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MOLECULAR BIOLOGY OF MINERALOCORTICOID METABOLISM

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ABSTRACT

Mineralocorticoids are adrenal steroid hormones that regulate the retention of sodium by the kidney and, hence, are crucial in the regulation of sodium balance, intravascular volume, and blood pressure. The molecular biology of mineralocorticoid biosynthesis and action has only recently been elucidated. The genes encoding the various enzymes that convert cholesterol to mineralocorticoids have now been cloned. This has revealed the molecular basis of several inherited forms of mineralocorticoid excess, which cause hypertension, and several forms of mineralocorticoid deficiency, which cause salt loss. The cloning of the mineralocorticoid receptor revealed a paradox. Both the mineralocorticoid and the glucocorticoid receptor are activated equally by cortisol, even though cortisol has very modest mineralocorticoid activity. This is explained by the cloning of two genes for the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). Type-II 11βHSD, found primarily in the kidney, irreversibly converts cortisol to cortisone, which does not activate the mineralocorticoid receptor. Type-II 11βHSD thus defends the mineralocorticoid receptor from being activated by the very high concentrations of cortisol in the blood. Recent studies in genetically hypertensive rats suggest that other enzymes or factors that regulate salt balance may remain undiscovered. Thus the study of mineralocorticoid biosynthesis and action remains one of the most promising approaches to understanding hypertension.

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INTRODUCTION

Mineralocorticoids are adrenocortical steroids that exert crucial roles in salt and water metabolism and in the regulation of blood pressure. The terms mineralocorticoid and glucocorticoid were coined by Hans Selye in 1946 to distinguish the salt-retaining actions from the carbohydrate-mobilizing actions of the secretions of the adrenal cortex in response to stress (reviewed in 66). In considering salt and water metabolism, one should remember that mineralocorticoids act by promoting the retention of salt (i.e. Na^+) by the kidney, while the retention of water is stimulated by vasopressin (antidiuretic hormone) produced by the posterior pituitary.

MINERALOCORTICOID BIOLOGY

Biosynthesis

Mineralocorticoids and other adrenal steroids are synthesized from cholesterol according to the pathway shown in Figure 1. The first step in the biosynthesis of all steroid hormones is the conversion of cholesterol, a 27-carbon steroid, to pregnenolone, a 21-carbon (C21) steroid. The enzyme responsible for this reaction was once referred to as the 20,22 desmolase complex because it was thought that multiple enzymes were required for the sequential 20-hydroxylation and 22-hydroxylation, and for the subsequent cleavage of the single bond between carbons 20 and 22. However, there is only a single mitochondrial cytochrome P450 enzyme, termed P450scc (where scc denotes side-chain cleavage), that is responsible for all three of these catalytic activities on a single active site (for review see 63, 66). The same P450scc enzyme encoded by a single gene on chromosome 15 is responsible for the initiation of adrenal mineralocorticoid, glucocorticoid, and sex steroid synthesis, as well as for the synthesis of gonadal steroids and some placental and brain steroids as well. This enzyme catalyzes each of the three steps in the side-chain cleavage reaction in response to the receipt of three pairs of electrons donated by

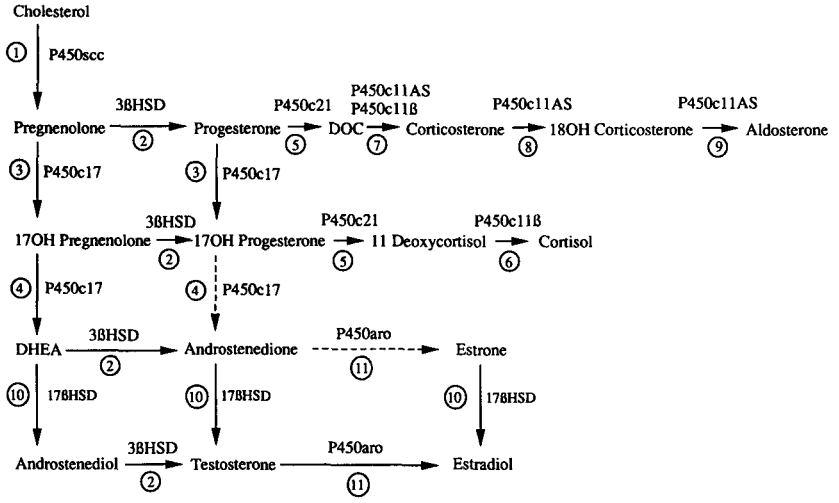

Figure 1 Principal pathways of human adrenal steroid hormone synthesis. Other quantitatively and physiologically minor steroids are also produced. The chemical identities of the enzymes are shown by each reaction. (*Reaction 1*) Mitochondrial cytochrome P450scc mediates 20α-hydroxylation, 22-hydroxylation, and scission of the C20–22 carbon bond, to convert cholesterol to pregnenolone. (*Reaction 2*) 3βHSD, a short-chain dehydrogenase bound to the endoplasmic reticulum, mediates 3β-hydroxysteroid dehydrogenase and isomerase activities. (*Reaction 3*) P450c17 catalyzes the 17α-hydroxylation of pregnenolone to 17OH-pregnenolone and of progesterone to 17OH-progesterone. (*Reaction 4*) The 17,20-lyase activity of P450c17 converts 17OH-pregnenolone to dehydroepiandrosterone (DHEA), but very little 17OH-progesterone is converted to Δ^4 androstenedione. (*Reaction 5*) P450c21 catalyzes the 21-hydroxylation of both progesterone and 17OH-progesterone. (*Reaction 6*) P450c11β converts 11-deoxycortisol to cortisol. (*Reactions 7, 8, and 9*) In the adrenal zona glomerulosa, 11-deoxycorticosterone (DOC) is converted to corticosterone and then to 18OH-corticosterone and finally to aldosterone by a single enzyme, P450c11AS. DOC may also be converted to corticosterone by P450c11β in the zona fasciculata. The final two reactions, 10 and 11, are found principally in the testes and ovaries. (*Reaction 10*) Several isozymes of 17β-HSD, a short-chain dehydrogenase, mediates both 17-ketosteroid reductase and 17β-hydroxysteroid dehydrogenase activities, converting DHEA to androstenediol, androstenedione to testosterone, and estrone to estradiol. (*Reaction 11*) Testosterone is converted to estradiol by P450aro (aromatase).

NADPH through the intermediacy of the electron transport chain NADPH →adrenodoxin reductase→adrenodoxin→P450scc (63, 66). Although this one enzyme initiates both glucocorticoid and mineralocorticoid synthesis, its regulation in these two pathways is distinctly different.

