PRIMARY ALDOSTERONISM: CHANGING DEFINITIONS AND NEW CONCEPTS OF PHYSIOLOGY AND PATHOPHYSIOLOGY BOTH INSIDE AND OUTSIDE THE KIDNEY

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I. INTRODUCTION

The last two decades have seen a resurgence of clinical and research interest in primary aldosteronism (PA). This shows no sign of waning, stimulated by appreciation that 1) contrary to previous long-held belief, PA is not uncommon and is currently the most common specifically treatable and potentially curable cause of hypertension; 2) aldosterone excess has harmful cardiovascular, renal, central nervous, and psychological effects, that are at least partly independent of its effects on blood pressure, have had a profound influence on raising clinical and research interest in PA. Such research on patients with PA has, in turn, furthered knowledge regarding aldosterone synthesis, regulation, and effects. This review summarizes current progress in our understanding of the physiology of aldosterone, and towards defining the causes (including genetic bases), epidemiology, outcomes, and clinical approaches to diagnostic workup (including screening, diagnostic confirmation, and subtype differentiation) and treatment of PA.
II. SYNTHESIS AND REGULATION OF ALDOSTERONE PRODUCTION

A. Aldosterone Synthesis

Biosynthesis of aldosterone in humans appears to take place almost entirely in the zona glomerulosa (ZG), the outermost layer of the adrenal cortex, and involves the action of three cytochrome P-450 enzymes (heme-containing proteins that perform oxidative conversions by accepting electrons from NADPH) and one hydroxysteroid dehydrogenase (FIGURE 1). Like other steroid hormones, the primary precursor for synthesis is cholesterol. The initial and rate-limiting step involves the transport of cholesterol from the outer to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR, encoded by STAR) (23, 306). Evidence from recent studies in mice has raised the possibility that StAR works in conjunction with other proteins, including translocator protein (TSPO), to facilitate this shuffling process (141). At the inner mitochondrial membrane, cholesterol is converted to pregnenolone by the cholesterol side chain cleavage enzyme (P450scc, encoded by CYP11A1) (468). Pregnenolone then enters the endoplasmic reticulum (ER) where it is converted by 3β-hydroxysteroid dehydrogenase (encoded by HSD3B2) to progesterone (470). Also in the ER, progesterone undergoes hydroxylation by 21-hydroxylase (encoded by CYP21) to deoxycorticosterone. The final three steps of aldosterone biosynthesis, in which deoxycorticosterone progressively undergoes 11-hydroxylation, 18-hydroxylation, and 18-oxidation, occur in the mitochondria, and in humans are now thought to be mediated by a single enzyme, aldosterone synthase (encoded by CYP11B2) (222). Although earlier reports suggested that, in humans, the first of these three steps was likely to have been primarily the result of 11β-hydroxylase activity (391), later studies suggest that the gene encoding that enzyme (CYP11B1) is not expressed in ZG but instead is confined to zona fasciculata (ZF; the middle layer of the adrenal cortex) and zona reticularis (ZR; the innermost layer) (371). A similar pattern is seen in rats (380) but not in bovine adrenals, in which CYP11B2 expression appears to be absent, and CYP11B1 is responsible for aldosterone production.

FIGURE 1. Biosynthetic pathways of aldosterone and cortisol formation. Pathways occurring in zona glomerulosa (ZG) alone are shown in the blue shaded region, those in zona fasciculata (ZF) alone in the pink region, and those in both ZG and ZF in the purple region. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; STaR, steroidogenic acute regulatory protein; P450scc, cholesterol side chain cleavage enzyme; 3βHSD, 3β-hydroxysteroid dehydrogenase; 21OHase, 21-hydroxylase; aldo synthase, aldosterone synthase; 17OHase, 17α-hydroxylase; 11βOHase, 11β-hydroxylase. The genes encoding these enzymes are shown in parentheses.
in all three cortical zones (330). In rats and humans, expression of CYP11B2 occurs only in ZG, where it appears to be distributed diffusely in rats (380) but discontinuously (occurring in ZG cell clusters) in humans (371, 372).

Biosynthesis of cortisol also utilizes cholesterol as the primary precursor and shares with the aldosterone synthetic pathway its conversion to pregnenolone by P450scc. Unlike aldosterone synthesis, however, production of cortisol requires that pregnenolone, and/or its 3β-dehydrogenased product progesterone, undergo hydroxylation by 17α-hydroxylase (encoded by CYP17). 3β-Hydroxysteroid dehydrogenase then converts the 17-hydroxypregnenolone product to 17-hydroxyprogesterone, which subsequently undergoes conversion by 21-hydroxylase to 11-deoxycortisol. The final step is the hydroxylation of 11-deoxycortisol by 11β-hydroxylase to form cortisol.

In normal adrenals, biosynthesis of aldosterone occurs only in ZG and that of cortisol only in ZF, a phenomenon known as “functional zonation.” The underlying mechanisms are thought to include 1) confinement of CYP11B2 expression (required for aldosterone synthesis) to ZG (120, 380), 2) confinement of CYP17 (363) and CYP11B1 (371) expression (both required for cortisol synthesis) to ZF and ZR, and 3) lack of HSD3B2 expression (required for both aldosterone and cortisol synthesis) in ZR (371).

Regulators of aldosterone production can act acutely (within minutes) by affecting the activities of StAR and P450scc (the “early regulatory step”) or more chronically (within minutes) by affecting the activities of StAR and aldosterone synthase (the “late regulatory step”) (222). Chronic regulation may also occur by 11-deoxycortisol. The final step is the hydroxylation of 11-deoxycortisol by 11β-hydroxylase to form cortisol.

On the other hand, chronic stimulation of aldosterone production by ANG II results primarily from increased expression of aldosterone synthase and possibly of other enzymes involved late in aldosterone biosynthesis (2, 222, 526), but also possibly in part from ZG expansion via cell hypertrophy and hyperplasia (522). Chronic stimulation of the biosynthetic pathway by ANG II appears to involve similar second messengers to those involved in acute stimulation, particularly the PLC-mediated generation of IP3 and DAG (222). This results in increased intracellular calcium and CaMK activity and increased transcription of CYP11B2, mediated by increased expression of transcription factors including CREB and nuclear receptor related 1 protein encoded by NURR1, also known as NR4A2 (nuclear receptor subfamily 4, group A, member 2) (40, 41).

The formation of the active octapeptide ANG II follows 1) enzymatic cleavage predominantly in the liver by renin (produced mainly in the juxtaglomerular cells of the kidney) of the apparently inactive precursor protein angiotensinogen to produce the decapetide angiotensin I (ANG I, also inactive) and 2) the subsequent cleavage, this time mainly in the lungs, of ANG I to ANG II by angiotensin converting enzyme (ACE).

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**Table 1. Regulators of aldosterone synthesis**

<table>
<thead>
<tr>
<th>Stimulators</th>
<th>Inhibitors</th>
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<tr>
<td>Angiotensin II</td>
<td>Atrial natriuretic peptide</td>
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<tr>
<td>Potassium</td>
<td>Dopamine</td>
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<tr>
<td>Adrenocorticotropic*</td>
<td>Somatostatin</td>
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<tr>
<td>Serotonin</td>
<td>Estrogen (via ERβ receptor)</td>
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<td>Vasopressin</td>
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<td>Endothelin-1</td>
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<td>Estrogen (via GPER-1 receptor)</td>
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<td>Urotensin-II</td>
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<td>Parathyroid hormone</td>
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<td>Leptin</td>
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*Acutely only.
To understand factors that regulate aldosterone production via the renin-ANG II pathway, it is necessary to examine regulators of renin itself. Principal stimulators of renin release include 1) reduced sodium chloride delivery to the macula densa (specialized cells of the distal convoluted tubule close to the glomerulus) of the juxtaglomerular apparatus, 2) reduced perfusion pressure within the afferent arteriole of the glomerulus, and 3) sympathetic activation of juxtaglomerular cells via β-adrenergic receptors (153). Renin release is also under negative feedback regulation via ANG II, and its release is inhibited by atrial natriuretic peptide (ANP) (153). Situations commonly associated with enhanced renin production are therefore 1) sodium/volume depletion due to habitual or recent, significant dietary salt restriction, treatment with diuretics, gastrointestinal fluid losses and salt-wasting nephropathies; 2) renal artery stenosis and other forms of renovascular disease; 3) acute falls in blood pressure due to hemorrhage or vasodilatation; 4) activation of the sympathetic nervous system due to assumption of upright posture or stress; and 5) pharmacological blockade of the renin-ANG II system by ACE inhibitors or AT1 receptor blockers (ARBs), resulting in reduction of ANG II-associated negative feedback. The rise in renin would be expected to promote aldosterone production via increased ANG II in each of these situations except for the last (in which ZG cell AT1 receptor activation is reduced). Conversely, renin production is inhibited in states of excessive sodium retention and volume expansion including habitually high dietary salt consumption, acute sodium loading as in the aldosterone suppression tests for confirmation of PA (for example, intravenous saline suppression tests), excessive administration of intravenous fluids, PA itself, and renal conditions associated with abnormal retention of sodium including Liddle syndrome and familial hyperkalemic hypertension (Gordon syndrome), chronic kidney disease and aging, in which loss of functioning juxtaglomerular cells leads to reduced renin-producing capacity, and reduced sympathetic activation due to beta-adrenergic blockers or agents such as clonidine and α-methyldopa which inhibit central sympathetic output. Aldosterone production would be expected to fall due to reduced ANG II consequent on reduced renin in each of these situations with the exception of 1) PA, characterized by excessive adrenal aldosterone synthesis that occurs autonomously of renin/ANG II); 2) chronic kidney disease with a tendency to potassium retention and hyperkalemia, which will stimulate aldosterone synthesis; and 3) familial hyperkalemic hypertension, also associated with hyperkalemia (499).

The assumption of upright posture has a significant stimulatory effect on renin/ANG II and hence aldosterone (32, 48, 528). This occurs as a result of sequestration by gravity of 600-1,000 ml blood in the lower limbs, with the consequent reduction in venous return leading to sympathetic nervous system stimulation (to prevent blood pressure falling and syncope) of renin production. Reduced hepatic clearance of renin, as the liver shares in the generalized vasoconstriction, may also contribute to the rise in renin.
Because renin secretion peaks at around 0200-0800 h as part of a diurnal rhythm which is evident in recumbent subjects (211), the postural stimulus is more marked at this time of day and hence best assessed by comparing overnight recumbent renin and aldosterone levels at 0700-0800 h with upright levels after 2-3 h standing (198, 491, 503, 528).

Renin is also expressed in tissues other than the kidney, including the adrenal gland, and adrenal renin expression can be enhanced under experimental pathological conditions, including in nephrectomized rats (397) and CYP11B2 knockout mice (276). Markedly increased intra-adrenal expression of renin has also been observed in Task 3 potassium channel knockout mice and may at least partly explain the severe hyperaldosteronism associated with this model (34).

C. Regulation by Plasma Potassium

Increased aldosterone synthesis in response to increases in plasma potassium is thought to be mediated, like ANG II, via cellular calcium influx (FIGURE 2) (266, 477). Rises in potassium cause depolarization of the ZG cell membrane, resulting in activation of T- and L-type calcium channels. The rise in intracellular calcium and consequent activation of CaMKs leads to both acute (via increased StAR activity) and chronic (via increased expression of CYP11B2) stimulation of aldosterone synthesis (40, 222, 526).

Just as rises in plasma potassium can stimulate aldosterone production, so can falls to hypokalemic values reduce aldosterone production. Patients with PA, in which aldosterone production is relatively autonomous to renin/ANG II, may retain normal responsiveness to other regulators including potassium. This has the potential to result in false-negative screening and confirmatory tests for PA, as both rely on measurement of aldosterone (as a ratio to renin for screening, and following sodium loading for confirmatory testing). Hence, where possible, plasma potassium levels should be normalized by oral slow-release KCl supplements before screening is performed and by the time of completion of confirmatory testing (198, 491, 503). In addition to differences in the degree of autonomy to potassium versus ANG II, rises in aldosterone following potassium supplementation in patients with PA may result, at least in part, in stimulation of the contralateral gland in patients with APA, direct stimulatory effects on renin-producing cells (538), and reduced sodium resorption via potassium-dependent inhibition of the sodium chloride cotransporter (NCC) in the distal convoluted tubule of the kidney (519).

