thus, it was proposed that deficient A-ring reduction might be the primary defect in both forms of AME (97). *A priori*, this was unlikely, considering that the precursor-product ratio for 11β-dehydrogenation was an average of 10-fold greater than that for 5β-reduction (Fig. 3). Subsequent genetic analysis (see below) confirmed that type 1 AME is a primary genetic defect in 11β-dehydrogenation, but the basis of type 2 AME has yet to be elucidated.

V. Functional Roles of 11-HSD

This section summarizes studies of 11-HSD enzymatic activity in various tissues. Much of this work has been reviewed in greater detail elsewhere (98). Studies of the distributions of specific isozymes using immunological or molecular genetic techniques are summarized in *Sections VI and VII*.

A. Liver

As discussed elsewhere in this review, exogenously administered cortisone is largely reduced to cortisol by the reductive activity of 11-HSD in the liver (6, 7). Considering the steroid profiles in the rare patients with an apparent deficiency of this activity (91, 92), it seems that the enzyme expressed in the liver counterbalances dehydrogenase activity present elsewhere in the body to maintain adequate circulating levels of bioactive glucocorticoids. The reductase activity of liver 11-HSD may also have local effects; inhibition of this enzyme *in vivo* by carbenoxolone increases whole body insulin sensitivity, presumably by decreasing intrahepatic levels of glucocorticoids (99).

An $NADP^+$-dependent 11-HSD isozyme has been purified to homogeneity from rat liver (100).

B. Kidney and other mineralocorticoid target tissues

In addition to patients with AME or licorice intoxication, other patients with renal disease have decreased ratios of plasma cortisone to cortisol, supporting the idea that most cortisone is produced in the kidney (101). This activity is concentrated in the distal convoluted tubules and cortical collecting ducts; except for the rat, most mammals have little activity in the proximal tubules (102–104). This distribution of enzymatic activity parallels expression of the mineralocorticoid receptor and thus implies an intracellular mechanism of protection of this receptor. This activity is strongly NAD^+-dependent, functions only as a dehydrogenase, and has a very high affinity for glucocorticoids (105). Similar activity has been expressed in a number of cultured renal epithelial cell lines (106, 107) and has also been detected in both human (108) and rat (109) colon.

C. Brain

Although initial reports suggested that the hippocampus had very low levels of 11-HSD activity (24, 25), high levels of $NADP^+$-dependent 11-HSD activity were subsequently found in most areas of the rat brain including the hippocampus, cortex, cerebellum, and pituitary, with lower levels in the hypothalamus and brain stem (110, 111). Reductase activity is present in a similar distribution. At this time, NAD^+-dependent activity has not been directly assayed in the brain (but see *Section VII* for *in situ* hybridization studies). Experiments on cultured rat fetal hippocampal cells suggests that reductase activity predominates over dehydrogenase activity in this part of the brain (112). This activity is induced by glucocorticoids, suggesting that it may potentiate hippocampal stress responses *in vivo*.

A detailed review of actions of adrenal steroids in the brain (113) is beyond the scope of this article. Both glucocorticoid and mineralocorticoid receptors have been documented in many areas of the brain. The mineralocorticoid receptor may respond mainly to glucocorticoids in parts of the brain such as the hippocampus because adequate 11-HSD activity is lacking (114). However, aldosterone does have specific effects on the central nervous system (reviewed in Ref. 115). In rats, intraventricular infusions of doses of aldosterone that have no peripheral effects increase resting blood pressure, an effect that is blocked by low doses of mineralocorticoid antagonists. Corticosterone does not have this effect but seems to be essential to it, because the effect is not seen in adrenalectomized animals but returns if the animals are given systemic corticosterone. Aldosterone also increases salt appetite. Evidently, mineralocorticoid receptors must be specific for aldosterone in portions of the brain mediating these effects.

D. Circulatory system

Glucocorticoids have significant effects on the peripheral vasculature such as potentiating pressor responses to catecholamines and decreasing production of vasodilator prostaglandins and kallikreins (116). However, aldosterone has distinct interactions with vascular mineralocorticoid receptors. This suggests that a mechanism to maintain specificity of the mineralocorticoid receptor must be present in the vasculature (117, 118). Mineralocorticoid receptors and 11-HSD are coexpressed in cultured vascular smooth muscle

cells (119). In rats, 11-HSD activity that is predominantly $NADP^+$-dependent is found in resistance vessels such as the mesenteric artery and, to a lesser degree, in the aorta (120, 121). This activity is decreased in Dahl salt-sensitive hypertensive rats as compared with Dahl salt-resistant or Sprague-Dawley rats (122), suggesting that 11-HSD activity in the vascular wall might be involved in the pathogenesis of hypertension in this rat model. Similar effects have been proposed for humans (90).

Although initial studies did not demonstrate 11-HSD activity in the rat heart (24, 25), low levels of this activity were subsequently documented (120, 121). Studies in rats suggest that aldosterone has direct effects on cardiac myocyte collagen synthesis and myocardial fibrosis, effects that can be prevented by blockade of mineralocorticoid receptors (123, 124). Mineralocorticoid receptors have also been documented in the human heart. For aldosterone to have a specific effect in the context of much higher circulating levels of glucocorticoids, 11-HSD should be present in the heart. Human hearts indeed contain 11-HSD activity which seems to be NAD^+ rather than $NADP^+$-dependent (125); levels are approximately 1% those in the kidney.

E. Skin

In humans, both epidermis and sweat glands have 11-HSD activity. Activity in the epidermis is $NADP^+$-dependent (126). *In vivo*, it functions mainly as a dehydrogenase (126), but cultured human skin fibroblasts have predominantly reductase activity (127). This activity is up-regulated by glucocorticoids. In contrast, sweat glands express higher levels of an activity that is mainly NAD^+-dependent. Corticosterone is a more effective substrate for this activity than is cortisol.