The adrenal cortex may be divided anatomically and functionally into three zones. The outermost, the zona glomerulosa, is the principal site of mineralocorticoid synthesis, and the two inner zones, the fasciculata and the reticularis, are the principal sites of glucocorticoid and adrenal androgen synthesis. The

enzymology and cell biology of the zona glomerulosa is distinct in three respects. First, its cells abundantly express the type-I receptor for angiotensin II (AII), whereas the cells of the other adrenal zones have few AII receptors. Second, glomerulosa cells fail to express the enzyme P450c17, which can both hydroxylate steroid carbon 17, as required for glucocorticoid synthesis, and it can cleave the single bond between carbons 17 and 20 to convert 21-carbon steroids to 19-carbon sex steroids. Third, glomerulosa cells uniquely express the aldosterone synthase enzyme, P450c11AS.

Thus, the biosynthesis of mineralocorticoids from pregnenolone requires the sequential action of only three enzymes: 3ß-hydroxysteroid dehydrogenase (3ßHSD), P450c21, and P450c11AS. 3ßHSD, which converts pregnenolone to progesterone, is a short-chain dehydrogenase enzyme, distantly related to two other steroidogenic enzymes, 11ßHSD and 17ßHSD, and to alcohol dehydrogenase. Human beings have multiple 3ßHSD genes and express at least two distinct forms of this enzyme. The type-II enzyme is expressed in the adrenals and gonads and is involved in the principal biosynthetic pathways of mineralocorticoids, glucocorticoids, and sex steroids. The type-I enzyme is expressed in the placenta and in peripheral, extraglandular tissues (52) and may modify steroids secreted by the adrenals and gonads (11). Progesterone in the zona glomerulosa is then hydroxylated at carbon 21 by the adrenal 21-hydroxylase, P450c21, to yield 11-deoxycorticosterone (DOC). Although there are two P450c21 genes, one is nonfunctional, so that there is only one adrenal P450c21, which 21-hydroxylates the precursors to both mineralocorticoids and glucocorticoids. However, other as-yet-unidentified enzymes can catalyze the 21-hydroxylation of steroid hormones in peripheral, extraglandular tissues (15, 62), and possibly also in the adrenal cortex itself (107). DOC itself is a potent mineralocorticoid, and overproduction of DOC, e.g. in congenital adrenal hyperplasia due to 11ß-hydroxylase (P450c11ß) deficiency, can cause salt retention and hypertension. However, the subsequent metabolism of DOC results in a much more potent mineralocorticoid, aldosterone.

The conversion of DOC to aldosterone occurs in three steps, all catalyzed by a single mitochondrial enzyme, P450c11AS. Two closely related genes that have 93% nucleotide sequence identity lie within about 40 kb of each other on chromosome 8q22 (119) and encode two similar enzymes. The CYP11B1 gene encodes P450c11ß, and CYP11B2 encodes P450c11AS. P450c11ß appears to be expressed in all three zones of the adrenal cortex. It has very high levels of 11ß-hydroxylase activity but very little 18-hydroxylase activity and virtually no 18-oxidase (aldosterone synthase activity) (128). Thus, P450c11ß catalyzes the conversion of 11-deoxycortisol to cortisol in the human zona fasciculata, and the conversion of DOC to corticosterone in the rat zona fasciculata, and is the last enzyme in the pathway to glucocorticoid biosynthesis (Figure 1). By contrast, P450c11AS, whose synthesis is confined to the zona

glomerulosa, has three activities. It first 11ß-hydroxylates DOC to yield corticosterone, then 18-hydroxylates corticosterone to 18-hydroxycorticosterone, and finally 18-oxidizes the latter steroid to aldosterone. The 18-hydroxylation may also precede the 11-hydroxylation to produce small quantities of 18-hydroxy DOC. The molecular biology of these two related genes and enzymes has been reviewed recently (120).

Regulation

The two principal regulators of mineralocorticoid biosynthesis and secretion are the renin/angiotension system and potassium ion, although other factors, such as adrenocorticotropic hormone (ACTH) and sodium ion, play minor roles. Renin is a serine protease secreted by the juxtaglomerular cells of the kidney in response to decreases in blood pressure and/or intravascular volume. Renin attacks angiotensinogen, a blood-borne glycoprotein produced by the liver, and cleaves off its first ten amino acids. This decapeptide, known as angiotensin I, is biologically inactive until converting enzyme, found in the lung, cleaves off two carboxy-terminal amino acids to form the active octapeptide, AII. Inhibition of converting enzyme with agents such as captopril is one of the more successful approaches to the treatment of hypertension.

AII directly stimulates arteriolar vasoconstriction within seconds, thus increasing blood pressure, and also acts to stimulate the biosynthesis of aldosterone by the adrenal zona glomerulosa. AII binds to cell surface receptors linked to the protein kinase C/Ca^{2+} second messenger pathway. This directly increases aldosterone biosynthesis within minutes and also directly stimulates the transcription of the gene for P450scc. AII first stimulates, then supresses, P450scc gene transcription through the intermediacy of the Ca^{2+}/PKC pathway and using P450scc gene promoter regions distinct from those used by cAMP (69). Potassium ion apparently exerts similar effects on the zona glomerulosa by depolarizing the cell membrane, which results in the uptake of extracellular Ca^{2+}, which then activates steroidogenesis in much the same way as AII. Thus, AII and potassium activate the same intracellular second messenger pathway at different levels but exert actions fundamentally different from ACTH. Recent work has shown that the adrenal zona glomerulosa is also able to produce renin in situ (25), which appears to maintain basal cellular levels of P450 c11AS, but it is not clear whether or not AII is involved in this activity (103).

Receptor Structure

The human mineralocorticoid and glucocorticoid receptors are closely related members of the superfamily of nuclear zinc-finger transcription factors. This family includes ligand-dependent receptors, such as those for thyroid and steroid hormones, vitamin D, and retinoic acid and a group of ligand-inde-

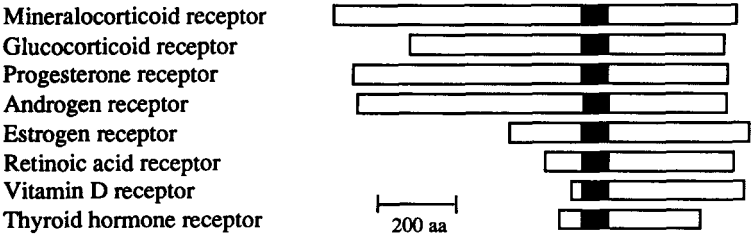

Figure 2 Structure of various zinc-finger nuclear transcription factors. The black box in the center designates the highly conserved DNA-binding domain. The large carboxy-terminal region contains the ligand-binding (steroid-binding) domain, whereas the amino terminal region, which is absent in several members of this family, contains the steroid-dependent phosphorylation transactivation domain, which enhances transcriptional activity of the hormone/receptor complex. Only a few representative receptors are diagramed; there are at least two dozen such receptors.

pendent (orphan) receptors (Figure 2). The human mineralocorticoid receptor (hMR) gene encodes a protein of 984 amino acids (5) significantly larger than the 777 residues of the human glucocorticoid receptor (hGR). This size discrepancy is primarily due to the large amino terminus, which bears no homology to hGR. The cDNA cloning and comparison of the deduced amino acid sequences of different hormone receptors show that this family of receptors has a similar structural and functional organization (for review see 29). The variable N-terminal region is followed by a short, well-conserved cystiene-rich DNA-binding domain, and then by a less-well-conserved C-terminal steroid-binding domain. These three main domains are separated by less-well-defined regions and are probably subdivided into shorter structural motifs.