D. Regulation by ACTH

ACTH can stimulate aldosterone production acutely but, unlike renin/ANG II and potassium, not chronically (222). Acute stimulation involves the binding of ACTH to its receptor, causing activation of adenylate cyclase and consequent production of cAMP. This in turn increases the activity of protein kinase A (PKA) which increases both expression (via CREB family transcription factors) and activation (through phosphorylation) of StAR (46, 305). ACTH-induced activation of PKA also contributes to acute aldosterone production by increasing calcium influx (170, 461).

Under normal circumstances, ACTH secretion demonstrates a diurnal rhythm, peaking between 0500 and 0900 h and falling thereafter (368). Hence, dynamic studies assessing aldosterone responses to maneuvers such as posture stimulation or intravenous saline suppression are unlikely to be confounded by rises in ACTH provided they are conducted after this peak has occurred. Because ACTH rises in response to stress, however, aldosterone levels may rise transiently in association with apprehension, discomfort, or other stressful stimuli, and this may contribute towards an apparent response to upright posture, or prevent suppression during saline loading. Stress-induced rises in ACTH can cause aldosterone to rise at the start of a procedure such as adrenal venous sampling (AVS), only to fall into a trough during sample collection (463). This may explain some of the occasional instances where the AVS study is unsatisfactory because of the apparent lack of secretion by either gland at the time of sampling (571). Another potential contributing factor to this phenomenon is the pulsatile nature of ACTH secretion, resulting in alternating peaks and troughs of both cortisol and aldosterone. Measures that can be employed to combat these effects of variable ACTH secretion include 1) performing AVS in the early morning hours when ACTH levels are at their highest; 2) in addition to careful explanation of the procedure by the treating physician, employing a clinical psychologist (where feasible) to teach patients relaxation before their procedure; and 3) performing AVS under ACTH stimulation (following bolus injection or during intravenous infusion of synacthen) (198, 432, 499, 587).

While a transient increase in gene expression of CYP11B2 is seen in the first few hours of chronic ACTH exposure in isolated rat cells, this is subsequently replaced by repression (235). Similarly, in humans, low-dose infusion of ACTH causes an initial rise in plasma aldosterone during the first 24–36 h, followed by a fall over subsequent days to below baseline values (154). The mechanisms behind this inability of ACTH to induce chronic stimulation of aldosterone secretion by ZG cells remain poorly understood.

E. Other Regulators

Renin/ANG II, potassium and ACTH are the “major” regulators of aldosterone, but significant but relatively minor effects have been documented for a number of other substances.
1. ANP

In addition to inducing natriuresis, diuresis, extravascular volume shift, and vasorelaxation and directly inhibiting renin release by juxtaglomerular cells, ANP has direct inhibitory effects on both basal and stimulated (by ANG II, potassium, or ACTH) aldosterone secretion (57, 65, 245, 311, 365). These effects appear to be mediated via the ANP-A receptor on ZG cells and include 1) activation of the large-conductance, calcium- and voltage-activated maxi-K potassium channel which stabilizes the membrane potential (preventing depolarization) (178); 2) a direct effect on voltage-gated T-type calcium channels leading to a reduction in calcium conductance (38); and 3) a direct inhibitory effect on StAR expression (93) and/or phosphorylation (65).

Through these mechanisms, ANP has counterregulatory effects to aldosterone on blood volume and blood pressure. As PA is a volume-expanded, hypertensive state and ANP is secreted in response to atrial stretch and raised systemic blood pressure, it is perhaps not surprising that levels of ANP are raised in PA and fall in response to unilateral adrenalectomy or during spironolactone therapy (91, 124, 295). The evidence that this leads to a reduction in aldosterone secretion in PA is not convincing, however, and if ANP does not achieve the degree to which volume expansion and hypertension occur in PA, it may be predominantly through one of its other (renal and/or vascular) actions.

2. Serotonin

It is well established that serotonin (5-hydroxytryptamine) acutely stimulates synthesis of aldosterone by ZG cells, an effect that has been demonstrated both in vitro [using preparations of ZG cells removed from both animals (313, 355, 422, 514) and humans (414)] and in vivo (310). Stimulation of aldosterone secretion in humans appears to be via the serotonin 5-HT4 receptor (279), and is not dependent on ACTH, which was suppressed by dexamethasone administration during these studies (279). The action appears to involve stimulation of cAMP production (157, 422) as well as influx of calcium into ZG cells (but not mobilization of intracellular stores) (109, 176, 422). The demonstration of serotonin-containing mast cells in the human adrenal cortex (278) and of 5-HT4 receptor expression in human ZG and ZF cells (78) has raised the possibility that serotonin may regulate aldosterone secretion in a paracrine fashion. Interestingly, 5-HT4 mRNA expression was found to be upregulated in aldosterone-producing adenoma (APA) tissue (78), and serotonin detected in both clusters of steroidogenic cells within APAs and mast cells (sometimes hyperplastic) near or within some of these tumors (125), suggesting a possible pathogenetic role for serotonin in some states of excessive aldosterone production.

3. Dopamine

Dopamine exerts a tonic inhibitory action on aldosterone secretion via type 2 dopamine (DA2) receptors (4, 73, 323, 329, 375) which are present in ZG cells (410, 577). The fact that DA2 receptor antagonists (most commonly metoclopramide) induce a brisk rise in plasma aldosterone, but administration of dopamine or specific DA2 agonists (such as bromocriptine) usually fail to suppress aldosterone under basal, sodium replete conditions, suggests that this tonic inhibition is virtually complete in the basal, unstimulated state (70, 72, 375). In addition, dopamine appears to selectively oppose stimulation of aldosterone secretion induced by ANG II as evidenced by its ability to blunt the rise in plasma aldosterone in response to ANG II infusion (71, 100, 476, 560), assumption of upright posture (302, 560) or dietary sodium depletion (100, 124, 295) but not by ACTH (71, 100, 476, 560) or K+ (569). Recent studies performed by Chang and co-workers have demonstrated that APAs express lower levels of DA2 receptor mRNA compared with normal adrenocortical tissue (87) and also that dopamine appears to have antiproliferative effects on adrenocortical tissues (88). These observations raise the possibility that reduced inhibition of aldosterone secretion and cell growth by dopamine contributes to the excessive production of aldosterone by, and the development of, these tumors (88). Low tumor DA2 expression, however, would not explain why patients with APA have been reported to demonstrate brisk and sometimes even exaggerated responses of plasma aldosterone in response to metoclopramide (332, 364, 570). Adrenal cortical cellular responses to dopamine that may contribute to its inhibitory actions on aldosterone production include blockade of T-type calcium channels (389) and reductions in PKC isoforms (89), intracellular calcium (89, 169), and cAMP (329).

4. Estrogen

In addition to acting via the classical nuclear ERα and ERβ receptors, estrogen can also exert actions through a much more recently described, G protein-coupled, seven-transmembrane receptor, GPER-1 (previously known as GPR30) (149). This receptor is strongly expressed in ZG and adrenal medulla, but not in ZF or ZR (35). In normal adult human adrenal cortex and in APAs, both ERβ (74, 111) and GPER-1 (74) are much more abundantly expressed than ERα. In a series of elegant studies, Caroccia et al. (74) recently demonstrated that, in both normal ZG cells and APAs, estrogen has differential effects on aldosterone production depending on which receptor it activates, exerting a tonic inhibitory effect through ERβ and a stimulatory effect through GPER-1 which is “unmasked” when ERβ is blocked (74). The GPER-1-mediated stimulation of aldosterone production was associated with upregulation of CYP11B2, and appeared to be PKA/cAMP dependent (but did not involve a rise in intracellular calcium) (74).
5. Endothelin-1

At least in vitro, endothelin-1 (ET-1) stimulates aldosterone production by adrenocortical cells (104, 229, 428, 429, 591) via ET\textsubscript{A} and ET\textsubscript{B} receptors (429), the genes for both of which (along with ET-1 itself) are expressed in ZG cells (427, 431). Rises in intracellular calcium and of CYP11B2 expression have been reported to accompany the increase in aldosterone secretion (428). ET-1 also potentiates the actions of ANG II (103) and ACTH (425) on aldosterone production in vitro. The relevance of these effects in vivo and in patients with PA is less clear, however. In a study involving healthy control subjects, Vierhapper et al. (545) reported ET-1 infusion to have a potentiating effect on ACTH-stimulated plasma aldosterone levels but no effect on unstimulated levels. Veglio et al. (540) found no difference in ET-1 levels in patients with PA versus normal controls. Morello et al. (341) reported a lack of lateralization of cortisol-corrected ET-1 during AVS in patients with APA, and did not find components of the ET system to be upregulated in microarrays of APA tissue. While the genes encoding ET-1, ET\textsubscript{A}, and ET\textsubscript{B} appear to be expressed in APA tissue (126), studies assessing the ability of ET-1 to stimulate aldosterone production by APA cells in vitro have yielded inconsistent results, with one reporting an effect equipotent to ANG II (431) while another found no effect (591). ET-1 may have growth-promoting actions on ZG cells, being reported in one study to stimulate the proliferation of rat adrenal ZG cells, acting through ET\textsubscript{A} receptors coupled with protein kinase C- and tyrosine kinase-dependent signaling pathways (319). ET-1-(1–31), a product of an alternate chymase reaction on big-ET (the precursor of ET-1), is also produced in the adrenal and stimulates aldosterone production in vitro but appears to be less potent than ET-1 itself, possibly because it acts only via ET\textsubscript{A} (and not ET\textsubscript{B}) (430).

6. Vasopressin

Vasopressin has been shown to stimulate aldosterone production both in vivo in rats (392) and in vitro using animal (392, 574) and human (218) ZG cell preparations. This effect is mediated via the V\textsubscript{1} subtype of vasopressin receptors (168, 395) coupled to PLC, and involving phosphoinositide metabolism, intracellular calcium mobilization, calcium influx, and PKC translocation (171). Mitotic effects on ZG cells have also been reported (102, 392). Vasopressin has been detected in adrenal chromaffin cells and cortical cells (395), and in cells within APAs (some of which express V\textsubscript{1} mRNA) (274, 395), raising the possibility of a paracrine effect on aldosterone secretion. Patients with PA were recently reported to have higher plasma levels of vasopressin (measured as the COOH-terminal portion of the vasopressin pro-peptide) compared with healthy controls and patients with essential hypertension (373). These workers also described a downward shift in the relationship between urinary osmolality and plasma peptide concentration in patients with PA, suggesting a state of renal vasopressin resistance [possibly in part due to potassium depletion (417)] which may underlie the increased plasma levels (373).

7. Urotensin-II

Urotensin-II (UII), a vasoactive peptide which binds to a specific G protein-coupled UII receptor (UT-R), stimulates production of aldosterone and expression of CYP11B2 in ZG cells when chronically infused into rats (184). These workers reported UII to be overexpressed in pheochromocytoma but underexpressed in APA compared with normal human adrenal medulla and cortex (which showed similar expression) while the converse was true for UT-R (184). Morimoto et al. (343) reported somewhat different results, with UII strongly expressed in pheochromocytoma, medulla, and APA but only weakly in cortex, while UT-R was clearly expressed in all four tissue types (343). The clinical significance of these findings is unclear, but it has been suggested (184) that excessive production of UII by some pheochromocytomas and the consequent stimulation of aldosterone production by adjacent cortical tissue may underlie the coexistence, reported by several groups, of pheochromocytoma with PA (199, 515).

8. Somatostatin

In murine studies, somatostatin has been reported to inhibit basal and stimulated (by ANG II, ACTH, or K\textsuperscript{+}) aldosterone production both in vitro and in vivo (5, 53, 223, 318, 415, 421) via somatostatin receptors that are present in ZG cells (6). Chronic administration also inhibits ZG cellular growth (415, 421). In humans, somatostatin appears to inhibit aldosterone production stimulation by ANG II (251) but not by ACTH (246). All five known human somatostatin receptor subtypes are expressed in normal adrenal cortex and in APAs (312, 532). Exposure of adrenal cortical cells to a somatostatin analog (SOM230) was associated with a reduction in steroid hormone production but not cell viability (312), suggesting a potential therapeutic role in adrenocortical hormone excess states such as PA.