Both mineralocorticoid and glucocorticoid actions in the skin may be modulated by 11-HSD. Both the epidermis and sweat glands express mineralocorticoid receptors (126), and it is reasonable to speculate that aldosterone could affect the electrolyte composition of sweat. Glucocorticoids enjoy wide therapeutic use for many skin disorders. They also have effects on skin vasculature, causing vasoconstriction when applied topically (128). Hypertensive individuals have a greater vasoconstrictor response to hydrocortisone than normotensive subjects do, suggesting that this response may be correlated with small blood vessel reactivity elsewhere in the body (90). The vasoconstrictive effect of hydrocortisone is potentiated by the simultaneous application of glycyrrhetinic acid (128), which presumably acts by inhibiting 11-HSD. The related compound, carbenoxolone, indeed does inhibit 11-HSD in sweat glands (126). Topical 11-HSD inhibitors might be a useful way to increase the local effects of glucocorticoids without increasing their systemic absorption (128).

F. Ovary

Rat ovaries have 11β-dehydrogenase but not 11-reductase activity (129). Activity can be demonstrated in human ovarian granulosa cells isolated from ovarian follicles obtained from women undergoing *in vitro* fertilization (130). The amount of activity varies between women and even between different follicles obtained from the same woman (131). This activity is a consistent predictor of the success of *in vitro* fertilization. The presence of detectable activity (seen in a narrow majority of patients) is invariably associated with failure to achieve a pregnancy, whereas fertilized ova from follicles with absent 11-HSD activity develop into pregnancies 64% of the time (130, 131). However, 11-HSD activity has no significant effect on the gross rate of *in vitro* fertilization.

It is not obvious why detectable 11-HSD activity predicts an unsuccessful outcome of *in vitro* fertilization. Because actual fertilization rates are similar whether or not 11-HSD is present in the follicle, the lack of successful pregnancies must reflect a defect in oocyte maturation such that the early embryo cannot implant or is otherwise not viable. Possibly cortisol has a positive direct effect on oocyte maturation. Because cortisol also inhibits ovarian steroidogenesis (132), high 11-HSD activity may also alter the relative levels of various steroids. This might adversely affect oocytes. Finally, 11-HSD activity may simply be a marker for some other regulatory factor that adversely affects oocytes.

G. Placenta

Most cortisol circulating in the human fetus is synthesized by the fetal adrenal gland, whereas the majority of circulating fetal cortisone is of maternal origin (133). This implies that the placenta expresses 11-HSD that oxidizes maternal cortisol to cortisone, preventing it from reaching the fetus (134).

Cultured human endometrial stromal cells express 11-HSD activity that can utilize either $NADP^+$ or NAD^+. Medroxyprogesterone and estradiol act synergistically to increase these activities in a time- and dose-dependent manner (135). These cultured cells are a model for decidualization, the process by which the uterine lining reacts to the invading trophoblast during early gestation to form the placenta. This finding suggests that expression of 11-HSD in the placenta occurs very early in gestation. This may reflect a critical need to protect the embryo from maternal glucocorticoids. Alternatively, it may indirectly modulate invasion by the trophoblast. This process involves degradation of the extracellular matrix by trophoblastic cells, and expression of both extracellular matrix components and proteases is influenced by glucocorticoids (136).

In human term placenta, dehydrogenase activity strongly predominates over reductase activity. This activity is apparently heterogenous. After differential centrifugation, NAD^+-dependent dehydrogenase activity is more prominent in crude nuclear and mitochondrial fractions, whereas $NADP^+$-dependent activity is concentrated in the microsomal fraction (137). Dehydrogenation of cortisol has a Michaelis Menten constant (K_m) in the micromolar range whereas the K_m for corticosterone is 10 times lower. In contrast, the K_m for reduction of either cortisone or 11-dehydrocorticosterone is in the micromolar range.

Studies of the nuclear plus mitochondrial fraction from homogenates of human or rat placenta confirm that the activity present in this fraction has a strong preference for NAD^+ as a cofactor and has a high affinity for glucocorti-

coids, with K_m values of 14 nM for corticosterone and 55 nM for cortisol (138).

Similarly, JEG-3 human choriocarcinoma cells express both NAD^+- and $NADP^+$- dependent 11-HSD activities. In either case, the activity has a high affinity for glucocorticoids. These activities are found in crude nuclear, mitochondrial, and microsomal fractions, with NAD^+-dependent activity being somewhat more prominent in the nuclear fraction (139). It is not certain whether these high-affinity NAD^+- and $NADP^+$-dependent activities represent distinct isozymes.

In the midterm baboon placenta, reductase activity predominates over dehydrogenase activity, but the reverse is true at term. This is due to increased expression of dehydrogenase activity in trophoblast, a change that is accentuated by estrogens (140), whereas the activity expressed in decidua is primarily in the reductive direction (141).

H. Other fetal tissues

Most midgestation fetal tissues have 11-HSD activity that, with the exception of chorionic membranes, acts predominantly as a dehydrogenase rather than a reductase. This activity decreases in most tissues by birth. Reductase activity in the liver increases during late gestation and early childhood (142). Reductase activity in the human fetal lung also increases during gestation (143), and similar changes have been noted in rabbits and rats (*e.g.* Ref. 144). Glucocorticoids promote maturation of the fetal lung and are used to treat respiratory distress syndrome in premature infants. Because of the high level of dehydrogenase activity in the placenta, reductase activity in the lung is presumably necessary to maintain levels of bioactive glucocorticoids that are sufficient for normal lung maturation.

VI. The Type 1 (Liver) Isozyme of 11-HSD

A. Terminology

Two distinct isozymes are discussed in this section and *Section VII*, respectively. The first was originally isolated from liver, catalyzes both dehydrogenation and reduction, utilizes $NADP^+$, and has a relatively low affinity for steroids. It has been referred to as the liver (L) or type 1 isozyme; we prefer the former term but use the latter in the present article by editorial request. The second isozyme was first documented in the kidney, catalyzes only dehydrogenation, utilizes NAD^+, and has a relatively high affinity for steroids. We have previously referred to this isozyme as the kidney (K) isozyme; it is here termed the type 2 isozyme.