The 250-amino-acid steroid-binding domain of the hMR shares 57–60% sequence identity with the hGR (5, 13), consistent with the similarities of the steroid structures that bind to both receptors and with the fact that most steroids bind both receptors equally well. In addition to its role in hormone binding and nuclear translocation, the C-terminal domain may also participate in *trans*-activation and dimerization. The sequence of the DNA-binding domain is very highly conserved, with 94% amino acid identity in 68 residues (7, 13). This domain recognizes short, highly conserved DNA sequence motifs within steroid-responsive genes (7). The 602-amino-acid amino-terminal immunogenic domain of the hMR is the least conserved (5, 13). There is considerable heterogeneity of size and sequence for this region among the receptors for various classes of steroids (13, 29). There is less than 15% identity in this region of the hMR and hGR; consequently most receptor antibodies are directed against this region.

The inactive hMR is found as a 9S form that includes a 90-kDa heat shock

protein (hsp 90) (9), and possibly other protein components that can be associated with other steroid receptors (89, 100, 106). Binding of steroid hormone activates or transforms the 9S form to a 4S form by the release of hsp90 and other proteins from the receptor and make it able to bind DNA (27, 84, 104). However, the hMR can also bind DNA as a large oligomer, suggesting that the interaction between the different subunits of the hMR (in particular between hsp90 and the steroid/DNA-binding domain) may be different from that of the GR (4). Thus, in the case of GR, hsp90 appears to mask the DNA-binding domain of the receptor so that dissociation of hsp90 must occur for the receptor to promote transcription. By contrast, hsp90 may not dissociate from the hMR before binding to DNA and may actually be involved directly in regulating the receptor function as a DNA-binding transcriptional factor (4, 82).

Receptor Affinities

The substantial amino acid sequence identity (57%) in the ligand binding domains of the hMR and hGR lead to substantial overlap in their binding activities. These binding activities are so similar that MR and GR are frequently referred to as type-I and type-II GRs, respectively. As discussed below, mineralocorticoid activity is a consequence of both receptor binding and the susceptibility of steroids to inactivation by the enzyme 11ß-hydroxysteroid dehydrogenase. The steroid-binding activities of the hMR (type I) and the hGR (type II) have been determined by transfecting COS-1 cells with vectors expressing either the hMR or the hGR, and then examining the ability of the expressed receptors to *trans*-activate a cotransfected promoter-reporter construction consisting of the glucocorticoid-sensitive mouse mammary tumor virus (MMTV) linked to the luciferase reporter gene (LUC). Using this procedure, Arriza et al (5) first showed that aldosterone, cortisol, and corticosterone have similar affinities for the MR. A more recent study compared the K_i values for 11 different natural and synthetic steroids (97). These data, summarized in Table 1, show that many steroids not classically regarded as mineralocorticoids, such as progesterone and cortisol, bind to the hMR as well as, or better than, aldosterone. Thus, the findings of Arriza et al raised fundamental questions about the mechanisms by which mineralocorticoids and the MR exert effects distinct from those exerted by glucocorticoids and the GR.

High-affinity binding sites for [^{3}H]aldosterone are found in both classic mineralocorticoid target tissues (e.g. kidney, parotid, and colon) and in nontarget tissues (e.g. hippocampus and heart). In the absence of transcortin, these sites show equal affinity for aldosterone, cortisol, and corticosterone, typical of type-I receptors, to distinguish them from the type-II, classic dexamethasone-binding glucocorticoid receptors. However, in vivo the classic mineralocorticoid target tissues are selective for aldosterone and show little [^{3}H]corticosterone binding

Table 1 Receptor binding activity of various steriods[a]

Steroids	MR	GR
9α-Fluorocortisol	41	1000
Cortisol	24	800
DOC	16	260
Dexamethasone	13	900
Aldosterone	12	180
Tetrahydro DOC	4	1
Progesterone	2	40
RU28362	1	800
RU38486	1	35
Spironolactone	1	1
Tetrahydro progesterone	1	1
No steroid	1	1

[a]Plasmid vectors expressing the human cDNAs for either the mineralocorticoid receptor (MR) or glucocorticoid receptor (GR) were transfected into cells together with a reporter vector consisting of the hormone-responsive long terminal repeat of the mouse mammary tumor virus linked to the Luciferase reporter gene (MMTV-LTR) as described by Arriza et al (5). Data (in arbitrary units) are taken from those reported by Ruprecht et al (97). DOC, 11-Deoxycorticosterone.

(38). In the kidney, the MR is most abundant in the collecting ducts, but it is also found in glomeruli, proximal tubules, and the thick ascending limb, consistent with the predicted receptor distribution derived from biochemical studies (116). MR are also found in the small and large intestine. In the central nervous system, MR are particularly abundant in the hippocampus, amygdala, and lateral septum (36) and have also been found in the pituitary and hypothalamus (60) as well as in vascular smooth muscle cells (77).

Mineralocorticoid Action: 11ßHSD

11ß-Hydroxysteroid dehydrogenase (11ßHSD) is a microsomal enzyme that interconverts the active steroids, cortisol and corticosterone, to their inactive metabolites, cortisone and 11-dehydrocorticosterone, respectively. There are at least two 11ßHSD enzymes: the type-I enzyme is expressed predominantly in glucocorticoid target tissues such as liver, testis, lung, and proximal convulated tubule; and the type-II enzyme is expressed in mineralocorticoid target tissues.

The type-II enzyme is crucial in regulating mineralocorticoid action. As the concentrations of cortisol in human blood are nearly 1000 times higher than those of aldosterone, the discovery that glucocorticoids could bind to MR immediately posed the problem of how aldosterone could exert a specific and separate action from cortisol. It is now generally agreed that type-II 11ßHSD

inactivates glucocorticoids in mineralocorticoid target tissues and thus defends the MR from being overwhelmed by glucocorticoids (38). Thus, when there is congenital absence of type-II 11ßHSD (the syndrome of apparent mineralocorticoid excess) (108) or when the enzyme's activity is inhibited by licorice (26) or carbenoxolone (109), the protective mechanism fails and cortisol gains inappropriate access to MR, resulting in hypertension and hypokalemia. By contrast, type-I 11ßHSD activity in the liver favors conversion of cortisone to cortisol, thus maximizing tissue concentrations of cortisol in tissues where NADPH is abundant (68, 70), as the NADPH-NADP (nicotinamide adenine dinucleotide phosphate) ratio determines the balance between oxidation (conversion of cortisol to cortisone) and reduction (conversion of cortisone to cortisol) activities of the type-I enzyme.