9. Parathyroid hormone

Experimental and clinical data support the concept that parathyroid hormone (PTH) stimulates adrenal aldosterone synthesis (61, 244, 317, 384). Mechanistically, this may involve both direct effects of PTH acting via PTH receptors [which have been identified in adrenal cortex (535)] within ZG cells and leading to rises in cAMP, IP\textsubscript{3}, and cytosolic calcium (317), and a secondary response to PTH-induced stimulation of renin production by JG cells. Clinically, aldosterone and renin concentrations have been reported to be higher in patients with primary hyperparathyroidism and to fall following parathyroidectomy (61, 267), and...
both aldosterone (240) and renin (214) levels appear to rise in response to PTH infusion. In patients with PA, PTH infusion was associated with an exaggeration in the rise of plasma aldosterone during ANG II infusion, suggesting that PTH sensitizes the adrenal to stimulation by ANG II (137). There is also evidence to suggest that aldosterone may induce PTH secretion. This may help to explain why patients with PA have been reported to have higher levels of PTH than those with essential hypertension, and that levels fall following either surgical correction of PA by unilateral adrenalectomy or during treatment with an MR antagonist (400, 426). These observations have led to the suggestion that PTH-induced rises in aldosterone may contribute to the excess in cardiovascular morbidity that has been reported among patients with primary hyperparathyroidism, and that, conversely, aldosterone-induced rises in PTH may do likewise for patients with PA (523).

10. Leptin

Huby et al. (239) recently reported coexpression of CYP11B2 and leptin receptors in human adrenal cross-sections and adrenocortical cells. In vivo murine studies demonstrated that increases in leptin (either endogenous in obese rats or by infusion) dose-dependently raised CYP11B2 expression and aldosterone without elevating plasma ANG II, potassium, or corticosterone, and this was not prevented by blockers of receptors for ANG II or α- or β-adrenergic receptors. Leptin increased intracellular calcium and elevated calmodulin and calmodulin-kinase II expression in adrenocortical cells. Furthermore, mineralocorticoid receptor (MR) blockade blunted leptin-induced endothelial dysfunction and increases in cardiac fibrotic markers, suggesting that leptin-induced aldosterone secretion may play a role in causing cardiovascular disease in some (for example, obese) subjects (239).

11. Aberrant adrenocortical G protein-coupled receptor expression as a potential mechanism for abnormal regulation and excessive, renin/ANG II-independent aldosterone synthesis

Several studies have raised the possibility that, at least in part in a minority of patients with PA, excessive aldosterone production could result from abnormal expression of G protein-coupled receptors in aldosterone-secreting cells. In almost all cases, this has appeared to involve increased expression of receptors normally found in the adrenal (eutopic receptors) rather than expression of receptors not normally expressed in the adrenal (ectopic receptors) (320). Examples of the former (some of which have been discussed earlier in this section) include receptors for ACTH (24), serotonin (277), somatostatin (312), vasopressin (395), glucose-dependent insulinotropic peptide (GIP) (274), gonadotropin releasing and luteinizing hormones (320), and thyroid stimulating hormone (596).

12. Circulating autoantibodies in PA

At least two groups have reported on the presence and potential pathophysiological role of activating autoantibodies to the AT1R receptor (AT1AA) in patients with PA. Rossitto et al. (444) found higher AT1AA titers in patients with APA versus those with BAH or essential hypertension. Furthermore, plasma aldosterone levels fell to a greater degree after captopril administration in AT1AA-positive compared with AT1AA-negative PA patients, suggesting a functional role in terms of promoting aldosterone secretion. Li et al. (285) detected AT1AA by an AT1 receptor-transfected cell-based bioassay in 9 of 12 patients with BAH and 6 of 13 with APA but in none of 15 normotensive control subjects (285). In vitro studies performed by this group showed that the AT1AA directly stimulated basal and enhanced ANG II-induced aldosterone production by cultured adrenal cells in an AT1 receptor-dependent manner (as evidenced by blockade of these effects with candesartan) (259). Further replication of these observations and additional studies confirming a pathological role of these autoantibodies in PA are awaited with interest.

III. ACTIONS OF ALDOSTERONE

A. Actions on Epithelial Tissues

Aldosterone is the main mineralocorticoid hormone in humans (216). The classical action of aldosterone is stimulation of sodium retention in the transporting epithelia particularly the distal nephron, but also in the distal colon and sweat glands. Sodium retention is associated with increased secretion of potassium and hydrogen ions (158). Hence, the principal functions of aldosterone are regulation of extracellular volume and blood pressure and control of potassium homeostasis (118). These classical effects of aldosterone are mediated by the MR, located in the cytosol of the cells responsible for sodium reabsorption and potassium secretion (101, 216), and are termed “genomic” in that they involve the effects of ligand bound MR on transcription of genes within these cells. In the distal nephron, the apical (luminal facing) membranes of these cells contain the amiloride-sensitive epithelial sodium channels (ENaC) (179) which mediate sodium reabsorption across the apical membrane from urine to the cytosol (101). Transport of sodium from cells to blood across the basolateral membrane is an active process driven by the Na⁺-K⁺-ATPase pump. In exchange for this resorbed sodium, Na⁺-K⁺-ATPase pumps potassium into the cell from the blood and, driven by a lumen-negative electrochemical gradient created by sodium resorption through ENaC, this then deports the cell and enters the tubular lumen through luminal K⁺ channels (101, 423). ENaC is made up of three subunits: α, β, and γ. The availability of α-subunits dictates the level of ENaC activity in the distal nephron, and the expression of
this subunit is under the transcriptional control of the MR (521). The amount of ENaC at the plasma membrane is reduced by Nedd4-2 (neuronal precursors cells-expressed developmentally downregulated protein 4-2)-dependent ubiquitylation, which designates ENaC for degradation. Aldosterone enhances the membrane abundance of ENaC by increasing expression of serum glucocorticoid kinase (sgk) which phosphorylates Nedd4-2 and thereby prevents Nedd4-2 from interacting with ENaC (292). Aldosterone also increases the total numbers of the channels by increasing expression of the α-subunit of ENaC (101, 293, 483, 521).

Although the major tubular site of aldosterone action is ENaC in the collecting duct, it also has a minor action in the distal convoluted tubule where it increases the expression of the thiazide sensitive Na–Cl cotransporter (264). In states of potassium excess (for example, a high dietary potassium intake), when not accompanied by volume depletion, aldosterone release (mediated at least in part by sgk) also leads to increased expression of the renal outer medullary potassium channel (ROMK) at the apical surfaces of distal convoluted and collecting duct cells, thereby enhancing tubular capacity to excrete potassium (555).

Although MR has similar binding affinity for (and can be activated by both) aldosterone and cortisol, inactivation of cortisol to cortisone by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) confers relative specificity of the MR for aldosterone within target cells (480) and thereby protects against the development of a mineralocorticoid excess state that would otherwise occur, given that plasma concentrations of cortisol are much (~1,000-fold) greater than those of aldosterone.

**B. Nonepithelial and Nongenomic Actions**

In addition to epithelial cells, the MR has been identified in a wide variety of nonepithelial tissues including endothelial cells, vascular smooth muscle, macrophages, adipocytes, cardiomyocytes, and the hippocampus of the brain (69, 112, 164, 296, 321, 325, 393, 516, 583). Experimental studies have demonstrated that activation of MR in at least some of these tissues (especially vascular and brain) appears to promote elevation of blood pressure (by increasing vascular reactivity via a combination of endothelial and direct smooth muscle effects, and by increasing sympathetic nervous system output, respectively) (381, 516). As will be discussed in a subsequent section, aldosterone excess brings about cardiovascular damage (inflammation, fibrosis and remodelling) through direct mechanisms that are at least in part independent of its effects on blood pressure. With the exception of blood vessels, all the above listed nonepithelial tissues found to express MR (that is, macrophages, adipocytes, cardiomyocytes, and hippocampal cells) lack 11βHSD2 (161, 224). Because of this, and given the much higher concentrations of cortisol than aldosterone that exist in plasma and locally in these tissues, it has been argued that MR are likely to be almost completely occupied by cortisol in those tissues than by aldosterone (159). While under basal conditions cortisol is thought to have a tonic inhibitory effect on MR activity in cardiomyocytes, experimental evidence suggests that it acts as an MR agonist under conditions of inflammation and hypoxia with reactive oxygen species generation, driven by the consequent change in intracellular redox, and leading to further myocardial injury (161, 327). MR within cardiac endothelial cells and macrophages are also thought to play a role in mediating cardiac inflammation and damage that is associated with MR activation (584).

Nongenomic effects of aldosterone in cardiovascular and renal tissues have also been reported. These effects are not mediated by gene expression but appear to involve activation of different signaling pathways including PKC, phosphoinositide 3-kinase, and c-Src and of transporters including the Na+/H+ exchanger, the Na-K-2Cl cotransporter, and a Mg2+ transporter transient receptor potential melastatin 7 cation channel (TRPM7) (216, 582, 593). The nongenomic effects are thought to be mediated by interaction with MR or other receptors (including G protein-coupled estrogen receptor 1, GPER1) which are located in the cell membrane (unlike the classical cytosolic/nuclear receptors) (101). Unlike the genomic actions, the nongenomic actions are rapid, insensitive to transcription and translation inhibitors, and insensitive to classical receptor inhibitors (595).

**IV. EFFECTS OF ALDOSTERONE EXCESS**

**A. Effects Via Actions on Distal Renal Tubular Mineralocorticoid Receptors**

Aldosterone production that is excessive for the body’s prevailing sodium/volume status leads to excessive distal tubular resorption of sodium and volume expansion, eventually resulting in hypertension. In PA, a condition first described by Jerome Conn in the mid 1950s (98) in which excessive adrenal aldosterone production occurs autonomously of the renin/ANG II system, the increase in sodium chloride delivery to the macula densa and rise in systemic blood pressure sensed by the specialized afferent arteriolar cells of the juxtaglomerular apparatus can result in profound chronic suppression of renal renin secretion (97). Sodium retention in the distal tubule is associated with urinary potassium loss and this, if sufficiently severe and prolonged, can result in hypokalemia (96). As well, metabolic alkalosis can result because the increase in sodium resorption is also accompanied by an increase in urinary excretion of hydrogen ions (96).
B. Effects Via Actions on Mineralocorticoid Receptors in Other Tissues

In addition to effects resulting from excessive distal renal tubular sodium/volume retention and potassium loss, it has become apparent in more recent years that aldosterone excess has adverse effects resulting from excessive MR activation in other tissues (FIGURE 3).

1. Pro-hypertensive effects on vascular and neural tissues

Evidence that aldosterone excess can induce endothelial dysfunction and vascular remodeling (58, 506), enhance sympathetic outflow (238, 506), and impair baroreflex function (333, 581) has led to the concept that these may be additional mechanisms by which hypertension develops in PA. In rodent models at least, there is evidence to suggest that activation of brain MR may even be more important than distal renal MR in the development of mineralocorticoid-induced hypertension (194, 195), although questions remain as to whether, in the absence of 11β-HSD2 in most brain regions, aldosterone is able to gain access to and activate the MR (which is thought to be primarily occupied by corticosterone).

2. Adverse cardiovascular effects

In addition to causing hypertension, aldosterone excess in PA induces injury (inflammation, remodeling, and fibrosis) in cardiovascular tissues in ways that are at least partly independent of its effects on blood pressure. First demonstrated in rodent models (553), this has now been confirmed in humans by cross-sectional studies assessing markers of cardiovascular disease in PA. Hence, compared with essential hypertensives matched for blood pressure levels, patients with PA have exhibited increased left ventricular (LV) dimensions (403, 438) and myocardial backscatter (268, 440) (as a marker of fibrosis) on echocardiography, increased carotid intima-media thickness (44, 233) and femoral pulse wave velocity (44), and, most importantly, increased rates of cardiovascular events including arrhythmias, myocardial infarctions, strokes, and mortality (82, 328, 349, 416, 453).