B. Biochemistry

Rat liver expresses high levels of 11-HSD activity. In homogenates, both dehydrogenase and reductase activities could be demonstrated in the presence of $NADP^+$ and NADPH, respectively. These activities were concentrated in the microsomal fraction. They could be differentially solubilized using detergents and had different stabilities upon heating, suggesting that the two activities were catalyzed by different enzymes (145, 146). The dehydrogenase activity was purified to homogeneity by solubilization with Triton DF18 followed by NADP-agarose affinity chromatography (100). It was a glycoprotein with a molecular mass of 34 kDa that preferred $NADP^+$ as a cofactor. The K_m for corticosterone was 1.8 μM. Based on gel filtration experiments (100) and the behavior of related enzymes (147), it is most likely that this enzyme aggregates in solution as a tetramer.

The rabbit enzyme is glycosylated at asparagines N122, N161, and N206 (148); the latter two sites are conserved in other mammalian species (149, 150). The attached glycans have a high mannose content, implying that the enzyme is normally oriented to the luminal side of the endoplasmic reticulum (148).

C. Molecular biology

1. Isolation of cDNA and genes. A cDNA clone encoding this enzyme was isolated using an antiserum to the purified rat protein (149). The full-length cDNA was 1.4 kb long including an open reading frame of 876 bp, predicting a protein of 292 amino acids. The corresponding human cDNA was then isolated (150). The amino acid sequences predicted from the rat and human cDNA clones are 77% identical, but the human enzyme is slightly larger; the amino terminus of the rat enzyme corresponds to M4 of the predicted human enzyme. Mouse (151), sheep (152), and squirrel monkey (153) cDNA clones have subsequently been isolated.

The human gene for this isozyme, *HSD11B1* (*HSD11L*), is located on chromosome 1 and contains six exons with a total length of more than 9 kb (150) (Fig. 4).

2. Structure-function relationships. A search of sequence databases revealed that this isozyme of 11-HSD is a member of a large class of structurally related "short-chain dehydrogenases" (149). Such enzymes all have molecular masses of about 30 kDa. Highly conserved amino acids in these enzymes are clustered near the amino terminus in a region proposed to constitute part of the binding site for the cofactor, which in the case of 11-HSD1 is $NADP^+$ or NADPH. Absolutely conserved tyrosine (Y) and lysine (K) residues are located toward the carboxyl terminus (154). In rat 11-HSD1, these are Y179 and K183. Even conservative substitutions of either of these residues destroy enzymatic activity (155). Similar results have been obtained from mutagenesis studies of related enzymes (147, 156, 157).

x-Ray crystallographic studies of a related enzyme, 3α,20β-hydroxysteroid dehydrogenase from *S. hydrogenans*,

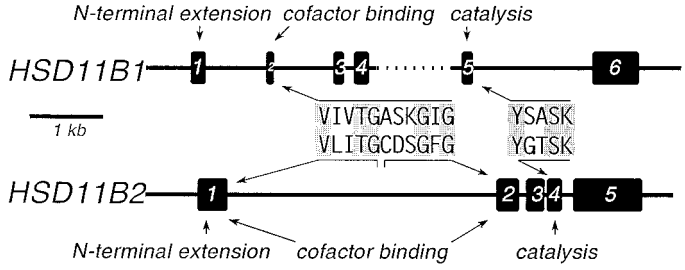

FIG. 4. Genes encoding 11-HSD isozymes. *Numbered black boxes* represent exons, and those exons that encode domains of functional importance are indicated. Highly conserved predicted amino acid sequences are shown in *single letter code*; identical residues within these regions are *shaded*.

demonstrated that the residues corresponding to Y179 and K183 are located near the pyridine ring of the cofactor in a cleft presumed to be the substrate-binding site. They could therefore be involved in catalytic activity (147). These two residues may facilitate a hydride ion (a proton plus two electrons) transfer from the 11α position of corticosterone to $NADP^+$. It is hypothesized that the ϵ-amino group of K183 facilitates deprotonation of the phenolic group of Y179 (deprotonation of a phenolic group in aqueous solution normally has a pK_a of $\sim$ 10). The deprotonated phenolic group then removes a proton from the 11β-hydroxyl group of the steroid, leaving a negative charge on the 11 position of the steroid nucleus. This allows transfer of the 11α-hydrogen (as a hydride) to the pyridine group of the cofactor (Fig. 5).

The identity of the substrate-binding site was confirmed by further x-ray crystallographic studies of 3α,20β-hydroxysteroid dehydrogenase that had been cocrystallized with the 11-HSD inhibitor, carbenoxolone (158).

There is a highly hydrophobic N-terminal domain that is presumed to anchor the enzyme in the membrane of the endoplasmic reticulum. A mutant enzyme lacking this domain was inactive, unstable, and apparently not glycosylated (159).

3. Activity. Although the enzyme purified from rat liver functioned only as a dehydrogenase, the recombinant enzyme expressed from cloned cDNA exhibited both 11β-dehydrogenase and the reverse oxoreductase activity (conversion of 11-dehydrocorticosterone to corticosterone) when expressed in mammalian cells (149). At physiological pH in cell lysates, the kinetic constants for dehydrogenation and reduction (K_m of 1.1 and 1.4 μM, respectively) were almost identical (160). These findings implied that this isozyme actually catalyzes a fully reversible reaction and that reductase activity was destroyed during purification from the liver. This may have been caused by the $NADP^+$ added during purification to stabilize the enzyme. Similarly, the ability of purified enzyme to bind a 2′,5′-ADP affinity column was destroyed by a previous affinity chromatography step using $NADP^+$ to elute the enzyme (148). Moreover, the reductase activity of the recombinant enzyme was irreversibly abolished by incubating cell lysates with $NADP^+$ (160). These findings all suggest that loss of reductase activity may be due to a permanent conformational change in the enzyme induced by $NADP^+$.