There are at least two 11ßHSD enzymes that differ genetically, structurally, and enzymatically. The human type-I 11ßHSD gene on chromosome 1 consists of six exons spanning about 7 kb and encodes a protein of 292 amino acids (114). This enzyme catalyzes both the oxidase and reductase reactions using either NADP or NADPH as a cofactor and has a high K_m (1 M) (2, 70), so that only abundant steroids will be metabolized. This type-I 11ßHSD is widely distributed in multiple tissues (70, 114). The type-II 11ßHSD gene on chromosome 16q22 consists of five exons spanning about 6.2 kb and encodes a protein that is only 14% identical to the type-I enzyme (1, 3). This enzyme catalyzes only the oxidase reaction (cortisol to cortisone), uses NAD as a cofactor, and has a low K_m (10–100 nM) (10, 99). This enzyme is most abundantly expressed in mineralocorticoid target tissues such as kidney and colon and is also expressed in pancreas, placenta, prostate, and gonads (3). The rat type-II enzyme is dose-dependently inhibited by its end product (11-dehydrocorticosterone), while the liver enzyme is not (99). Thus, the 11ßHSD type II found in the renal collecting ducts possesses all of the features required to protect the MR from occupancy by endogenous glucocorticoids: location in mineralocorticoid target cells, very high affinity for its substrate, and an ability to reduce steroids drastically to irreversible dehydrogenation. Consistent with this, patients with mutations in this enzyme (but not the type-I enzyme) have the syndrome of "Apparent Mineralocorticoid Excess" (AME), in which glucocorticoids act as mineralocorticoids (73, 123).

GENETIC DISORDERS OF MINERALOCORTICOID SYNTHESIS

Salt-Wasting Syndromes

CONGENITAL LIPOID ADRENAL HYPERPLASIA Congenital lipoid adrenal hyperplasia (lipoid CAH) is the most severe form of CAH. Affected individuals can

synthesize no steroid hormones and hence are all phenotypic females with a severe salt-losing syndrome that is fatal if not treated early. Although it was long thought that the disorder was in the cholesterol side-chain cleavage enzyme (P450scc), a role for P450scc was ruled out by molecular genetic analysis of affected individuals (54). The molecular lesion in this disorder was recently found in the Steroidogenic Acute Regulatory Protein (StAR) (55), which facilitated the demonstration that StAR acts by promoting the flow of cholesterol into mitochondria where it is converted to pregnenolone by P450scc (55).

As cholesterol can be converted to pregnenolone only in mitochondria, the transport of cholesterol from cytoplasmic stores into mitochondria is of paramount importance. Early work showed that cycloheximide and other inhibitors of protein synthesis can block ACTH-induced steroidogenesis in the adrenal or gonadotropin-induced steroidogenesis in ovarian granulosa cells (34, 39). This led to the search for various cycloheximide-sensitive labile factors that are required for the transport of cholesterol into mitochondria. Various candidate factors have included sterol carrier protein 2, steroidogenesis activator peptide, and endozepine, which is the endogenous ligand of the peripheral-type benzodiazepine receptor (PBR) (for review see 65). There is good evidence that binding of endozepine to PBR facilitates increased cholesterol transport into mitochondria and increases steroidogenesis (79), but none of the components of this system appear to exhibit the exquisitely rapid response to cAMP and inhibition by cycloheximide that characterize the acute response.

A series of 37-, 32-, and 30-kDa proteins is rapidly induced in adrenal and gonadal cells in response to tropic stimulators of steroidogenesis (28, 110). This protein family is very rapidly induced by cAMP and inhibited by cycloheximide; they are localized to mitochondria and are found in the adrenal cortex, testicular Leydig cells, and ovarian granulosa cells. The cDNA for this protein was recently cloned from mouse MA-10 cells and was dubbed StAR (20). The kinetics of induction, sensitivity to cycloheximide, and action to increase steroidogenesis all suggested that StAR was the acute regulator of steroidogenesis. However, it was not until its role in lipoid CAH (a gene knockout of nature) was determined that it was established that StAR is this long-sought regulator.

StAR is encoded by an 8-kb gene on chromosome 8p11.2 that encodes a small pre-protein of 285 amino acids; the nucleotide sequence of the complete gene and pseudogene have been determined (111, 112). StAR promotes the flow of cholesterol from the outer to the inner mitochondrial membranes (55), where it then becomes accessible to any available form of P450; thus, StAR can increase the activity of either P450scc or cholesterol 27 hydroxylase (112). Three different StAR mutations have been described in four patients with lipoid CAH: the nonsense mutations R193X and Q258X (55) and a splicing mutation

that eliminates exon 5 (115). Transfection of nonsteroidogenic COS-1 cells with StAR and with a fusion protein consisting of P450scc, adrenodoxin, and adrenodoxin reductase (46) demonstrates that native StAR, but not the mutants, promotes cholesterol transport and steroidogenesis. Thus, StAR is essential for the synthesis of all steroid hormones.

3ß-HSD 3ßHSD deficiency is an autosomal recessive disorder of the type-II 3ßHSD gene. In its classic form, 3ßHSD deficiency impairs cortisol, aldosterone, and testosterone synthesis and usually causes ambiguous genitalia in newborn males. Two human 3ßHSD enzymes have been identified by cloning of both their cDNAs and genes. These are short-chain dehydrogenases, unrelated to P450 enzymes, that are 371 amino acids long and 93.5% identical to one another. The type-I gene is expressed in the placenta and in peripheral tissues such as the skin and mammary gland, whereas the type-II gene is expressed in the adrenals and gonads. Both genes are about 7.8 kb long and consist of four exons and three introns and are found on chromosome 1p13 (8, 51, 52, 57, 90).

3ßHSD is a membrane-bound short-chain dehydrogenase that catalyzes 3ß-hydroxysteroid dehydrogenation and isomeration of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids) in both adrenals and gonads. Thus, the plasma concentrations of pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone (DHEA), and DHEA sulfate are elevated in affected patients. Plasma levels of 17-hydroxyprogesterone may also be increased as a result of the action of peripheral type-I 3ßHSD, which will still convert 17-hydroxypregnenolone to 17-hydroxyprogesterone when the type-II enzyme is disordered in the adrenals and gonads (11). However, the plasma 17-hydroxypregnenolone/17-hydroxyprogesterone ratio will remain strikingly elevated, permitting the diagnosis to be made. Levels of urinary 17-ketosteroids are also elevated due to the overproduction of DHEA and its sulfate.