The existence of a familial variety of PA with a known and readily detectable genetic mutation has provided a unique opportunity to examine the concept of blood pressure-independent effects of aldosterone excess. In familial hyperaldosteronism type I (FH-I, otherwise known as glucocorticoid-remediable aldosteronism), inheritance of a CYP11B1/CYP11B2 “hybrid gene” mutation leads to PA and, eventually in most individuals, hypertension of variable severity (287, 419, 489). With the screening of members of affected families, it has been possible to detect individuals with PA (confirmed biochemically) before the onset of hypertension, and hence to assess effects of aldosterone excess without the confounding effects of blood pressure elevation. Echocardiographic studies in eight such normotensive individuals with FH-I demonstrated increased LV wall thicknesses and reduced diastolic function compared with 24 age- and sex-matched normotensive controls with similar 24-h ambulatory blood pressure levels (502). These individuals also had higher serum levels of interleukin-6, which supported the notion that inflammation is a key component of the cardiovascular damage that occurs with aldosterone excess (479).
3. Adverse renal effects

In addition to cardiovascular effects, experimental and clinical data suggest that aldosterone excess also leads to renal damage, again through mechanisms that are at least partly independent of hypertension. In rat models, mineralocorticoid excess leads to renal vascular and tubular inflammation and fibrosis, and this is preventable by administration of an MR antagonist (50). Patients with PA have higher urinary albumin excretion than matched essential hypertensives (220, 436, 464). This has been reported to occur in association with higher creatinine clearance and sonographic evidence of decreased intrarenal vascular resistance (464), suggesting that the albuminuria at least partly reflected a state of glomerular hyperfiltration resulting from aldosterone-induced sodium retention and volume expansion. This was supported by the observation that all findings were reversed by unilateral adrenalectomy or spironolactone treatment (418, 464).

Evidence for an association between PA and various components of the so-called “metabolic syndrome” has been reported and could help to explain at least some of the excess cardiovascular morbidity that has been observed. Positive correlations between aldosterone levels and body mass index have been reported for both normotensive (43) and hypertensive (434) individuals. Fallo and co-workers, having previously reported metabolic syndrome (and in particular hyperglycemia) to be more prevalent among patients with PA than in essential hypertensives (139), more recently found both higher levels of insulin resistance, defined by the homeostasis model assessment index, and lower adiponectin levels among 40 patients with PA than in a similar number of matched subjects with low-renin “essential” hypertension (135). Giacchetti et al. (182) observed resolution of gluco-metabolic abnormalities in patients with APA post adrenalectomy. Both direct effects of aldosterone (acting possibly by interfering with insulin receptor function or by other means) and effects of hypokalemia have been suggested as potential pathogenetic mechanisms for these apparent associations. In contrast to these findings, Matrozoiv et al. (314) found no differences in fasting blood levels of glucose or lipids, nor in the prevalence of diabetes mellitus in a retrospective comparison of patients with PA and essential hypertensives.

4. Effects of specific treatment against aldosterone excess

If aldosterone excess in PA is associated with organ damage and morbidity that is excessive for the degree of hypertension, does specific treatment against excessive aldosterone action provide more benefit than just lowering blood pressure? Put another way, is nonspecific antihypertensive treatment good enough to provide optimal organ protection in these patients, or should we be addressing the other effects of aldosterone as well? An additional, related question is whether very early PA (detected, for example, during screening among pedigrees with familial forms) should be treated even before hypertension has developed. Available evidence indicates that specific surgical or medical treatment of PA is substantially superior to nonspecific antihypertensive medication in terms of clinical outcomes, with some data to suggest that surgery is more effective than medical MR blockade. In a study reported by Tsuchiya et al. (527), both unilateral adrenalectomy and spironolactone treatment led to improvements in endothelial function in patients with PA (which was worse than in essential hypertensives despite similar blood pressure levels on nonspecific antihypertensives). Strauch et al. (504) reported unilateral adrenalectomy but not spironolactone to result in reduced arterial stiffness (measured as carotid-femoral pulse wave velocity and augmentation index) in PA patients studied ~1 yr following surgery or commencement of medical treatment. Catena et al. (81) similarly found surgery but not spironolactone to result in significant reduction in left ventricular mass after one year. However, following that time point, changes were greater in the medically treated group so that by a mean follow-up period of 6.4 yr, the overall reduction from baseline was comparable in the two groups. Whether this apparent “lag” in effect of medical treatment is associated with clinical important differences in cardiovascular outcomes is uncertain. Importantly, the excess rate of cardiovascular events (see above) that was reported by this group in these same patients prior to treatment of PA was reversed following treatment, a finding which provides compelling support for early detection of individuals with PA who can then benefit from improved cardiovascular outcomes afforded by specific surgical and medical management (82).

5. Adverse psychological effects and impaired quality of life

PA brings about adverse psychological effects and is associated with reduced quality of life. PA has been associated with depression (28, 262, 303), anxiety (21, 270, 473, 474), and even episodic rage (which resolved after unilateral adrenalectomy for APA) (236). Patients with PA attending our centre frequently report lethargy, fatigue, reduced ability to concentrate, and proneness to feelings and demonstrations of anger. Resolution of these symptoms is frequently rapid and marked following adrenalectomy for unilateral PA, and slower and less complete following commencement of specific medical treatment. Sukor et al. (509) found quality of life, measured across eight domains by the Short Form (SF)-36 questionnaire (a 36-item patient-reported survey of patient health), to be reduced compared with general Australian population norms in 22 Australian patients with unilateral PA before, but not 3 and 6 mo after unilateral adrenalectomy. Ahmed et al. (9) repeated this study in 21 patients with bilateral PA treated medically with spironolactone and/or amiloride and found similar reductions in quality of life scores to those of patients with unilateral...
V. EPIDEMIOLOGY OF PA

Throughout the 1970s to early 1990s, PA was considered a rare cause of hypertension (accounting for <1% of patients) and not worth looking for unless patients were hypokalemic. This is despite the fact that as early as 1964, <10 years after first describing PA, Conn (97) recognized that patients with PA (including those with APA) need not be hypokalemic. By 1967, he was able to report that 14 of 45 patients diagnosed by his group as having PA and found to have adrenal cortical tumors at the time of surgery were normokalemic preoperatively (96). Thirteen (93%) of these normokalemic patients demonstrated either cure or improvement of hypertension postoperatively. These observations led Conn (96, 97) to propose that hypokalemic PA represents only the tip of the iceberg for this condition with existing diagnostic methods “only diagnosing those cases that finally arrive at this stage of the disease,” and that normokalemic PA may be a common cause of hypertension.

In 1981, Hiramatsu et al. (231) reported on the use of the plasma aldosterone-to-renin ratio (ARR) to detect and remove APAs from 9 (2.6%) of 348 “unselected” hypertensives. Only three of the nine were hypokalemic. It is very likely that the true prevalence of PA in this hypertensive cohort would have been much higher as only patients with very high ratios were selected for further investigation, and only adrenal venography and scintigraphy (which would have missed most APAs <1 cm and those patients with bilateral PA) were used to confirm the diagnosis.

At the Greenslopes Hospital Hypertension Unit (GHHU), adoption by Gordon in the early 1990s of the policy to apply the ARR to all referred hypertensives, and not just those with hypokalemic or resistant hypertension, led to a 10-fold increase in the detection rate of PA [confirmed in each case by fludrocortisone suppression testing (FST)] from 5–10 to 50–90 per year (204, 207, 494). Only 21% of patients diagnosed since 1992 have been hypokalemic, the rest being normokalemic and thus masquerading as having “essential hypertension” (204, 207, 494).

How common is PA among the hypertensive population? In studies reported by the GHHU in the early 1990s, 6 (12%) of 52 respondents to advertisements seeking volunteers for antihypertensive drug trials and who were screened by ARR testing were found to have PA (confirmed by FST) (212), and PA was confirmed in 19 (8.5%) of 199 consecutively referred normokalemic hypertensive patients who underwent ARR testing (208). Although the patients in the studies were “selected” in that they had either selected themselves to volunteer for drug trial participation or had been referred to a hypertension unit, none was known to be hypokalemic or suspected of having PA at the time of presentation, and the majority were receiving no more than one antihypertensive agent.

Since these initial reports, many groups of investigators have similarly and independently reported evidence based on the results of ARR (and, where positive, confirmatory) testing of hypertensives to suggest that PA is common, and probably the commonest specifically treatable and sometimes curable cause of hypertension. Most have reported prevalence rates between 5 and 15%, with the majority of patients being normokalemic (142, 288, 294, 351, 370, 435, 589). In resistant hypertensive cohorts, the prevalence rates have been even higher and up to 20% (64, 128, 167, 505).

VI. ROLE OF GENETICS IN THE ETIOLOGY AND PATHOPHYSIOLOGY OF PA

Although the etiology of PA in most affected patients is currently unknown, considerable evidence exists to suggest a genetic basis in many, and possibly even the majority. This comes from studies assessing the nature of abnormal adrenal morphology in PA, the existence of familial forms (some with already identified underlying mutations), population genetics, and more recently the identification of somatic (within APAs) and germline mutations in genes encoding ion channels in patients with PA.

A. Evidence From Studies on Adrenal Morphology in PA

Traditionally, PA has tended to be divided into three main histopathological subtypes, namely, APA, bilateral adrenal hyperplasia (BAH), and the rare aldosterone-producing carcinoma. In reality, however, there exists a much more diverse array of abnormal adrenal morphology among patients with PA (206, 496). For example, in adrenals containing an APA, the remaining cortex frequently contains additional, smaller nodules. In the great majority of cases, the ZG paradoxically shows diffuse hyperplasia rather than atrophy that might be expected with chronically suppressed circulating renin/ANG II levels (366). In bilateral PA, the hyperplasia affecting each adrenal may be diffuse, mi-
cronodular, or macronodular. These findings have parallels with other syndromes of endocrine hyperfunction (including multiple endocrine neoplasia types 1 and 2) characterized by a generalized predisposition towards the development of diffuse and/or nodular hyperplasia and/or neoplasia within target endocrine tissues (for example, parathyroid glands, pancreas, pituitary, adrenal medulla, and medullary T cells of the thyroid), and for which genetic bases underlying such disturbances of cell growth regulation have been firmly established. As discussed in Section XIA, the application of newly available, highly specific antibodies for aldosterone synthase and 11β-hydroxylase in immunohistochemical studies of adrenal tissues removed from patients with PA has yielded observations that have further challenged traditional thinking regarding adrenal histopathology in this condition (183).

**B. Population Genetics**

Data from the Framingham Offspring Study have been highly relevant in further highlighting the expanding role of aldosterone excess in hypertension development and in demonstrating evidence for a genetic basis (367). Among normotensive individuals in the Framingham cohort, both a rise in blood pressure and the incident development of hypertension over 4 years of follow-up were positively correlated with aldosterone and the ARR and negatively correlated with renin, as would be expected if aldosterone secretion was autonomous. Not only did the ARR also show significant heritability ($h^2 = 0.34$) in this population, but a genome-wide linkage analysis of the ARR identified three loci with suggestive linkage, strongly supporting a genetic basis for some, if not all, forms of PA.

**C. Familial Hyperaldosteronism Type I**

Considerable new knowledge regarding the pathogenesis of PA, molecular basis for aldosterone regulation, and the nature and basis of phenotypic diversity among affected pedigrees has emerged from the study of familial forms of PA. The first to be described [by Sutherland et al. in 1966 (512)] was a glucocorticoid-remediable variety of PA inherited in an autosomal dominant fashion, now referred to as FH-I. The genetic basis for FH-I was elucidated in 1992 by Lifton et al. (286), representing the first time a form of human hypertension had been fully explained at the molecular level. The underlying defect is a “hybrid gene” mutation which is composed of 11β-hydroxylase gene (CYP11B1) sequences at its 5’ end and aldosterone synthase gene (CYP11B2) sequences at its 3’ end (286). Like CYP11B2, the “hybrid gene” encodes an enzyme that synthesizes aldosterone. However, unlike CYP11B1, the gene is regulated by ACTH and not by ANG II because its regulatory sequences are derived from CYP11B1, which is primarily ACTH (and not ANG II) responsive. Aldosterone production in FH-I is therefore regulated by ACTH rather than by ANG II (286). As a result, small doses of glucocorticoids (for example, dexamethasone 0.25 mg daily), by suppressing ACTH, suppress the expression of the hybrid gene, leading to resolution of aldosterone excess and hypertension.