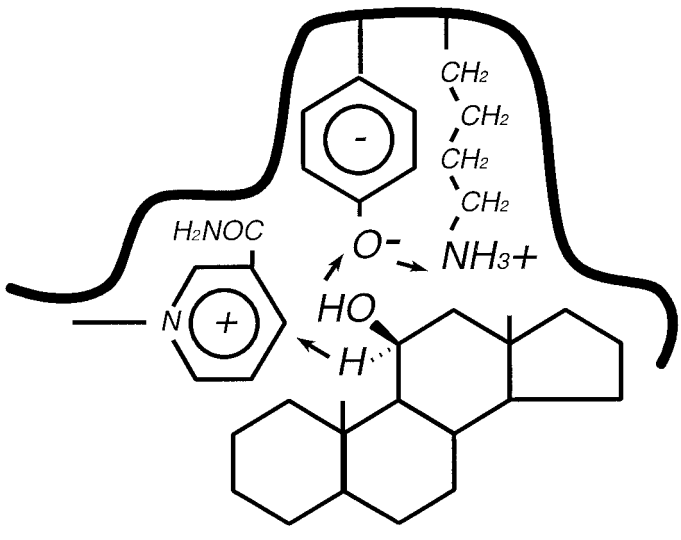

FIG. 5. Proposed catalytic mechanism for both isozymes of 11-HSD. The ϵ-amino group of lysine deprotonates the phenolic hydroxyl group of tyrosine, which in turn removes a proton from the 11β-hydroxyl group of the steroid, facilitating a hydride transfer from the 11α-position of the steroid to the pyridine ring of the cofactor (NAD^+ or $NADP^+$).

The relative amounts of reductase and dehydrogenase activities expressed from transfected cDNA varied between mammalian cell lines (149, 160, 161); moreover, when the same cDNA was transfected into cultured toad bladder cells, the expressed enzyme functioned only as a reductase (162). The reasons for these variations have not been elucidated. Differences in intracellular pH or in the relative levels of NADP+ and NADPH might be responsible. Altered post-translational modifications might also play a role. This isozyme of 11-HSD is a glycoprotein, and modifying glycosylation patterns of the recombinant enzyme by incubation of transfected cells with tunicamycin (160) or by mutagenizing glycosylation sites (163) differentially affects dehydrogenase and reductase activities.

D. Expression

1. Tissue distribution. In the rat, 11-HSD1 cDNA hybridized to RNA from a wide range of tissues including liver, kidney, testis, lung, heart, and colon, with strength of hybridization in approximately that order (149, 164). Immunoreactivity was similarly distributed (165). In the rat brain, hybridization was strongest in the hippocampus and cortex but was also found in the pituitary, hypothalamus, brain stem, and cerebellar cortex (110). The predominant RNA species was 1.7 kb long, but additional bands of 1.9 and 1.5–1.6 kb were noted in the kidney (164). The distribution of expression in mouse RNA samples was similar (151). In humans, a 1.5-kb RNA band was observed in samples from liver, testis, lung, foreskin fibroblasts, ovary, colon, and kidney (150). Of the tissues tested, by far the highest level of expression was in the liver, whereas (in contrast to the rat) expression was much lower in the kidney.

In general, the distribution of expression of this isozyme more closely paralleled that of the glucocorticoid receptor than that of the mineralocorticoid receptor, suggesting that this isozyme might, at least in some tissues, modulate levels of glucocorticoids reaching the glucocorticoid receptor (166).

One of the multiple mRNA species in rat kidney arises from initiation of transcription within the first intron (159, 167, 168). In the human gene, the first 30 amino acids (in the rat, the first 26 amino acids) of this enzyme are encoded by the first exon, and the first codon in the second exon is ATG, encoding methionine. Based on alignment of this enzyme with other related enzymes, it was proposed (150, 160) that initiation of translation at this codon might yield a functional enzyme. Although the ATG encoding M27 is indeed a functional initiation codon (159), cells transfected with the truncated cDNA apparently expressed low levels of the corresponding polypeptide and had no 11-HSD activity (159, 169).

Thus, the functional significance of transcripts originating in the first intron is unclear.

2. Immunohistochemistry and in situ hybridization. Whereas renal mineralocorticoid receptors are concentrated in distal convoluted tubules and cortical collecting ducts, immunohistochemical studies using polyclonal (24, 170) or monoclonal (171) antibodies localized this isozyme to proximal tubules and interstitial cells in rat kidneys. These findings were difficult to reconcile with the hypothesis that 11-HSD protected mineralocorticoid receptors from high concentrations of glucocorticoids in an autocrine manner, and it was thus hypothesized that the enzyme might be acting in a paracrine manner to prevent cortisol from reaching the distal nephron. Nevertheless, mRNA encoding this isozyme was found in all rat renal tubular epithelia by *in situ* hybridization although it was concentrated in juxtamedullary areas (172, 173). The reason for the discrepancy between the immunohistochemistry and *in situ* hybridization studies has not been determined. As discussed below, a second isozyme is expressed in distal convoluted tubules and cortical collecting ducts, but it is sufficiently different in sequence that it would not have hybridized to the probes used in the *in situ* hybridizations.

In fetal rat testis, the enzyme was not detected by immunofluorescence until day 26 of gestation, but the entire interstitial region was stained in adult testis. This pattern coincides with Leydig cell development (174). In the rat ovary, the enzyme was localized to oocytes and luteal bodies by both immunohistochemistry and *in situ* hybridization experiments (129). These findings were consistent with the idea that this isozyme modulates levels of glucocorticoids in the gonads.