At least 14 lesions in the type-II gene have been reported that cause the classic form of 3ßHSD deficiency. Nine were single amino acid replacements (91, 92, 101, 102), one was a nonsense mutation, three were frame-shifts, and one was a splicing error (16, 60a, 105, 105a). Only one small deletion and one insertion leading to a premature termination codon at positions 279 and 202, respectively, have been described (105). In the so-called nonclassic form of 3ßHSD deficiency, no mutations have been reported in either the type-I or -II 3ßHSD genes, suggesting that the classic hormonal diagnostic criteria do not truly identify a disorder of 3ßHSD (17, 127). The lack of described mutations in the type-I 3ßHSD gene probably correlates with the expression of the type-I, but not the type-II, gene in the placenta. Thus, any mutation that would substantially inactivate 3ßHSD type I would inhibit placental progesterone biosynthesis, which would lead to spontaneous abortion.

21-HYDROXYLASE DEFICIENCY Steroid 21-hydroxylase deficiency comprises about 95% of all cases of CAH and has an over-all incidence of about 1 in 12,000 persons. About two thirds of patients have salt loss, making it the most common congenital salt-losing disease. Adrenal 21-hydroxylase activity is catalyzed by P450c21, encoded by a gene termed CYP21B or P450c21B, to distinguish it from the duplicated but nonfunctional P450c21A gene. P450c21 is found only in the adrenal cortex, although there is 21-hydroxylase activity in many other tissues catalyzed by unknown enzymes (62). The genetics of P450c21 are unusual and complicated. Random deletions and point mutations almost never occur; instead, gene conversions account for about 85% of all mutant P450c21 alleles. In these gene conversions, all or part of the P450c21B gene is replaced by, or converted to, the sequence of the corresponding sequence of the P450c21A gene (for review see 64, 71).

P450c21 catalyzes the 21-hydroxylation of progesterone to DOC and of 17OH-progesterone to 11-deoxycortisol (Figure 1). Consequently, a complete absence of P450c21 will lead to a deficiency of both aldosterone (causing salt loss) and cortisol (causing a variety of symptoms). Decreased or absent secretion of cortisol induces the production of ACTH by the pituitary, which in turn stimulates adrenal growth and steroidogenesis. The various steroidal precursors shown in Figure 1 accumulate and, in the absence of 21-hydroxylation, are diverted to the synthesis of androgens. During fetal development, these excess adrenal androgens have no detectable effect of 46,XY males, but 46,XX females become virilized, with varying degrees of labial fusion and cliteromegaly that can occasionally lead these girls to be mistaken for boys at birth.

Because adrenal steroids are not needed for human fetal development, these fetuses do well until birth. After birth, newborns with a severe defect in P450c21 may experience a salt-losing crisis, consisting of hyponatremia, hyperkalemia, metabolic acidosis, and cardiovascular collapse. However, these symptoms are usually seen after the first one or two weeks of life, so they may not be diagnosed and may lead to death. Most affected girls are diagnosed while newborns because the genital ambiguity alerts medical personnel, but as many as half of the affected males die without diagnosis. Thus, understanding and recognizing disorders of mineralocorticoid metabolism can be lifesaving.

The adrenal synthesis of cortisol normally exceeds that of aldosterone by nearly 1000-fold. Thus, genetic disorders in P450c21 that destroy up to 99% of its activity may still permit the synthesis of enough aldosterone to prevent the clinical manifestations of salt loss. However, the defect in cortisol synthesis will still be severe, resulting in ACTH excess, adrenal hyperplasia, hyperandrogenism, and virilization of female fetuses. Again, the females are usually diagnosed because of the genital ambiguity, but the males typically elude diagnosis. In the absence of salt loss they do rather well, often growing to be

larger and stronger than their peers because of the overproduction of testosterone by the adrenals. However, this early growth extracts a high price, as the hyperandrogenism accelerates bony maturation. Thus, these boys with non-salt-losing CAH may be tall children but become short adults. Although these patients do not have the clinical signs of salt loss, they do have a defect in mineralocorticoid synthesis, as evidenced by inappropriately high plasma renin activity. Thus, the treatment of both the salt-losing and non-salt-losing varieties of CAH usually consists of replacement of glucocorticoids and mineralocorticoids.

CORTICOSTERONE METHYL OXIDASE DEFICIENCIES The term corticosterone methyl oxidase (CMO) deficiency refers to a group of rare autosomal recessive defects in mineralocorticoid biosynthesis (118). The term is somewhat archaic, as it derives from a time when the enzymes needed for mineralocorticoid synthesis had not yet been identified as discrete molecular entities; nevertheless, the classification remains useful. Two different syndromes are generally recognized: CMO-I deficiency refers to patients who have salt wasting associated with high serum concentrations of corticosterone but low concentrations of 18OH-corticosterone and aldosterone; CMO-II deficiency is similar except that 18OH-corticosterone concentrations are high. The aldosterone deficiency leads to hyponatremia, hyperkalemia, and metabolic acidosis. The clinical severity of the disorder varies inversely with the age: Infants and newborns tend to be severely affected whereas older children and adults may have normal serum electrolytes, even without treatment. Plasma renin activity is markedly elevated in very young patients but may be normal in adults (95). In both forms of CMO deficiency, aldosterone synthesis is impaired while the adrenal zona glomerulosa continues to produce corticosterone and deoxycorticosterone in response to the renin-angiotensin system.

Because plasma 18OH-corticosterone is low in CMO-I and high in CMO-II deficiency, but aldosterone is low in both disorders, the ratio of 18OH-corticosterone to aldosterone is the usual diagnostic hallmark. However, this is not always reliable in CMO-I deficiency; the ratio of corticosterone to 18-hydroxycorticosterone should also be increased in CMO-I deficiency, and decreased in CMO-II deficiency, corresponding to the absence or presence of 18-hydroxylase activity, respectively (118).

Recent studies have examined the CYP11B2 gene encoding P450c11AS in the CMO deficiencies. Two different lesions have been described in CMO-I deficiency. One was a deletion of five nucleotides, causing a frame shift and premature stop codon in exon 1 that prohibited the expression of any P450 c11AS protein (67). The other was the mutation R384P, which still resulted in low levels of 18-hydroxycorticosterone (40). The type-II deficiency has been studied extensively in Jews of Iranian origin, among whom the incidence