Like CYP11B1 (but unlike CYP11B2), the “hybrid gene” is expressed in ZF (391). Hence, with regards to aldosterone synthase activity, normal adrenal functional zonation in FH-I is breached. One outcome of this is that cortisol, normally “protected” by this functional zonation against the effects of aldosterone synthase, is now exposed to its 18-hydroxylase and oxidase activities, leading to excessive production of 18-hydroxy- and 18-oxo-cortisol (“hybrid steroids”) in FH-I (533) (FIGURE 4).

Hypertension severity in FH-I varies greatly, even within the one family, with some remaining normotensive into adulthood while others may develop hypertension in early childhood and suffer such severe elevations in blood pressure as to succumb to early death from stroke (419, 489). No matter how severe the hypertension, however, control can be readily achieved by the administration of glucocorticoids in small, well-tolerated doses (485, 512). Such dramatic disease-specific treatment responses make accurate diagnosis of affected family members paramount. Elucidation of the genetic basis of FH-I has been a major step forward in this respect. Before this, diagnosis depended primarily on dexamethasone suppression testing, with affected patients typically demonstrating marked, sustained plasma aldosterone suppression (to <4 ng/100 ml and often to undetectable levels) during 4 days administration of dexamethasone (0.5 mg every 6 h) (354, 486, 512). While reasonably reliable, this was not infallible (354, 486, 512). Furthermore, this approach required multiple blood testing and was therefore relatively taxing, especially for young children. In contrast, genetic testing has proven highly reliable and required a single blood test which can be easily obtained for subjects of all ages (even at birth, by the collection of cord blood) (252, 489). Originally available as a Southern blot method (286), a polymerase chain reaction-based method later developed at GHU (252) is faster and highly reliable. Genetic testing allowed us to test over 200 members of a large Australian family, a task which would not have been feasible with dexamethasone suppression testing, and to identify 24 (including 3 newborns) as positive for the hybrid gene mutation (252, 489). Early identification of FH-I by genetic testing, including at prehypertensive stages during childhood and teenage, permits timely institution of treatment by targeting detected family members for regular blood pressure assessments from the point of detection. Given the critical role for salt in the development of both hypertension and non-blood pressure-dependent effects of aldosterone excess in PA (see sect. XIVF), it is also possible that early institution of dietary sodium restric-
tion in such individuals may help delay or even prevent the onset of these sequelae.

D. Familial Hyperaldosteronism Type II

In 1991 we first reported on the familial occurrence of PA that was not glucocorticoid-suppressible and not associated with the hybrid gene mutation, and subsequently labeled this familial hyperaldosteronism type II (FH-II) to differentiate it from the glucocorticoid-suppressible form. Additional families were subsequently also reported from other countries (140, 297). Our first report included 6 patients from 3 families while our current series stands at 115 patients from 47 families. Like FH-I, the phenotype in many of these families is transmitted in an autosomal dominant (“vertical”) fashion, with others having too few identified affected members to allow the mode of transmission to be clearly defined.

Clinical, biochemical, and morphological characteristics of patients with FH-II do not differ significantly from those with apparently non-familial PA (204, 493, 495). Among the patients identified by our Brisbane group, ages at presentation have ranged from 14 to 78 yr, and ~50% were female and 25% were hypokalemic. As with apparently sporadic PA, ~30% have undergone unilateral adrenalectomy (the great majority of these after lateralization on AVS). Two other patients have undergone bilateral adrenalectomy, one for bilateral giant macronodular hyperplasia causing autonomous adrenal aldosterone and cortisol production, and the other in another institution for severe, medically unresponsive, hypokalemic PA associated with bilateral macronodular hyperplasia. In the remaining 70% of patients (most with bilateral PA confirmed by AVS or in whom definitive AVS results are lacking), hypertension has been treated medically with agents that block aldosterone action.

Interestingly, affected members of the same pedigree may have differing forms of PA. For example, in one family, a 63-yr-old hypertensive, normokalemic father with a 0.5-cm right APA had a 37-yr-old hypokalemic, hypertensive daughter with a 2.2-cm right APA. Preoperatively, the father’s plasma aldosterone levels showed normal rises in re-

FIGURE 4. Biosynthetic pathways of aldosterone and cortisol formation in patients with familial hyperaldosteronism type I. The pink shading indicates that these pathways all occur in zona fasciculata (see FIGURE 1). OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; STaR, steroidogenic acute regulatory protein; P450scc, cholesterol side chain cleavage enzyme; 3βHSD, 3β-hydroxysteroid dehydrogenase; 21Oase, 21-hydroxylase; HG product, the hybrid gene product; 17Oase, 17α-hydroxylase; 11βOase, 11β-hydroxylase. The genes encoding these enzymes are shown in parentheses.
response to either the assumption of upright posture or an intravenous infusion of ANG II, consistent with the ANG II-responsive variety of APA, whereas the daughter’s APA was ANG II-unresponsive. While the father’s tumor was virtually entirely composed of ZG-like cells and hybrid cells (cells having morphological characteristics of both ZG and ZF), the daughter’s tumor was composed predominantly of ZF-like cells. This is consistent with our previously reported observations that ANG II-responsive APAs usually demonstrate a predominance (at least 80%) of non-ZF-like cells, while ANG II-unresponsive APAs tend to be composed mainly (at least 50%) of ZF-like cells (530) [finding which, however, was not confirmed in two other studies (133, 335)]. Subsequently, the daughter’s 46-yr-old hypertensive, normokalemic sister was diagnosed as having PA due to BAH. In our largest family of at least seven affected individuals and two others with “borderline PA” among five generations, all six who have undergone AVS showed bilateral adrenal aldosterone production.

Because screening (by ARR testing) of family members of patients found to have PA is rarely undertaken, the prevalence of FH-II has not yet been accurately defined. In the few studies that have examined this issue, FH-II does appear to be considerably more common (at least 7-fold in the Brisbane experience) than FH-I. From 1992 to 2004, of 912 patients diagnosed in Brisbane as having PA after screening all newly referred hypertensives by ARR testing and confirming the diagnosis by FST, 29 (3.2%) represented new families with FH-II (their affected relatives being subsequently identified) and only 4 (0.4%) were from new families with FH-I (despite all undergoing either dexamethasone suppression testing or genetic testing for the hybrid gene mutation). Mulatero et al. (353) assessed prevalence rates for familial PA among 300 patients diagnosed in their institution. Hybrid gene testing yielded only two (0.7%) with FH-I. Of the remaining 298, 12 had at least 1 affected relative, giving a prevalence of FH-II of at least 6% (almost 10 times that of FH-I) in that study. The prevalence may have been higher given that only 199 had relatives available for, and consenting to, biochemical screening and (where positive) confirmatory testing, and in 54 of these families a definite call on affection status could not be made, either because some hypertensive members did not undergo ARR testing or because of equivocal results in some who did (353).

Unlike in FH-I, the genetic defects underlying the great majority of patients with non-glucocorticoid-suppressible familial PA have not yet been elucidated. Early candidate gene approaches found no evidence for causative mutations in CYP11B2 (524), CYP11B1 (140), the ANG II type 1 receptor gene (AT1) (525), the p53 tumor suppressor gene (33), or the multiple endocrine neoplasia type 1 gene (MEN1) (524). Although linkage studies employing microsatellite markers appeared to implicate a locus at chromosome 7p22 with a highly significant combined logarithm of odds (LOD) score of 5.29 among 5 families (2 Australian, 2 Italian, and 1 South American) (471, 511), an extensive search in this region by both direct Sanger sequencing of multiple candidate genes (76, 131, 249, 472, 508) and next generation sequencing of the entire linked locus (unpublished observations) has so far failed to identify causative mutations. Because of this, the search has been widened and results of intensive whole exomic sequencing analyses (including our largest affected family) are awaited with great interest.

As described in section VI, E and F below, germline mutations in KCNJ5 (encoding a potassium channel) (90, 94, 337, 352, 459), CACNAID (456), and CACNAIH (460) (both encoding subunits of calcium channels) have been identified in small number of individuals (some with affected relatives) with early-onset PA. Those with KCNJ5 mutations have been referred to as having familial hyperaldosteronism type III (FH-III) (345), making it likely that a similar approach to terminology (FH-IV, FH-V, etc.) will be applicable as more new germline mutations are found. That being the case, the term FH-II might be best viewed as one of exclusion (that is, all forms of familial PA for which the underlying mutation has yet to be identified) and the proportion of familial PA that remains in this category will almost certainly wane as the molecular bases for an increasing number of pedigrees are identified.

E. Familial Hyperaldosteronism Type III

In 2008, Geller et al. (181) described an American family with an affected father and two daughters with PA which, like FH-II, was not glucocorticoid-suppressible and not associated with the hybrid gene mutation, but, unlike FH-II, was characterized by severe, childhood-onset hypertension and very florid PA, 18-hydroxy- and 18-oxo-cortisol levels which were markedly elevated (to a much greater degree than in FH-I), and resected adrenals showing marked, diffuse hyperplasia of ZF, with the combined adrenal weight in one daughter reaching 81 g (normal <12 g). The description of this family led Mulatero to coin the term familial hyperaldosteronism type III (FH-III) (345). As is discussed in the section VIF, the affected members of this family were later found by Choi et al. (94) to carry a germline mutation in KCNJ3, which encodes a potassium channel. Since that report, several other families with PA and germline KCNJ5 mutations have been reported, some of them demonstrating much milder phenotypes (see sect. VIF).

F. The “New” Genetics: Somatic and Germline Mutations in Genes Encoding Ion Channels and Pumps

The last few years have seen major developments in understanding of molecular mechanisms involved in the patho-
genesis of PA. In 2011, Choi et al. (94) reported somatic mutations (causing G151R and L168R amino acid substitutions) in KCNJ5, encoding an inwardly rectifying potassium channel (GIRK4 or Kir3.4), in 8 of 22 APAs removed from Swedish patients with apparently nonfamilial PA. In the same report, a third, germline mutation (causing T158A) in KCNJ5 was identified in the American family with FH-III described in section VI E and first reported by this group in 2008 (181).

Subsequent reports confirmed a high prevalence of APAs bearing somatic KCNJ5 mutations. Prevalence rates among Caucasian cohorts from Europe, the United States, and Australia have been remarkably consistent at ~40% (15, 30, 54, 94, 145, 282, 336, 565). For as yet uncertain reasons, however, studies performed in Japan, China, and Taiwan have yielded higher rates (65–80%) (336, 513, 550, 578, 593). The two somatic mutations originally described by Choi et al. (G151R and L168R) have since emerged as accounting for the great majority occurring among APA cohorts, and while several others (including T158A, dell157, and E145Q) have been reported, they appear to be much less common.

Compared with patients with APAs that lacked KCNJ5 mutations, those bearing mutations have tended to demonstrate a female predominance, younger age, somewhat more florid PA and larger mean tumor size, although these characteristics have not been invariable across different reported studies (15, 30, 54, 94, 145, 282, 336). These features, and the predilection for ZF and elevated levels of hybrid steroids observed in the American FH-III family (94, 181), are also shared by the ANG II-unresponsive (versus the ANG II-responsive) variety of APA (202, 203, 501, 530). It was therefore with considerable interest that in a group in 2008 (181). In normal adrenals, expression of KCNJ5 is restricted to ZG cells (94). Compared with cardiomyocytes, the physiological function of GIRK4 in the adrenal is poorly understood. Experimental studies suggest that these channels promote an outward current (thereby promoting more negative membrane potentials) under basal and stimulated conditions. Overexpression of wild-type KCNJ5 in human adrenocortical cells was reported by Oki et al. (382) to be associated with reduced (more negative) cell membrane potential, cytosolic Ca^{2+}, expression of StAR and CYP11B2, and synthesis of aldosterone both basally and in response to ANG II. Similarly, Williams et al. (334) reported smaller increases in cytosolic Ca^{2+} in response to high extracellular K^{+} in adrenal cells overexpressing KCNJ5 compared with from infancy to late teens), the severity of phenotype has otherwise shown considerable diversity. Interestingly, a striking genotype-phenotype correlation has been observed with subjects carrying T158A, I151S, and G151R tending to have more florid PA (often requiring bilateral adrenalectomy to control hypertension) and marked adrenal enlargement, and those with G151E mutations having milder PA (with hypertension control usually achievable with medical therapy including an MR antagonist) and adrenals that appeared normal on imaging studies (90, 94, 337, 352, 459). On the basis of this observation, Lenzini and Rossi (281) have proposed subclassifying FH-III into types A (more severe, resistant to medical treatment) and B (less severe, responsive to medical treatment).