In vascular smooth muscle and the heart, both immunoreactivity and mRNA were localized to smooth muscle cytoplasm but were not found in endothelium (120). These findings were consistent with the distribution of enzymatic activity in these tissues.

3. Regulation. Expression of 11-HSD1 mRNA in the rat liver is much higher in males than in females. Sexual dimorphism of hepatic enzyme expression is not unusual in the rat. In this case, as for other enzymes, it results from sex-specific patterns of GH secretion in the rat, with males having a pulsatile pattern of secretion, and females a more continuous pattern (175, 176). In addition, estrogens directly repress 11-HSD1 mRNA in rat liver (177). These interventions have similar effects on gene expression in rat kidneys, but in contrast, estrogens increase renal 11-HSD activity. This is presumably due to increased expression of the 11-HSD2 isozyme in the kidney. Expression in the hippocampus is unaltered by peripherally administered GH or estrogen. The sexual dimorphism in gene expression is of uncertain functional significance, considering that it is not observed in mice (151).

Glucocorticoids significantly increase gene expression in fetal sheep liver, but slightly decrease it in adult sheep (178). They also increase expression in cultured human lung cells (179).

E. Lack of involvement in the syndromes of AME or 11-reductase deficiency

The availability of sequence data on the human *HSD11B1* gene made it feasible to analyze the corresponding genes from patients with AME to determine whether mutations in this gene were responsible for the disease. No mutations were identified in affected alleles from four unrelated patients with AME or both parents of a deceased patient with AME (92).

Other lines of evidence also suggested that the 11-HSD1 isozyme did not play a significant role in conferring ligand specificity on the mineralocorticoid receptor. This isozyme was expressed at highest levels in the liver, which does not respond to mineralocorticoids, and although it was expressed at high levels in the rat kidney (149), it was expressed at much lower levels in human (150) and sheep (152) kidneys. Even in rat kidney, immunoreactivity to the protein was observed primarily in proximal tubules and not in distal convoluted tubules and collecting ducts, the sites of mineralocorticoid action (170). Accordingly, a second isozyme was sought in mineralocorticoid target tissues. This is discussed in the following section.

Because 11-HSD1 is expressed at high levels in the liver, an organ in which 11-reductase activity predominates, and because the corresponding cDNA confers mostly reductase activity when transfected into certain lines, it seemed plausible that mutations in *HSD11B1* might cause 11-reductase deficiency (*Section IV.E.1*). Only one patient suspected to have this condition has been studied thus far, and no mutation in this gene was identified (92). This might mean that there is an additional isozyme that catalyzes 11-reduction or that the diagnostic criteria for 11-reductase sufficiency are not sufficiently precise, but it seems most likely that the causative mutation was in a region of the gene that was not characterized. Study of additional kindreds with this putative syndrome should distinguish among these possibilities.

VII. The Type 2 (Kidney) Isozyme of 11-HSD

A. Biochemistry

Evidence for an additional isozyme that was active in mineralocorticoid target tissues was quickly obtained. In a histochemical procedure, a nonphysiological substrate, 11β-hydroxyandrostenedione, was oxidized by rat kidney tissue slices in the presence of NAD^+ and used to reduce nitroblue tetrazolium to diformazan. This stained distal convoluted tubules and cortical collecting ducts. $NADP^+$ and substrates that did not contain an 11β-hydroxyl group did not react in this assay (180).

More direct evidence for a distinct isozyme was obtained from biochemical studies of isolated rabbit kidney cortical collecting duct cells (103, 105). Activity of 11-HSD in the microsomal fraction was almost exclusively NAD^+- dependent and had a K_m for corticosterone of 26 nM. There was almost no reduction of 11-dehydrocorticosterone to corticosterone, suggesting that, unlike the 11-HSD1 (liver) isozyme, the 11-HSD2 (kidney) isozyme only catalyzed dehydrogenation. The enzyme in the human placenta had similar char-

acteristics (138); it was NAD^+-dependent and had K_m values for corticosterone and cortisol of 14 and 55 nM. Partial purification using AMP affinity chromatography suggested that this isozyme had a molecular mass of 40 kDa. Similar activities were noted in sheep kidney (181) and many human fetal tissues (182). Thus far, 11-HSD2 has not been purified to homogeneity in active form from any source. However, a homogenous preparation was recently obtained by a combination of affinity chromatography, affinity labeling, and preparative two-dimensional electrophoresis (183).

B. Molecular biology

1. Cloning of cDNA and predicted structure of the enzyme. Cloning of cDNA encoding 11-HSD2 was rendered more difficult by the unavailability of purified enzyme that could be used to produce an antiserum or to obtain amino acid sequence data. However, because sheep and human kidneys predominantly expressed this type of enzyme, it was feasible to clone the corresponding cDNA by expression-screening strategies in which pools of clones were assayed for their ability to confer NAD^+-dependent 11-HSD activity on *Xenopus* oocytes or cultured mammalian cells. Positive pools were divided into smaller pools and rescreened until a single positive clone was identified. Both sheep (184) and human (185) cDNA encoding this isoform were isolated in this manner. Subsequently rabbit (186), rat (187), and mouse (188) cDNAs were isolated.

The protein is predicted to contain 404 (sheep) or 405 (human) amino acid residues with a total molecular mass of 41 kDa [the published sheep sequence (184) contains a frameshift error near the 3′-end of the coding sequence]. The human and sheep predicted peptide sequences are 83% identical. A search of sequence databases revealed sequence similarity to members of the short chain alcohol dehydrogenase superfamily. The 11-HSD2 isozyme was most similar (37% sequence identity) to the type II (placental, NAD^+-dependent, microsomal) isozyme of 17β-hydroxysteroid dehydrogenase (189). It was only 20–26% identical to 11-HSD1. The relatively high degree of similarity between the 11-HSD2 isozyme and placental 17β-HSD (comparable to the similarity between cytochrome P450 gene family members) suggests that these two enzymes may be in the same gene family within the short-chain dehydrogenase superfamily.