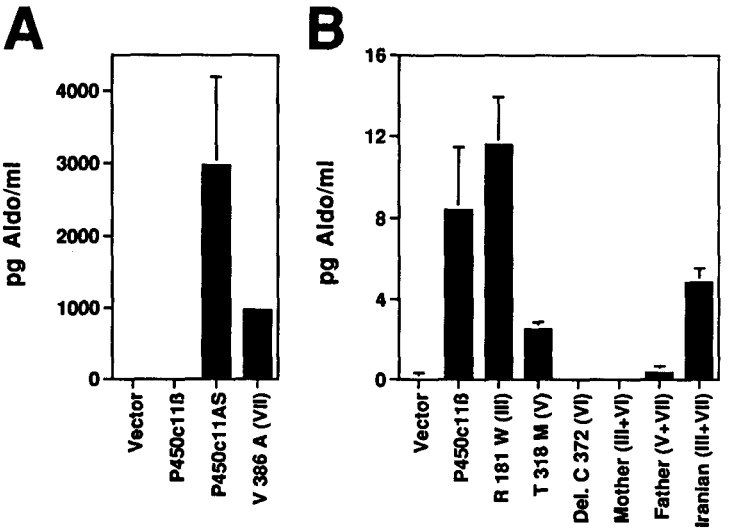

Figure 3 Aldosterone synthase activities of P450c11ß, P450c11AS, and P450c11AS mutants in CMO II deficiency. Vectors expressing each construction were transfected into mouse MA-10 Leydig tumor cells, which provide the adrenodoxin and adrenodoxin reductase needed by the P450c11 enzymes; after incubation with DOC, the production of aldosterone was measured by immunoassay. The Roman numerals refer to the exon in which each mutation is found. The mother had both R181W and ΔC372 on a single allele, and the father had T318M and V386A on a single allele. The compound mutation commonly found in Iranian Jews is R181W plus V386A. Data are mean standard deviation. Note the 250-fold difference in scale between panels A and B. From (128).

of this disorder is very high. Twelve such patients in seven affected families were all homozygous for two different point mutations, R181W and V386A (80). Family members homozygous for only one of these mutations were phenotypically and clinically normal; both mutations are required to produce disease. The mutation V386A may be due to a gene conversion, as P450c11ß has Ala at position 386 (80).

Recently, we reported another patient with a typical clinical and hormonal picture of CMO-II deficiency. Direct sequencing of patient and parent DNA showed that the father's allele contributed T318M and V386A, and the mother's allele contributed R181W and the deletion/frame-shift mutation Δ372. These mutants were recreated in cDNA expression vectors singly and in the parental pairs, showing that neither allele contributed any measurable activity (Figure 3). By amino acid sequence alignment (74), T318 in either P450c11AS or P450c11B corresponds to a conserved residue that receives protons from a nearby acidic residue, which in turn cleaves the dioxygen bond

of O_2 to create the iron-oxy intermediate required for P450 catalysis. Thus, any mutation of T318 would be expected to have profoundly deleterious effects on enzymatic activity (128).

Many questions remain unanswered. For example, in the case described above, each parent's defective alleles showed no detectable activity, so that the predicted clinical phenotype should have been CMO-I rather than CMO-II deficiency (128). Also, the R181W mutation retains only 0.38% of aldosterone synthase activity (128), but individuals homozygous for this mutation do not have salt loss (80). Furthermore, some patients with the clinical and hormonal findings of CMO-II deficiency either have mutations of P450c11AS that do not ablate activity or have no mutations of P450c11AS at all (31). These findings suggest that other enzymes may be involved in the synthesis of other mineralocorticoids. As discussed below, the finding of additional forms of P450c11 in the rat suggests that other human enzymes may remain to be identified.

Hypertensive Syndromes

11ß-HYDROXYLASE DEFICIENCY Steroid 11ß-hydroxylase deficiency is the second most common cause of CAH but still comprises fewer than 5% of patients with CAH. However, a large number of cases has been reported in Israel among Jews of Moroccon descent, among whom the incidence is 1/5000–1/7000 (96). 11ß-Hydroxylase deficiency is caused by mutations in the CYP11B1 gene that encodes P450c11ß; this will disrupt the conversion of 11-deoxycortisol to cortisol (Figure 1). As a consequence of deficient cortisol production, the pituitary secretes large amounts of ACTH, stimulating adrenal steroidogenesis. As a result, affected patients characteristically have very high serum concentrations of 11-deoxycortisol, the diagnostic hallmark of the disorder. The ACTH-driven adrenal hyperactivity leads to accumulation of steroidal precursors that are converted to androgens. Just as in 21-hydroxylase deficiency, this leads to virilization of female fetuses in utero but causes no anatomic changes in males. The unique aspect of 11ß-hydroxylase deficiency is that the patients also produce excess DOC, principally in the ACTH-responsive cells of the adrenal zona glomerulosa. As DOC is an effective mineralocorticoid, the excess DOC can cause retention of salt and water and consequent hypertension. This ACTH-driven overproduction of DOC also suppresses the renin-angiotensin system and the secretion of aldosterone so that the patients have mineralocorticoid-based hypertension in the absence of the principal mineralocorticoid, aldosterone. Although 11ß-hydroxylase deficiency is often termed the hypertensive form of CAH, the hypertension may not develop for several years, and affected newborns may actually present with a paradoxical salt-losing episode. This is probably due to two factors: the mineralocorticoid

resistance typical of newborns, and the lower potency of DOC as a mineralocorticoid.

Numerous mutations in P450c11ß can cause 11ß-hydroxylase deficiency. The high incidence in Jews from Morocco is due to the mutation R448H (121); at least ten other mutations have been identified in other populations, including two frame-shift mutations, four premature stop codons, and four missense mutations. The frame shifts include a one-nucleotide deletion in codon 32 and a two-nucleotide insertion in codon 394. The nonsense mutations include W116X, K174X, Q338X, and Q356X, and the four missense mutations are T318M, R374E, R384Q, and V441G (for review see 120). A comparison of the amino acid sequence of P450c11ß with the sequences and tertiary structures of several bacterial P450 enzymes whose structures have been solved crystallographically (47, 48, 88) shows that the identified missense mutations in P450c11ß are localized in regions of functional importance that abolish enzymatic activity. R448 is adjacent to C450, which is required for binding the heme group of P450c11ß; T318M modifies a residue essential for proton transfer to the bound oxygen molecule; R374Q mutates a highly conserved residue thought to affect binding of adrenodoxin; R384 appears to form part of the substrate binding pocket; and V441G is adjacent to the highly conserved heme binding region.

17α-HYDROXYLASE DEFICIENCY Deficiency of 17-hydroxylase activity is a rare form of CAH caused by defects in cytochrome P450c17, the single enzyme that has 17-hydroxylase and 17,20 lyase activities. More than 125 cases of 17-hydroxylase deficiency and 14 other cases of apparently isolated 17,20 lyase deficiency have been reported (125). 17-Hydroxylase deficiency is characterized by absent sex steroid synthesis and by impaired production of cortisol and compensatory hypersecretion of ACTH, which stimulates the synthesis of large amounts of DOC, 18-hydroxy DOC, corticosterone, and 18-hydroxycorticosterone. The increased concentrations of corticosterone and DOC produced by the zona fasciculata in response to ACTH can suppress the production of aldosterone in the zona glomerulosa, just as in 11ß-hydroxylase deficiency. However, some patients may have normal or elevated aldosterone with suppressed renin activity.

There have been several attempts to detect heterozygotes. In studies of obligate heterozygotes, slight increases in plasma DOC, corticosterone, 18-hydroxy-DOC, and 18-hydroxycorticosterone have been observed (23, 124). The study of several families also demonstrated exaggerated responses of 17-deoxysteroids to ACTH stimulation (23, 93, 124). However, the ratio of urinary metabolites of C21,17-deoxysteroids to C21,17 hydroxysteroids is the most accurate means of heterozygote detection and has predicted the zygosity of family members as confirmed by molecular genetic analysis (30).