Inwardly rectifying K^{+} channels are so named because they allow K^{+} to move more easily into than out of the cell. In cardiomyocyte cells, as with all inwardly rectifying potassium channels (227), GIRK4 channels (encoded by KCNJ5) can promote inward current (positive charge into the cell, which is the opposite of leak channels), but only do this when the resting membrane potential is more negative than the K^{+} equilibrium potential (EK). In this way, they correct hyperpolarization by allowing the flow of positively charged K^{+} ions into the cell, pushing the membrane potential back to the resting potential. Because this rarely occurs in vivo, an inward K^{+} current is usually only observed during electrophysiological studies when the membrane potential is clamped to very negative values. When the membrane potential is slightly more positive than the EK, GIRK channels show an outward current, and thereby contribute to the stabilization of the resting membrane potential. During an action potential (AP), the outward current progressively decreases because of the inward rectification, the channels close, prolonging the AP (227). Inwardly rectifying channels are composed of four subunits and can be homo- or heterotetrameric. GIRK4 predominantly forms heterotetrameric complexes with GIRK1 (encoded by KCNJ3) and more rarely with GIRK2 (KCNJ6) or GIRK3 (KCNJ9) (192, 227), and such heterodimerization is required for the channel to function normally.

In contrast to somatic mutations, germline KCNJ5 mutations have only rarely been reported and appear to be a rare cause of familial PA. We have not identified germline KCNJ5 mutations among any of our patients with FH-II (over 30 families so far studied), and Mulatero et al. (352) found only 1 of 21 pedigrees to be so affected. In addition to the original T158A mutation detected in the American family (94), several others (including G151E, G151R, I151S, and Y152C) have been identified (90, 337, 352, 458). While all but one (337) affected members of pedigrees so far described have shared an early onset of hypertension (ranging
nontransfected cells. Hence, it would appear that GIRK4 normally functions to stabilize the resting membrane potential in ZG cells (as in cardiomyocytes). Interestingly, however, in nontransfected human adrenal cells, administration of a GIRK inhibitor (tertapiin Q) had no effect on cell membrane current (263) and siRNA targeted KCNJ5 knockdown did not affect aldosterone production (382), suggesting that endogenously expressed KCNJ5 may be relatively inactive in normal ZG.

What are the functional effects of KCNJ5 mutations? In vitro studies have demonstrated KCNJ5 mutations reported in patients with PA to be associated with loss of selectivity of the mutated channels to potassium, allowing sodium conductance into the cell which predisposes to cell membrane depolarization. In turn, this leads to an influx of calcium, upregulation of genes (including CYP11B2 and two of its major transcriptional regulators, NR4A2 and NR4A3) involved in aldosterone synthesis, and ultimately an increase in aldosterone production (15, 29, 94, 336, 352, 383). How, and indeed whether, they lead to adrenal cell proliferation and tumor development remains less well understood and a fascinating area of ongoing research.

Surprisingly, the G151E mutated channels (inheritance of which leads to a relatively mild phenotype) were reported by Scholl et al. (459) to demonstrate markedly higher (that is, more abnormal) sodium conductance than G151R mutated channels. Furthermore, HEK 293T cells transfected with G151E mutated KCNJ5 demonstrated markedly reduced survival compared with cells with wild-type KCNJ5, whereas those with G151R mutated KCNJ5 showed only a modest reduction in survival. Survival was greatly enhanced by incubating in a low sodium medium, suggesting that the greater conductance of sodium was causal in conferring the greater cell lethality associated with the G151E mutation compared with G151R. The authors speculated that this increased lethality may explain the lack of adrenal hyperplasia occurring clinically in patients bearing germline G151E (versus G151R) mutations (459). Against this hypothesis, however, is the observation that patients bearing the Y152C germline KCNJ5 mutation also show a mild phenotype despite it having a relatively small effect on Na\(^+\) conductance (337).

Do somatic KCNJ5 mutations originate in ZG cells, ZF cells, or both? The occurrence of aldosterone secretion is clearly consistent with a ZG-like phenotype, yet APAs harboring KCNJ5 mutations are frequently predominantly composed of cells which histologically resemble ZF (16, 29, 113, 333, 457). Furthermore, the presence of very high levels of "hybrid steroids" in some patients with germline KCNJ5 mutations [whose adrenals have been reported to demonstrate marked hyperplasia of ZF (94, 181)] suggest the coexpression of CYP17 (permitting synthesis of cortisol and normally only expressed in ZF) and CYP11B2 (resulting in conversion of cortisol to 18-hydroxy- and 18-oxo-cortisol and normally only expressed in ZG) within the same cells. Nakamura et al. (359) demonstrated colocalization of CYB11B2 and CYP17 by immunohistochemistry in a mean of only 0.6% of APA cells among 27 APAs studied, but did not subdivide APAs according to KCNJ5 mutation status. If mutations arise in ZG, it is possible that they also lead to the acquisition of ZF-like phenotypic features, including ZF-like histological characteristics, derepression of CYP17, and loss of responsiveness of aldosterone to ANG II. Conversely, occurrence of somatic mutations in ZF cells may somehow lead to derepression of CYP11B2. A possible alternative explanation for the excessive production of "hybrid steroids" in some patients with FH-III is that the predominant ZF cells are interspersed with ZG cells, and that diffusion of excessive, locally produced cortisol from ZF into neighboring ZG cells allows its conversion to 18-hydroxy- and 18-oxo-cortisol. Indeed, the ability for either locally produced or even circulating cortisol to be converted into hybrid steroids has been demonstrated by Freel et al. (152). If this were the case in FH-III, however, markedly elevated peripheral levels of the substrate cortisol would be expected but were not observed in those patients (181). Further work in this fascinating area is awaited with interest.

Until recently, all reported KCNJ5 mutations were located within the selectivity filter of the GIRK4 channel. Kuppusamy et al. (272) described a novel somatic mutation (resulting in a T149 insertion) close to, but outside, the selectivity filter in an APA from a patient with resistant hypertension. When expressed in vitro, the mutation was associated with a strong Na\(^+\) inward current, a rise in intracellular Ca\(^2+\) (due to activation of voltage-gated Ca\(^2+\) channels and reduced elimination of Ca\(^2+\) by Na\(^+\)/Ca\(^2+\) exchangers) and increased expression of CYP11B2 and production of aldosterone. In a study of 251 Caucasian subjects with PA (almost all apparently nonfamilial), we identified 3 heterogeneous missense germline mutations (causing R52H, E246K, and G247R) and found that 12 (5% of the cohort) were carriers for the rare nonsynonymous single nucleotide polymorphism rs7102584 causing E282Q substitution (357). These three mutations and rare polymorphism were all remote from the selectivity filter. In vitro studies showed that R52H, E246K, and E282Q (but not G247) substitutions to be functional, affecting the inward current, a rise in intracellular Ca\(^2+\) (357). Hence, germline variations in the KCNJ5 gene may have a role to play in the common sporadic form as well as the much rarer syndromic forms of PA.

Somatic mutations within APAs have since also been described in other genes encoding ion pumps and channels, namely, ATP1A1 (encoding the α-subunit of Na\(^+\)-K\(^+\)-
ATPase), ATP2B3 (encodes a Ca\(^{2+}\)-ATPase calcium pump), and CACNA1D (encodes a voltage-gated calcium channel subunit), but with much lower frequency (31, 47, 145, 456, 565). In vitro studies suggest that ATPase mutations, like those in KCNJ5, also lead to ZG cell membrane depolarization, resulting in increased aldosterone production (31, 47, 565). Mutations in CACNA1D cause activation of the channel at more hyperpolarized potentials and some also impair channel inactivation (31, 456). Unlike patients with KCNJ5-mutated APAs, those with APAs carrying the newer mutations have tended to be more commonly males (31, 47), with smaller APAs (31, 145) containing predominantly ZG-like cells (31). Germline mutations in CACNA1D have been reported in two individuals with PA (456), both of whom also had seizures and functional neurological abnormalities resembling cerebral palsy.

Germline and somatic mutations in armadillo repeat containing 5 gene (ARMC5, thought to be a tumor suppressor gene and involved in steroidogenesis) have previously been found to be casually associated with hypercortisolism in the setting of primary macronodular adrenal hyperplasia (18, 145, 456).
26, 165). More recently, Zilbermint et al. (594) found germline variants of ARMC5 that were predicted by in silico analysis to be damaging in 6 (10.7%) of 56 patients with PA (594). All six patients were African-Americans. Although these variants were present in a statistically higher proportion among this cohort than in public databases, the clinical significance of these findings remains unclear and awaits confirmation.

Scholl et al. (460) recently identified a gain-of-function germline mutation (M1459V) in CACNA1H (encodes a T-type voltage-gated calcium channel subunit) in 5 of 40 subjects chosen for evaluation on the basis of hypertension and PA diagnosed at or before the age of 10 yr (460). Three of five additional relatives found by family screening to have inherited the mutation had early-onset hypertension. One required unilateral adrenalectomy, with histological examination revealing marked ZG hyperplasia, and hypertension recurred postoperatively. The other two were normotensive as adults. Expression of CACNA1H in ZG was confirmed by immunohistochemistry. The adrenal glands in mutation carriers showed no morphological abnormalities on imaging studies. In vitro studies demonstrated that the mutation caused a shift of activation to more hyperpolarized potential and loss of inactivation, which would be expected to result in increased intracellular calcium and increased aldosterone production (460).

G. Transcriptome Analyses

A rapidly growing body of gene expression data utilizing APA and nontumorous cortical tissue has provided further insights into the pathogenesis of APA and identified other genes with a potential role in aldosterone excess. Compared with adjacent or normal cortical tissue, APA tissue has repeatedly demonstrated increased expression of CYP11B2 (25, 39, 283, 551) and more variably of CYP21 (25, 39, 283) but not usually genes encoding enzymes further upstream in the steroidogenic pathway (25, 551), suggesting that upstream precursor availability is not limiting excessive aldosterone production. Other genes found to be overexpressed in APA have included 1) those encoding G protein-coupled receptors, including the luteinizing hormone receptor (LH-R), melanocortin 2 receptor (MC2R), gonadotropin releasing hormone receptor (GnRHR), and serotonin receptor 4 (HTR4) (580); 2) transcription factors, including paired related homebox 1 (PRRX1) (551) and Nur-related factor 1 (NURR1), nerve growth factor induced clone B (NGF1B), steroidogenic factor 1 (SF1) and dosage-sensitive sex reversal, adrenal hypoplasia congenital region of the X chromosome gene 1 (DAX1) (39); and 3) proteins involved with calcium signaling, such as calcium/calmodulin-dependent protein kinase 1 (CAMK1) (283), all of which may play a role in increasing aldosterone synthesis in APA cells. Assie et al. (25) also found increased expression of genes encoding the high-density lipoprotein receptor (raising a potential mechanism by which the APA cell may receive increased amounts of cholesterol in the initiation of steriodogenesis) as well as adrenodoxin and P-450 oxidoreductase (required to provide electrons required by mitochondrial steroid-synthesizing P450 enzymes).

Potassium current and membrane potential in ZG cells are dependent on the expression of TASK channels (106, 107). Genetic deletion of task1 and/or task3 in mice is associated with a PA phenotype (110, 217, 226). Lenzini et al. (280) analyzed the transcriptome and microRNA profiles of APAs and found lower expression than in normal adrenal cortex of TASK-2 and a higher expression of two microRNAs, hsa-miR-23 and hsa-miR-34, which were found to decrease TASK-2 mRNA expression by binding to its 3’-untranslated region (280). Transfection of adrenocortical H295R cells with a TASK-2 dominant-negative mutant construct significantly increased aldosterone production and gene expression of CYP11B2 and StAR. The authors concluded that aldosterone overproduction by APAs may, at least in part, be due to downregulation of TASK-2 as a result of overexpression of regulatory microRNAs (280).