Regions of sequence similarity between the two isozymes (Fig. 4) include part of the putative binding site for the nucleotide cofactor (residues 85–95 in 11-HSD 2) and the absolutely conserved tyrosine and lysine residues (Y232 and K236 in this enzyme) that function in catalysis. The region immediately to the N-terminal side of the catalytic residues forms part of a putative steroid-binding pocket in the short-chain dehydrogenase, 3α,20β-HSD, that has been analyzed by x-ray crystallography. This region is notably well conserved (10/18 identical residues) between the two isozymes of 11-HSD, consistent with a role in binding the substrate.

Hydropathicity plots suggest that 11-HSD2 has three successive hydrophobic segments of approximately 20 amino acids each in the N-terminal region before the cofactor-binding domain (184). These could function as transmembrane segments anchoring the enzyme to the membrane of the endoplasmic reticulum, although it is also possible that part of this region functions as a signal peptide that is cleaved when the newly synthesized enzyme is inserted into the endoplasmic reticulum.

2. Enzymatic analysis. Recombinant 11-HSD2 has properties that are virtually identical to the activity found in mineralocorticoid target tissues. The recombinant enzyme functions exclusively as a dehydrogenase; no reductase activity is detectable with either NADH or NADPH as a cofactor (184, 185, 187). The dehydrogenase activity of the humans, rabbits, and rats has an almost exclusive preference for NAD^+ as a cofactor. The sheep isozyme is able to utilize $NADP^+$ to oxidize corticosterone, but not cortisol, approximately 25% as well as it utilizes NAD^+. The enzyme has very high affinity for glucocorticoids, but corticosterone is the preferred substrate, with first order rate constants 10 times higher than those for cortisol, even in mammalian species in which cortisol is the predominant glucocorticoid. Reported K_m values for corticosterone are 0.7–10.1 nM and for cortisol, 14–47 nM.

3. Gene structure. The corresponding gene, termed *HSD11K* or *HSD11B2*, is located on chromosome 16q22 and contains five exons spaced over approximately 6.2 kb (190) (Fig. 4). The putative binding site for the NAD^+ cofactor (including the core sequence, GxxxGxG) is split between exons 1 and 2, whereas the putative catalytic residues, Y232 and K236, are encoded by exon 4.

The predicted amino acid sequence of 11-HSD2 is only 21% identical to the predicted sequence of human 11-HSD1 (150). When these sequences are aligned, the introns do not correspond in number or location. These data indicate that these two isozymes belong to different gene families.

Ribonuclease protection analysis showed that transcripts in the adult human kidney begin at −116 nucleotides (nt). This site is used to a minor extent in the placenta, in which transcription begins predominantly at −74 nt. There are no TATA elements upstream of either cap site (190).

4. Gene regulation. Using luciferase reporter constructs, the region from −2 to −330 nt relative to the initial ATG codon has been identified as an essential region for basal transcription of *HSD11B2* in JEG-3 human choriocarcinoma cells. Two segments in this region, −278 to −257 and −215 to −194, are protected in DNase I footprinting analysis. Both segments have consensus binding sites for the Sp1 transcription factor. Gel shift assays of these segments show several DNA-protein complexes using JEG-3 nuclear extract. Only the slowest migrating complex is competed by an antiserum to Sp1. These results suggest that the two Sp1 sites, either alone or in combination, are essential for transcription of *HSD11B2* gene in JEG-3 cells (191).

C. Expression

The tissue distribution of expression of 11-HSD2 has been examined by RNA blot hybridization in human adults (185) and fetuses (192) and in sheep (184), rats (187), and mice (188). In all species, this isozyme is expressed in placenta and mineralocorticoid target tissues, particularly the kidney, whereas it is not detected in the liver, heart, or adult testis.

It is also expressed at high levels in sheep and rat adrenal glands, but is not detected in the mouse or in human fetal adrenals. Whereas human fetal and adult tissues contain transcripts of 1.9–2.0 kb, fetal tissues also express transcripts of 5 and 7 kb. These may represent utilization of alternative polyadenylation sites or partially processed transcripts.

Expression within mineralocorticoid target tissues has been further localized by immunohistochemistry and *in situ* hybridization. In the kidney, 11-HSD2 is expressed in distal convoluted tubules and cortical collecting ducts and is thus colocalized with the mineralocorticoid receptor (186, 188, 193–196). In salivary glands, it is expressed in tubular elements with no or minimal expression in acini (197). In the colon, it is found in the mucosa but not in the submucosa or the muscularis (196, 197). Among tissues that are not classic targets of mineralocorticoids, 11-HSD2 is strongly expressed in the syncytiotrophoblast of the placenta (195, 197). In the rat (197) and sheep (198) adrenal cortex, it is expressed in the zona fasciculata and, to a lesser extent, in the zona reticularis, but not in the zona glomerulosa. In the rat ovary, it is expressed in the corpus luteum but not in follicles, and it is expressed in stromal cells in the rat oviduct and the lamina propria of the uterus.

Although nonexpression of 11-HSD2 was initially reported in rat brain (193), subsequent studies with a rat instead of a human probe found cells expressing mRNA for this isozyme in the commissural portion of the nucleus tractus solitarius, subcommissural organ, and ventrolateral and ventromedial hypothalamus (199). The role(s) of this isozyme in these locations are uncertain. Lesions of the commissural portion of the nucleus tractus solitarius rapidly cause hypertension, suggesting that this structure regulates the cardiovascular system. The function of the subcommissural organ is not known with certainty. It also contains angiotensin receptors (but not mineralocorticoid receptors), and it may help regulate salt and water balance.