The molecular genetic basis of 17-hydroxylase deficiency has now been studied in about two dozen patients, identifying at least 17 different lesions in the P450c17 gene. Five of these were nonsense mutations, three were deletions, three were small duplications, and six were single amino acid replacements (30). It is of interest that five lesions identified to date lie in the coding region of exon 8 and destroy both activities of P450c17 enzyme. These lesions include the 4-base pair (bp) duplication at codon 480 found in the Mennonite/Frisian population (49), an R496C mutation and Q461X mutation found in a Swiss patient (126), a 9-bp deletion at 487–489 (33), and the homozygous mutation R440H (30). Why these lesions in the carboxy terminal region of the protein should have such important enzymatic consequences is not clear, although sequences encoded by exon 8 are responsible for heme binding. For example, Arg 440 is in the heme-binding region very close to Cys442, which is the fifth ligand of the catalytic heme iron (18). Also, lesions in the carboxy terminal region may electrostatically influence the association with carboxylate residues of the microsomal P450 reductase (125). However, the activity of P450c17 is very sensitive to minor changes in its structure. Many point mutations, such as Y64S, S106P, and P342T, are far from the loci predicted to participate in the heme-binding region (74) or the active site (56, 81), and conservative mutations produced in vitro can greatly reduce the enzyme activity (53, 56).

MINERALOCORTICOIDS IN ESSENTIAL HYPERTENSION

Low-Renin Hypertension

Hypertension is the most commonly treated disease in the United States, affecting about 20% of the adult population. Although a great deal is known about the regulation of blood pressure, a specific causal abnormality can be found in only a small percentage of patients. Thus, about 95% of patients with hypertension are said to have essential, or primary, hypertension, which is better thought of as idiopathic hypertension. Mineralocorticoids act to retain salt in the kidney, thus increasing intravascular volume and blood pressure. The primary stimulator of mineralocorticoid synthesis and release is AII, which constricts arteriolar smooth muscles, and thus increases blood pressure. Thus, the renin/angiotensin/aldosterone system has been of substantial interest to investigators studying hypertension, although other hormonal and nonhormonal systems are also involved (for review see 6). Measurements of plasma renin activity (PRA) in hypertensive subjects can be low, normal, or high in a fairly smooth distribution. The absence of a clearly defined subpopulation with low PRA has suggested to some that the great variation in PRA reflects

various secondary bodily adaptations to the hypertensive state (6). Others have suggested that low-renin hypertension constitutes a distinct subgroup, consisting of about 15% of hypertensives (122); this is supported by the poor responsiveness of this group to treatment with various inhibitors of converting enzyme. The significance of low-renin essential hypertension thus remains controversial. Nevertheless, several rare, diagnosable forms of hypertension, such as aldosterone-producing adrenal tumors (Conn's Syndrome), or the syndromes of 11ß-hydroxylase and 17α-hydroxylase deficiency, are associated with low-renin hypertension and a demonstrable form of mineralocorticoid excess. Thus, there is substantial interest in a potential causal role for mineralocorticoids in low-renin hypertension.

Because plasma aldosterone concentrations are not elevated in most patients with low-renin hypertension, investigators began to search for other potential mineralocorticoids; indeed, there are reports of patients with elevated concentrations of several steroids, including DOC, 18OH-DOC, 18OH-corticosterone, 19nor-DOC, and 19nor-aldosterone (44, 50, 113). The elevated blood pressure in low-renin hypertension can be treated with spironolactone (an aldosterone antagonist), diuretics, or blockade of adrenal steroids, whereas hypertension with normal or high renin responds poorly to these drugs. Moreover, although there is no direct evidence for excess mineralocorticoids in patients with low-renin hypertension, it can be argued that their normal concentrations of mineralocorticoids are excessive relative to their suppressed plasma renin levels (50). Thus, the cause of hypertension in these patients has been speculated to be a relative excess of mineralocorticoids or an increased sensitivity to their action.

The link between mineralocorticoid excess and hypertension is further suggested by the fact that adrenocortical hyperplasia similar to that found in idiopathic hyperaldosteronism is frequently encountered during autopsies of hypertensive patients (98), particularly those with suppressed PRA (50). However, the absence of significant volume expansion and of hypokalemia argues against a role for mineralocorticoid excess in the pathogenesis of low-renin hypertension. Thus, low-renin hypertension could represent an altered set-point in the regulation of the renin/angiotensin/aldosterone axis.

The mineralocorticoids whose concentrations may be elevated in essential hypertension might increase the blood pressure directly, as precursors to more potent mineralocorticoids, or by amplifying the actions of other mineralocorticoids. For example, DOC and 18OH-DOC can bind to the mineralocorticoid receptor directly and increase the blood pressure (12). Similarly, adrenal 19OH-DOC, which is not a potent mineralocorticoid, can be metabolized by the kidney to 19nor-DOC, which has greater mineralocorticoid activity than does aldosterone (14, 24, 37, 41). Finally, 18OH-corticosterone, which is a weak mineralocorticoid that cannot provide adequate salt retention by itself,

may be able to amplify the actions of other mineralocorticoids, such as aldosterone and 19nor-DOC (94).

Involvement of the Renin/Angiotensin/Mineralocorticoid System

The reasons why these mineralocorticoids with hypertensinogenic activity are inappropriately elevated in patients with essential hypertension are unknown. Because the aldosterone response to AII or to upright posture is higher than expected in relation to the plasma renin levels, it has been suggested that low-renin hypertension reflects an enhanced sensitivity of the zona glomerulosa to AII (45). It is also possible that patients having an increased adrenal sensitivity to AII have increased sensitivity in the arterial vasculature as well. However, subtle alterations in the activity of P450c11AS could account for many of these observations. As described above, P450c11AS has 11ß-hydroxylase, 18-hydroxylase, and 18-oxidase activities. Recent work has shown that the hypertensive Dahl R rat has two mutations in P450c11AS that increase the aldosterone synthase activity of this enzyme 1000-fold (21). When we created corresponding mutations in human P450c11AS, this synthetic mutant produced four times as much 18OH-corticosterone and twice as much aldosterone as the wild-type enzyme (32). This suggests that point mutations in P450c11AS can either increase its aldosterone synthase activity, or possibly confer a novel activity, such as 19-hydroxylation, that could yield other hypertensinogenic mineralocorticoids. Thus, mutations in P450c11AS might be a cause of some cases of low-renin hypertension, but this has not yet been studied.