While mutation and transcriptome analysis have yielded valuable insights into the mechanisms of excessive aldosterone production by APAs, much less is understood about the pathogenesis of the development of the tumors themselves. Some authors have argued that mutations in ion channels and pumps lead to aldosterone excess, but not tumor development. In support of this notion, Dekkers et al. (113) found somatic mutations in KCNJ5, CACNA1D, or ATP1A1 in only one nodule in almost every adrenal removed from patients with multinodular adrenal hyperplasia who underwent unilateral adrenalectomy for PA. Mutations were invariably located in nodules staining positive for aldosterone synthase. These findings suggest that, while somatic ion channel and pump mutations almost certainly cause autonomous aldosterone overproduction, different genetic “hits” may be required to lead to APA formation. Boulkroun et al. (55) reported evidence from both in situ hybridization and transcriptome analysis of upregulation of the Sonic hedgehog (Shh, a marker of stem/precursor cells) pathway, which is essential for adrenal gland development and maintenance (265), in APAs. Some of the components of the pathway that were differentially expressed have been previously implicated in growth and tumor development. Interestingly, Shh was also overexpressed in the nontumorous but hyperplastic ZG, raising the possibility that reexpression of normally repressed fetal genes in the precursor cell layer of the adrenal cortex may lead to hyperplasia of the ZG and APA formation (55). These worker also demonstrated activation in the majority of APAs examined of the Wnt/β-catenin pathway (45), which is also important in the embryonic development, stem cell maintenance, and differentiation of many tissues, including the adrenal cortex (130), but also probably in
steroid (and particularly aldosterone) synthesis (225, 454). It is possible, therefore, that activation of this pathway may play pathogenic roles in both tumor development and excessive aldosterone production in APAs.

Williams et al. (564) demonstrated upregulation of teratocarcinoma-derived growth factor-1 (TDGF-1) in APA that inhibits apoptosis in adrenal cells. The gene encoding the calcium sensor protein visinin-like 1 (VSNL-1) was also shown to be upregulated by this group in a subset of APA (563). VSNL-1 is a target of the nuclear receptor SFI (147), which was also shown to be upregulated in APA (as noted earlier in this section).

Transcriptome analysis has also been used to compare gene expression patterns in APAs with or without KCNJ5 somatic mutations. Although Boulkroun et al. (54) found no difference in transcriptome profiles between these two groups of tumors, Monticone et al. (336) reported higher rates of expression (confirms by real-time PCR) of CYP11B2 in APAs bearing KCNJ5 mutations, and another 23 genes which exhibited differential expression (defined as an increase or decrease in mRNA levels of at least 2.5-fold). On the other hand, Akerstrom et al. (16) reported APAs bearing mutations in ATP1A1 or ATP2B3 to have higher rates of expression of CYP11B2 and NPNT (encodes nephrorectin) than KCNJ5-mutated APAs. In keeping with their ZF-like cellular morphology, APAs with KCNJ5 mutations were reported by Azizan et al. (29) to demonstrate higher rates of expression of CYP17A1 than those without. This provides a possible biochemical explanation for the high levels of “hybrid steroids” (18 hydroxy- and 18 oxocortisol) associated with these tumors (566), with CYP17A1 expression in APA cells permitting the generation of cortisol, which can then be converted to hybrid steroids by the action of aldosterone synthase (encoded by CYP11B2).

Murakami et al. (355a) employed a combination of methylation and transcriptome analysis and demonstrated global CpG hypomethylation in APA relative to adjacent adrenal cortex, and that 34 genes were upregulated with CpG hypomethylation in APA. Of these, three (CYP11B2, MC2R, and HPX) were linked to aldosterone production, and five are (PRRX1, RAB38, FAP, GCNT2, and ASB4) potentially to tumorigenesis.

Clearly the determination of genetic causes of PA is a rapidly expanding and exciting field, yielding fascinating new knowledge about the pathophysiology of this disorder and raising possibilities for innovative new treatments.

VII. SYMPTOMS AND SIGNS OF PA

While the great majority of patients with PA demonstrate hypertension, screening of members of families with genetic forms has allowed the recognition of subjects in whom, despite inheriting the causative mutation and manifesting biochemical hallmarks of PA, have normal blood pressure levels (136, 419, 493, 500). Presumably other genetic and/or environmental factors protect against (or conversely may aggravate) the development of hypertension to variable degrees among patients with PA. Hypokalemia, once thought to be a mandatory feature of PA, is in fact now known to be present in a minority (~20–25%) of patients and hence its absence does not exclude the diagnosis (351, 497, 586). When it is present, because of potassium’s role in muscle and neural function and in the kidney’s ability to concentrate urine, associated symptoms may include muscle weakness, cramping, tetany, paresthesias, palpitations due to arrhythmias (especially atrial fibrillation), polyuria, and nocturia. Nocturia is a common symptom even among patients with normal plasma potassium levels, possibly because of the rise in ANP levels that has been observed among patients with PA, or because an increase in potassium release from renal tubular cells (serving as an attempt to maintain normokalemia) may have led to an intracellular potassium deficit. Patients with PA also frequently describe feelings of lethargy, impaired mental concentration, and mood disturbances (9, 21, 28, 270, 473, 474, 509). During pregnancy, hypertension and symptoms may improve (49, 210, 424). This is thought to be due to the anti-mineralocorticoid effects of high circulating levels of placental progesterone, which antagonize aldosterone action at the MR. On the other hand, several case reports exist of PA presenting during pregnancy (17, 127, 466, 518). In one of these (518), APAs from both affected patients harbored somatic mutations in CTNNB1, encoding β-catenin in the Wnt cell-differentiation pathway (which may in part explain tumor development). They also expressed very high levels of mRNA for LHCGR (encoding luteinizing hormone-human chorionic hormone receptor) and GNRHR (encoding gonadotropin releasing hormone receptor), raising the possibility that high levels of human chorionic hormone during pregnancy led to stimulation of aldosterone production by these tumors via aberrant receptor activation (518). Plasma levels of estradiol increase as human pregnancy progresses (284). Caroccia et al. (74) reported that estradiol blunts aldosterone secretion by human adrenocortical cells in vitro via the type β estrogen receptor (ER β) but stimulates it (under ER β blockade) via GPER-1.

VIII. ASSAYS USED IN THE DIAGNOSTIC WORKUP OF PA

Highly reproducible assays are essential for accurate diagnosis and appropriate management of PA. This applies to all stages of the diagnostic workup, with screening by ARR measurement reliant on accurate assays of both aldosterone and renin, suppression testing (for confirmation of PA) on precise quantification of aldosterone (and to a lesser extent renin and cortisol), and adrenal venous sampling (crucial
for determining whether a patient is a candidate for unilateral adrenalectomy, or, alternatively, treatment with aldosterone antagonist medication) dependent on reliable assessment of aldosterone and cortisol levels (198).

A. Aldosterone Assays

Because plasma aldosterone circulates at picomolar concentrations (<1% of those of cortisol), accurate measurement poses a challenge and requires highly sensitive and specific assays. Plasma (or serum) aldosterone is most commonly measured by radioimmunoassay (316). The assay requires a high-affinity, highly specific antibody and meticulous laboratory technique. Faster, more convenient methods of directly measuring aldosterone are available, using immunometric techniques and automated machinery (116, 117, 396). However, considerable work is required to validate and improve these methods before they can be considered accurate enough to use for diagnostic purposes. Concerns with the automated aldosterone assay have included 1) the potential for false-negative suppression tests. In one analysis, aldosterone levels were below the assay’s limit of detection for over half the samples collected from normal subjects and nearly half those from patients with essential hypertension (455). 2) Nonspecific interference, possibly due to the brevity of the “wash” immediately prior to chemiluminescence, could lead to an unacceptably high “blank” value in bilaterally adrenalectomized and Addisonian patients. 3) Weaknesses of the assay system is a concern: it is calibrated by only a two-point recalibration against a stored master curve, and the two calibrators are reconstituted lyophilized aldosterone. Even the very well established radioimmunoassays for aldosterone have demonstrated variability in performance. Schirpenbach et al. (455) compared four different aldosterone assay approaches and reported them to often give markedly different results. Concerns have been raised about the specificity of the assays used, which is particularly relevant during adrenal venous sampling in which plasma derived from adrenal venous blood may contain very high concentrations of steroids and their precursors and metabolites, with the potential for cross-reactivity with the aldosterone assay antibody. The need for dilution of such samples to achieve concentrations that fall within the assay’s limits of quantification is another possible source of measurement error.

A major recent step forward has been the development of highly accurate and reproducible methods of measuring aldosterone within the clinically relevant range (and without the need for dilution, even with adrenal venous samples) using high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) (228, 269, 394, 517, 537) (FIGURE 6). The incorporation of new semi-automated technology has meant that these methods can generate results rapidly and with relatively high throughput, and can therefore be applied to the clinical setting. While running expenses are relatively small, the initial cost outlay associated with the purchasing of the required equipment does represent a barrier to the wide application of LC-MS/MS.

B. Renin Assays

Renin can be measured in terms of its enzymatic activity (plasma renin activity, PRA), or its mass (active or “direct” renin concentration, DRC). PRA is measured by incubating plasma at 37°C without addition of angiotensinogen (renin substrate), relying instead on endogenous angiotensinogen in the plasma (219). Renin cleaves angiotensinogen to produce a decapeptide, angiotensin I, which can be measured by radioimmunoassay (576). PRA is expressed as the amount of angiotensin I generated per unit of time. The recommended incubation time, 90 min, should be extended up to 18 h for samples with levels <1 ng·ml⁻¹·h⁻¹, to permit enough generation of angiotensin I to ensure assay reproducibility at the lower end of the scale (462). Subtracting the amount of preformed angiotensin I in a control aliquot incubated at 4°C is suggested, but this may reduce the reproducibility of the test (462).
The plasma concentration of the active (cleaved) form of renin (DRC) can be measured by radioimmunoassay, but more commonly measured by automated immunometric assay (123, 146, 396). Since renin does not affect blood pressure directly, but only through angiotensin, PRA is a better reflection of angiotensin II concentrations. DRC can be influenced by substrate concentrations. As an example, with estrogen administration, substrate increases, and DRC falls to maintain angiotensin II concentrations in the normal range (67, 378).

Because they are faster and more convenient, measurement of DRC by immunometric techniques and automated machinery have been adopted in many large, busy laboratories, replacing the more laborious, well-established PRA radioimmunoassays that have reliably reflected angiotensin activity (146, 197, 396). Legitimate concerns exist regarding the validity of PRC measurement by automated immunometric techniques when concentrations of active renin do not reflect the level of activation of the renin/ANG II system during treatment with estrogen-containing compounds or with renin inhibitors (492). Perhaps more importantly, reliability and reproducibility of these assays, while reasonable in the middle part of the human reference range, appear to be poor at the lower end, which is especially concerning for the workup of PA where renin levels are typically suppressed. Because the ARR appears to be more dependent on renin than aldosterone (340), especially when renin levels are low, small absolute changes can result in large changes in the ARR, and it could be argued that it is more important to measure renin accurately than aldosterone when screening for PA. On the other hand, advocates for automated immunometric renin assays point out that this method is much less time consuming than PRA (and therefore more practicable), can be combined with automated immunometric aldosterone measurement performed simultaneously on the same sample, and appears to have better interlaboratory agreement (308, 342). A further theoretical concern regarding PRA is the limited availability of substrate in patients with heart failure and chronic liver disease (413).

Hopefully, development of mass spectrometric renin assays with the same capability for precision and specificity as those that have been observed for aldosterone will soon become a reality. Those reported so far have been based on PRA-style measurement of angiotensin I generated from angiotensinogen (77). Their requirement for the same time-consuming incubation steps of the traditional PRA radioimmunoassay may explain why they have yet to be widely adopted into the clinical setting. The answer may lie in the development of mass spectrometric assays for plasma ANG II which, unlike renin, directly regulates aldosterone synthesis by stimulating ZG cells via AT1 receptors, and is therefore perhaps more physiologically relevant in the clinical assessment of patients for the presence of ANG II-independent aldosterone overproduction.

The above assay issues have represented important impediments to diagnostic accuracy and standardization of diagnostic criteria, and have added emphasis to the need for individual laboratories to develop their own references ranges and employ careful quality control measures. For both renin and aldosterone assays, this would ideally include using aliquots from human plasma pools, carefully selected to cover the critical range of measurements, rather than the lyophilized controls provided by the manufacturer to monitor intra- and interassay reproducibility.

IX. SCREENING FOR PA

The diagnostic workup for PA comprises three phases: screening, confirmatory testing, and determining the subtype of PA. Because confirmatory testing and subtype differentiation involve procedures that are relatively invasive, time consuming, and expensive, it is important to first screen at-risk populations for the possible presence of PA to minimize the number of patients who should then be considered for subsequent diagnostic assessment.