In fetal mice, 11-HSD2 is widely expressed early in development starting at embryonic day 9.5 (E9.5), but by E13 expression is limited to placenta, kidney, hindgut, testis, bile duct, adrenal, and lung. In the fetal mouse brain, 11-HSD2 is expressed in the thalamus, cerebellum, roof of the midbrain, and regions of pontine neuroepithelia (200).

D. Mutations in HSD11B2 are detected in all patients with AME

1. Prevalence of mutations. With the structure of the *HSD11B2* gene elucidated, it was possible to look for mutations in DNA samples from patients with AME. This effort was successful, thus confirming in its entirety the hypothesis that 11-HSD protects the mineralocorticoid receptor from high concentrations of cortisol (45–47, 201). Mutations have been detected on both chromosomes in all patients examined thus far [although mutations were initially not detected in one kindred (46), a homozygous mutation was documented in subsequent studies (47, 202)]. At this time, 11 different mutations have been detected in 20 patients from 15 kindreds (Table 2 and Fig. 6).

Only one patient has been a compound heterozygote for two different mutations, whereas all other patients have carried homozygous mutations. This suggests that the prevalence of AME mutations in the general population is low, so that the disease is found mostly in limited populations in which inbreeding is relatively high. For example, three Zoroastrian kindreds from India and Iran are all homozygous for the same mutation (R337Δ3nt).

It is striking that six kindreds are of Native American origin. Three from Minnesota or Canada carry the same mutation (L250S, L251P), consistent with a founder effect, but the others are each homozygous for a different mutation. The reason for the relatively high prevalence of this very rare disease among Native Americans is not immediately apparent. It might represent a bias in access to physicians with an interest in, and ability to make, this diagnosis as compared with similarly inbred populations elsewhere in the world. Alternatively, heterozygosity for mutations in *HSD11B2* might confer a selective advantage. Few obligate heterozygotes have been studied in detail, and it is possible that such

TABLE 2. Mutations causing apparent mineralocorticoid excess

Mutation	% Activity[a] (cortisol/corticosterone)	Patient[b]	Reference
Y232Δ 9nt[c]	0[d]	22	(46)
G305Δ 11nt[c]	0[d]		
E356Δ 1nt	0[d]	2	(47)
R374X	0[d]	16a,b	(201)
L250P,L251S	0/0	4,19,20	(46)
R337Δ 3nt	0/0.4	13	(46)
		5, 23	(47)
R208C	1.5/1.2	18	(46)
		24a,b	(47)
R213C	3.6/2.2	11a,b	(46)
R186C	17/9	12a,b	(47)
R337C	53/82	17a,c	(45)
Intron 3	[e]	21	(46)

[a] Percent activity figures are in whole cells with cortisol and corticosterone as substrates, respectively (202).
[b] See Table 1.
[c] The patient is a compound heterozygote for these mutations.
[d] These mutants are presumed to be inactive.
[e] This mutation causes skipping of exon 4 during pre-mRNA processing. The percentage of normally spliced transcripts is small but has not been precisely quantitated.

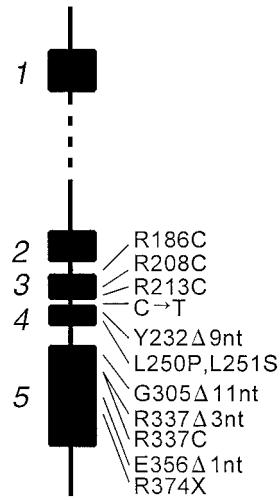

FIG. 6. Locations of mutations causing AME. Intron 1 is not drawn to scale.

individuals have an increased ability to conserve sodium under conditions of extreme sodium deprivation. Such conditions are not unlikely in inland Native American populations. A similar explanation has been proposed for the prevalence of hypertension among African Americans (203), although different genetic loci are presumably involved in that ethnic group.

2. Functional effects of mutations. Of the mutations identified thus far, two shift the reading frame of translation, a third deletes three amino acids including a crucial catalytic residue (Y232), and one is a nonsense mutation. These mutations are all presumed to completely destroy enzymatic activity. One mutation in the third intron leads to skipping of the fourth exon during processing of pre-mRNA (46). As the fourth exon encodes the catalytic site, the resulting enzyme is again presumably inactive. The other six mutations have been introduced into cDNA and expressed in cultured cells to determine their effects. One mutant (L250P, L251S) is completely inactive, and one (R337Δ3nt) has only a trace of activity. The others are all partially active in cultured cells with one, R337C, having greater than 50% of normal activity (202). Only R337C is partially active in lysed cells [although one group reported this mutation to be inactive in cell lysates (204), they did not utilize appropriate conditions to maximize enzyme stability (205)]. We believe that comparisons of activity are best made using the apparent first-order rate constant, V_{max}/K_m, which predicts reaction velocity at low substrate concentrations. Valid comparisons in whole cells require controls (Western blots or determinations of mRNA levels) for transfection efficiency. However, determinations of apparent K_m in whole cells must be interpreted cautiously, particularly when high concentrations of substrate are used, because many substrates including steroids are subject to active transport into or out of cells (206). Such mechanisms, which have their own kinetics, can confound kinetic measurements of enzymes.

Two of the mutations, R208C and R213C, lie within a region homologous to a probable steroid-binding domain in 3α,20β-hydroxysteroid dehydrogenase (147), and it was therefore speculated that these mutations might affect affinity of the enzyme for steroids (46). Consistent with this hypothesis, enzymes with these mutations indeed have high apparent K_m values for corticosterone when expressed in whole cells. However, apparent V_{max} is also affected and, moreover, these mutations do not have a strong effect on the apparent K_m for cortisol (202). Thus, these mutations do not affect steroid substrate binding in a straightforward way.

Two successive missense mutations, L250P, L251S, occur close to the catalytic domain. These mutations are predicted to alter the secondary structure from an α-helix to a turn in this region; they also make this region less hydrophobic.