The real relevance of mineralocorticoids in human hypertension remains unknown. However, recent work suggests that cryptic mineralocorticoid excess may not be uncommon in low-renin hypertension (42, 50). Although absolute elevations in plasma aldosterone concentrations are unusual, most patients with low-renin hypertension have inappropriately normal aldosterone values with elevated aldosterone/renin ratios, or with elevations of other mineralocorticoids (42, 50). Among 436 Japanese patients with idiopathic hypertension, 54 (12.4%) had low plasma renin activities; of these, 8 had an aldosterone-producing adenoma and 7 had idiopathic hyperaldosteronism. The remaining 39 had normal aldosterone, of which 30 also had elevated values of 18-hydroxycorticosterone, so that the ratios of aldosterone or 18OH-DOC to PRA were significantly elevated (50). Similarly, Gordon et al found that 12% of hypertensive patients had primary aldosteronism, as indicated by a consistently elevated aldosterone/PRA ratio (>30) and confirmed by the inability to suppress the patients' elevated aldosterone concentrations by treatment with salt and a synthetic mineralocorticoid.

The aldosterone/PRA ratio is not routinely used as a screening test, and most patients with mineralocorticoid excess are normokalemic and have normal

aldosterone. Hence, the portion of hypertensive patients with functional mineralocorticoid excess remains undetermined. Similarly, other mineralocorticoids such as 18OH-DOC and the urinary 19nor-DOC have not been studied systematically in hypertensive patients.

LESSONS FROM GENETICALLY HYPERTENSIVE RATS

When examined as a biological variable, blood pressure has a significant genetic component, which has been especially well studied in rodent models for genetic hypertension (86). In all species, blood pressure behaves as a polygenic trait, i.e. blood pressure shows a continuous variation, and discrete phenotypes are not generally discernible by studying the frequency distribution for blood pressure. Continuous variation is a consequence of multiple genetic loci that influence blood pressure by multiple biochemical and physiologic systems, each of which may have its own genetic inputs. The challenge is to identify and characterize these multiple genetic inputs.

Animal models of hypertension provide an important experimental and theoretical foundation for understanding human hypertension. There are many different strains of genetically hypertensive rats, including Okamoto's spontaneously hypertensive rats (SHRs), the Milan hypertensive strains (MHS), the Lyon hypertensive strains (LH), the New Zealand genetically hypertensive strain (GH), the Dahl's rats selected for sensitivity (S) and resistance (R) to dietary salt-induced hypertension, and the inbred strains (SS/Jr and SR/Jr) subsequently derived from the latter (87). Each of these may be a model for a different genetic factor regulating blood pressure. A powerful new tool is the use of transgenic mice and rats. In these animals, overexpression or suppression of individual candidate genes involved in hypertension permits a systematic examination of gene function, gene regulation, and a gene's relationship to a specific phenotype.

The Dahl S rat is the most widely studied genetic model of salt-sensitive hypertension. These rats were originally derived from outbred Sprague-Dawley stock by the selection of rats exhibiting high blood pressure when given a high-salt diet, whereas the Dahl R strain was similarly derived by selecting rats that retained relatively low blood pressures when given a high-salt diet (85). Plasma and renal renin activities measured both in vivo and in isolated perfused kidney are lower in Dahl S than in Dahl R rats, independent of dietary sodium, whereas the adrenal secretion of 18OH-DOC by the Dahl S rat is double that of the Dahl R rat (35). Physiologic concentrations of 18OH-DOC may induce increases in blood pressure similar to those induced by DOC (12). Differences in 19nor-DOC might also contribute to these differences in blood pressure of Dahl S and R rats in response to increased NaCl (24, 43). Thus,

mutation in the gene controlling the adrenal synthesis of 18OH-DOC might contribute to the differences in blood pressure in Dahl S and R rats.

The enzymes that are involved in the last steps in the biosynthesis of glucocorticoids and mineralocorticoids in rats are P450c11B and P450c11AS, just as they are in humans (58, 76, 103). Furthermore, the synthesis of 19OH-DOC, the substrate for the production of 19nor-DOC, may be catalyzed by one of the isozymes of P450c11 (75, 78). P450c11AS catalyzes 11ß- and 18-hydroxylations and 18-oxidation, converting DOC to 18OH-DOC, corticosterone, 18OH-corticosterone, and aldosterone, but it does not catalyze the 19-hydroxylation of DOC (22, 58, 83, 103, 117). However, the rat genome has two other P450c11 genes, called CYP11B3 and CYP11B4 (72). CYP11B4 is a pseudogene, but CYP11B3 encodes P450c11B3, which is 96% identical to P450c11ß. Rat P450c11B3 can convert DOC to corticosterone or to 18OH-DOC and can covert corticosterone to 18OH-corticosterone. Thus, P450c11B3 has 18 hydroxylase activity similar to P450c11AS but lacks detectable 18-oxidase activity and thus has a spectrum of activities midway between P450c11ß and P450c11AS (61). Recent work has also shown that the Dahl S rat has two mutations (E136D and E251R) in P450c11AS that increase the aldosterone synthase activity of this enzyme up to 1000-fold (21). Thus, P450c11AS is the first gene identified in an experimental animal model in which coding sequence mutations are enough to explain an alteration in the protein activity that could logically affect blood pressure.

The role of P450c11B3 in the development of hypertension in Dahl rats is unknown. However, P450c11B3 can produce more 18OH-DOC than can P450c11ß and hence might alter the blood pressure in the absence of changes in P450c11AS expression. P450c11B3 is only expressed in the adrenals of newborn rats between 6 and 32 days of age (61). Although a human counterpart for rat P450c11B3 has not been identified, it is tempting to speculate that overproduction of mineralocorticoids in childhood due to an abnormality in such a gene might have long-term effects, resulting in fixed primary essential hypertension.

Alterations in both 18OH-DOC and 19nor-DOC have been found in Dahl rats, suggesting that these differences in mineralocorticoid production might be attributable to P450c11ß. An RFLP for CYP11B1 cosegregates with both the pattern of mineralocorticoid synthesis and blood pressure in Dahl rats (19). The sequence of P450c11ß cDNAs from Dahl rats shows six nucleotide substitutions causing five amino acid changes (R127C, V351A, V381L, I384L, and V443M) in the Dahl R compared to normal control Sprague-Dawley or Dahl S rats (19, 59). Analysis of chimeric genes between P450c11B alleles from Dahl S and R rats expressed in COS cells indicates that the substitutions at amino acids 381 and 384 are the most important (59). However, these amino acid changes result in only a minimal reduction in the 18-hydroxylase activity

of P450c11B, which seems to be insufficient to explain the differences in steroid levels in these two strains; thus, it may be that these differences in CYP11B1 are not causal but instead are genetically linked to other causal loci.

SUMMARY AND FUTURE DIRECTIONS

Although most cases of hypertension remain idiopathic, the study of mineralocorticoid biosynthesis and action remains the most productive area of investigation into the basis of this disease. Studies of both human patients and hypertensive rats suggest that our understanding of this system remains incomplete. Future work integrating human epidemiologic studies, population genetics, molecular biology, and animal models should continue to advance our understanding of the regulation of blood pressure both in normal and in hypertensive subjects.

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