3. Genotype-phenotype correlations. Most inherited disorders exhibit allelic variation. The best studied disorder of steroid metabolism is congenital adrenal hyperplasia due to 21-hydroxylase deficiency, in which allelism accounts for approximately 80% of observed individual variations in both biochemical and clinical parameters of severity (207).

Although the number of patients with AME is small, sufficient data now exist to demonstrate a statistically significant correlation between degree of enzymatic impairment and biochemical severity as measured by the precursor-product ratio, (THF+aTHF)/THE (202) (Fig. 7). This correlation is most obvious for the partially active mutants. We assume in this analysis that R337C is the only significant mutation in the patients who carry it, even though only one exon of the gene was sequenced (45). If so, a 50% impairment of enzymatic activity is apparently sufficient to compromise metabolism of cortisol in the kidney, suggesting that there is very little excess capacity to metabolize cortisol in this organ. This seems to raise a paradox, because AME is a recessive disorder and heterozygous carriers, who would be expected to have 50% of normal activity, are asymptomatic. The same apparent paradox exists for 21-hydroxylase deficiency, which is also a recessive disorder; a clinically mild or nonclassic form of 21-hydroxylase deficiency is seen in individuals who are homozygous for mutations that are approximately 50% active when expressed in cultured cells (205). Altered stability or kinetic properties of the R337C mutant may be important, including alterations in enzyme inhibition by end product (*i.e.* cortisone or corticosterone) or by other circulating steroids.

The widest range of (THF+aTHF)/THE ratios is seen in patients who carry mutations that apparently completely destroy enzymatic activity. This is not very surprising; small variations in the very low levels of cortisone metabolites (*i.e.* THE) excreted by these patients will obviously lead to quite large differences in ratios in which this value is the denominator. It is possible that the normally low renal expression of 11-HSD1 (150) is sufficient to metabolize small amounts of cortisol when 11-HSD2 is absent. Thus, the relatively wide variations in precursor-product ratios seen in patients carrying inactive 11-HSD2 isozymes may reflect differences in expression or activity of 11-HSD1 in the kidney. Alternatively, a small amount of dehydrogenation may be catalyzed by the 11-HSD1 in the liver, even though the reaction in this organ is predominantly in the reductive direction.

Because of the small numbers of patients, and the possible

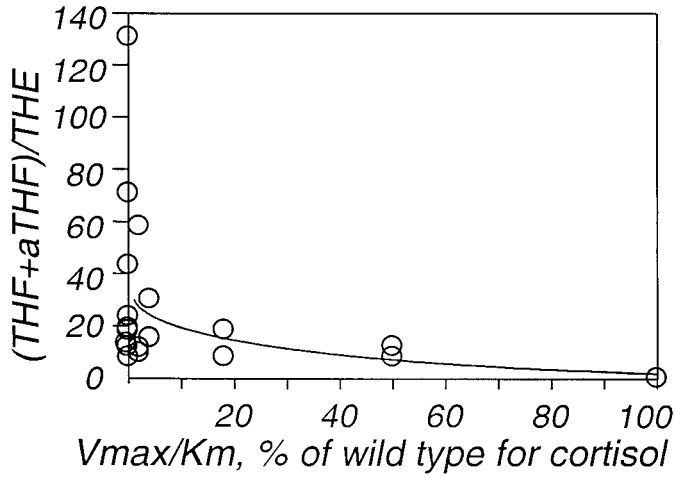

FIG. 7. Genotype-phenotype correlations in patients with AME. The precursor-product ([THF+aTHF]/THE) ratio for each patient is plotted against the percent of normal 11-HSD activity predicted from the mutation(s) carried by that patient.

confounding effects of prior antihypertensive therapy, it is difficult to correlate biochemical severity with measures of clinical severity, although anecdotal reports suggest that mutations that do not destroy activity may be associated with milder disease (45, 46). With the elucidation of the molecular genetic basis of this disorder, ascertainment of additional cases may permit these questions to be answered.

As previously discussed, there is equivocal evidence for alterations in 11-HSD activity associated with essential hypertension. Molecular studies (*e.g.* Ref. 208) of *HSD11B2* should unambiguously determine whether this gene is frequently involved in the development of hypertension. These might include linkage studies (looking for increased identity by descent in hypertensive sib pairs) and a search for frequent polymorphisms in *HSD11B2* that might be associated with the development of hypertension. Additional insights into the physiology of this important enzyme might be obtained by "knocking out" the corresponding gene in mice (209).

VIII. Summary

Whereas aldosterone is normally a much stronger mineralocorticoid than cortisol *in vivo*, mineralocorticoid receptors have identical *in vitro* affinities for these hormones. The *in vivo* specificity of the receptors is, at least in part, the result of activity of 11-HSD, an enzyme located in most mineralocorticoid target tissues that converts cortisol to cortisone. Cortisone is not a ligand for the receptor, whereas aldosterone is not a substrate of the enzyme. The syndrome of AME is a rare form of juvenile hypertension in which 11-HSD is defective. This deficiency allows mineralocorticoid receptors to be occupied by cortisol, leading to hypertension, because plasma concentrations of cortisol are much higher than those of aldosterone. Licorice, which contains 11-HSD inhibitors, causes a similar syndrome. There are two known isozymes of 11-HSD. The liver or type 1 isozyme is expressed at high levels in the liver, has a relatively low affinity for steroids (micromolar K_m), catalyzes both dehydrogenation and the reverse reductase reaction, and utilizes $NADP^+$ or NADPH as cofactors. The kidney or type 2 isozyme is expressed at high levels in the kidney and placenta, has a high affinity (nanomolar K_m) for steroids, catalyzes only dehydrogenation, and utilizes NAD^+ as a cofactor. Mutations in the *HSD11B2* (*HSD11K)* gene encoding the kidney isozyme of 11-HSD have been detected in all kindreds with AME studied thus far. This gene represents a candidate locus for the common, "essential" form of hypertension.

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