A. Who Should Be Screened?

An Endocrine Society Guideline has recommended screening for all but the mildest forms of hypertension, and for several other patient groups in whom the risk of PA is thought to be increased, including those with hypertension and spontaneous or diuretic induced hypokalemia, hypertension and adrenal incidentaloma, hypertension and sleep apnea, and (in recognition of familial forms) hypertension and a family history of early-onset hypertension or cerebrovascular accident at a young age (<40 yr) and all first-degree relatives of patients with PA (163). However, because PA is common among the hypertensive population, possibly accounting for as many as 5–13% of patients, an argument can be made for considering the diagnosis in all hypertensives (205, 491). The degree of benefit achieved from specific treatment of PA (and especially the chance of cure of hypertension following unilateral adrenalectomy for unilateral forms of PA) has repeatedly been shown to be inversely related to the preexisting duration of hypertension (95, 189, 300, 548, 590, 592), arguing for early diagnosis rather than waiting until hypertension becomes severe and resistant. Furthermore, screening patients before they are commenced on antihypertensive medications has the added advantage of avoiding potentially confusing effects of treatment on plasma renin and aldosterone levels which could lead to false-positive or -negative screening results. Screening is also relatively inexpensive, with combined measurement of plasma aldosterone and renin levels often bearing a similar cost to that of a common plasma lipid profile.
Although the presence of hypokalemia in a hypertensive patient (especially if not receiving potassium-wasting diuretics) would make PA highly likely, restricting screening to hypokalemic hypertensives (which was the traditional practice until the recent recognition of the high prevalence of PA among normokalemic hypertensives) would lead to almost 80% of patients with PA being missed.

**B. Methods of Screening**

As indicated above, given that only ~20% of patients with PA are hypokalemic, demonstration of hypokalemia by measurement of plasma potassium lacks sensitivity for PA. The presence of hypokalemia can also be missed if care is not taken to avoid factitious rises in potassium due to release from muscle and blood cells during collection. This involves 1) using fist clenching only to achieve venipuncture, 2) releasing the tourniquet after venipuncture has been achieved, 3) waiting for at least 10 s before gently withdrawing blood, 4) using a syringe and needle rather than a vacuumed sample container so that blood can be withdrawn slowly and carefully and then gently discharged down the side of the opened sample tube, and 5) centrifuging and separating the plasma from the cells within 30 min of collection.

Demonstration of frankly elevated plasma aldosterone levels also lacks sensitivity for PA, since many patients exhibit levels that lie within the wide normal range (447, 494, 497). A case of APA associated with low-normal (4.3–5.4 ng/100 ml; 119–150 pM) plasma aldosterone levels but with high ARR, lateralization of aldosterone to the left adrenal on AVS, and positive immunohistochemical staining for aldosterone synthase in a small adenoma within the removed left adrenal was recently reported by Rossi et al. (441). Such “normal” levels could be viewed as “inappropriately normal” in the face of suppression of the renin which, in individuals without PA, should result in suppression of plasma aldosterone to very low values. Reliance on the demonstration of hypokalemia and frankly elevated aldosterone were probably principal contributors to the earlier lack of recognition of the high prevalence of PA among hypertensive populations. Raised plasma aldosterone levels also lack specificity as they may occur secondarily to raised renin/ANG II (secondary hyperaldosteronism), as is observed in renovascular forms of hypertension or in patients receiving treatment with diuretic agents which promote natriuresis, both of which lead to increased renin production by juxtaglomerular cells as described above (see sect. II B).

Measurement of plasma renin provides a much more sensitive, but somewhat less specific, means of screening for PA than measuring plasma potassium or aldosterone. Renin levels are almost always suppressed in PA. Exceptions include patients who are habitually ingesting a low-sodium diet or receiving medications that can bring about increased renin production (see below and **Table 1**), or have “accelerated” or malignant hypertension (42, 256, 356), or concomitant renovascular hypertension (498). Lack of specificity, however, results from the possibility that renin levels may also be suppressed because of 1) treatment with agents (including β-adrenergic blockers, clonidine, or α-methyldopa) which reduce β-sympathetic stimulation of renin release (10, 62, 196, 387) or with nonsteroidal anti-inflammatory agents which promote salt retention (331); 2) high dietary sodium intake (198); 3) the gradual fall in renin that occurs as renal function declines with advancing age (105, 301); 4) chronic renal impairment, in which renal renin-producing capacity is reduced and salt-retention contributes to renin suppression (324); and 5) the presence of other salt-dependent, low renin forms of hypertension (22, 180, 200, 242, 258, 480, 549, 552, 558, 567). Among the latter conditions are 1) Liddle syndrome, caused by activation mutations of ENaC (552); 2) congenital or acquired (for example, through carbonic anhydrase administration or ingestion of licorice) deficiency of 11β-HSD2, which permits cortisol to gain access to and activate the MR (480); 3) hypertensive forms of congenital adrenal hyperplasia caused by mutations in either the 11β-hydroxylase or 17α-hydroxylase genes, which bring about reductions in cortisol production, leading to feedback stimulation of ACTH, which in turn causes increased production of the mineralocorticoid deoxycorticosterone (DOC) (258, 558); 4) primary glucocorticoid resistance, which is again associated with ACTH simulation and excessive DOC production (22); 5) ectopic ACTH syndrome, in which mineralocorticoid hypertension is thought to result from a combination of DOC excess and “overload” of the 11βHSD2 enzyme by very high levels of cortisol (549); 6) DOC-secreting tumors (242); 7) activating mutations of the MR (180); and 8) familial hyperkalemic hypertension (200), in which mutations in genes (WKIN, WNK4, CUL3, and KLCH3) regulating the NCC within the distal renal tubule are thought to lead to its activation and excessive retention of sodium and potassium (56, 187, 567). Unlike in PA, chronic suppression of renin/ANG II leads to suppression of aldosterone in all of these salt-dependent, low-renin forms of hypertension with the exception of familial hyperkalemic hypertension, in which elevated plasma potassium levels may prevent aldosterone suppression.

The ARR addresses many of the limitations described above in being much more sensitive for detection of PA than plasma potassium or aldosterone measured in isolation, and more specific that isolated renin measurements, in that if renin is suppressed and aldosterone is also suppressed, the ratio will not be elevated. For this reason, it has become the preferred method of screening by the great majority of centers worldwide. As will be discussed in section XII A, a growing list of physiological and pharmacological factors has been recognized to affect the ARR with the potential to cause false positives and negatives. However, provided rea-
sonable attempts are made to address control for these or at least to take them into account (see sect. XII B), the results of ARR testing are highly useful in selecting patients for further diagnostic workup, and careful ARR measurement is still probably the most reliable approach currently available. The ARR also shows good within patient reproducibility after medications known to interfere with results have been withdrawn where possible (443). Nevertheless, the ratio should still be regarded as a screening test only, and should be measured more than once (serially if conditions of sampling, including medications, are being altered) before deciding whether or not to go on to a suppression test to definitively confirm or exclude the diagnosis of PA.

Clearly there is considerable room for improvement over the ARR in terms of screening approaches for PA. As discussed above, the development of more accurate assay methods using high-throughput mass spectrometric techniques is already a reality for aldosterone and has been implemented for clinical use in a growing number of centers. Similar approaches to renin, and perhaps better still ANG II, measurement are eagerly awaited. Some investigators have reported on the use of multivariable approaches based on discriminant analysis which take into account absolute values of aldosterone and renin rather than just the ratio (247, 275, 442), but these are more cumbersome and may have limited reproducibility from center to center. The identification of KCNJ5, ATP1A1, ATP2B3, CACNA1D, and CACNA1H mutations has greatly enhanced knowledge regarding the roles of these channels and pumps in adrenal physiology and pathophysiology and has the potential to lead to new diagnostic and therapeutic strategies for PA. However, because these mutations are almost always somatic (requiring the examination of removed adrenal tumor tissue for their detection), and germline mutations are only very rarely found among patients with PA, the clinical utility of genetic testing as a future screening approach currently remains uncertain. This could change however as further inroads are made into elucidating the role of genetics in the development of this condition.

X. CONFIRMATION OF DIAGNOSIS OF PA

Because the ARR is not without occasional false-positive results, even under the conditions described in section XII B below, confirmatory testing is required before the diagnosis of PA can be definitively confirmed or excluded. This usually involves demonstrating an inability to completely suppress aldosterone production during maneuvers designed to bring about complete suppression of circulating renin, and therefore of aldosterone’s normal chronic regulator, ANG II. This permits confirmation of aldosterone production that is relatively autonomous of ANG II, the sine qua non of PA.

A. Importance of Confirmatory Testing

As will be described in the section XI, optimal management of PA involves determining 1) whether the patient truly has the condition and 2) if so, which particular subtype of PA is present. Confirmatory testing not only answers the first question, but selects patients who should then undergo further testing to answer the second (determining the subtype). This is very important as the major step in subtype differentiation, adrenal venous sampling (AVS), is expensive, difficult to perform, invasive, and not without risk of complications (most commonly, adrenal hemorrhage). Hence, one of the important functions of confirmatory testing is to exclude the diagnosis in patients without PA who can therefore be spared from having to undergo this procedure.

B. Selection of Patients for Confirmatory Testing

The starting point for selecting patients for confirmatory testing is the presence of repeatedly (at least two) raised ARR levels in a patient with hypertension, ideally in the absence of factors known to be associated with false-positive results. Confirmatory testing may also be considered as a means of “clearing the air” in occasional patients in whom confounding factors that may have led to a false-positive ARR cannot safely be withdrawn or modified (for example, in patients who require ongoing treatment with a β-adrenergic blocking agent because of coronary artery disease or women on an estrogen-containing oral contraceptive agent).

One limitation of relying solely on the ARR for selection for further workup is that, in the presence of extremely low renin levels (for example, a DRC of 2 mU/l or PRA 0.1 ng·ml⁻¹·h⁻¹ or less), the ARR may be elevated and thereby raise the possibility of PA even when plasma aldosterone is also very low (for example, 110 pM or 4 ng/dl) and clearly not consistent with PA. To avoid this problem, some investigators have suggested the inclusion of a minimum plasma aldosterone concentration (for example, 415 pM or 15 ng/dl) to accompany an elevated ARR as an additional selection criterion for confirmatory testing (589). This approach would have led many of our patients with PA (including some with APA) to have been missed because their plasma aldosterone levels fell below this cut-off level (494). For this reason, our approach is to proceed with diagnostic workup for PA in all patients with elevated ARR, except in those whose plasma aldosterone concentration is below the level used to define normal suppression during confirmatory fludrocortisone suppression testing (165 PM or 6 ng/dl). In those patients, we will periodically repeat the ARR and consider further diagnostic workup.

Where confirmatory testing involves salt loading (which is usually the case), exclusion criteria include severe hyperten-
sion or compromised cardiac or renal function because of the increased risk of potentially dangerous fluid overload. In patients with severe hypertension associated with marked left ventricular hypertrophy, for example, consideration may be given to a period of medical treatment with an agent that blocks aldosterone action (see sect. XIV) as an attempt to establish hypertension control and improve left ventricular dimensions, thereby reducing the risk of confirmatory testing to a more acceptable level.

The rationale for confirmatory testing followed (if positive) by AVS may also be questioned in patients for whom unilateral adrenalectomy is not likely to be a viable treatment option, either because they are poor candidates for surgery, or do not desire surgical intervention. An argument could be made in such circumstances for avoiding such invasive testing and instead initiating an empiric trial of medical treatment for PA (see sect. XIV).

C. Methods of Confirmatory Testing

Confirmation of PA involves demonstration of aldosterone production that is significantly autonomous of its normal chronic regulator, ANG II. This is usually accomplished by demonstrating evidence of ongoing aldosterone production in the face of maneuvers designed to bring about complete suppression of circulating renin. Of the various approaches described, the most common in use are oral sodium loading, saline infusion testing, and the captopril challenge test. However, although relatively labor-intensive, the FST is the approach used within our center as we have found it to be the most reliable (198, 499, 539). Patients undergoing FST are admitted to the hospital to ensure that the high-sodium diet and posture requirements of the test protocol are met, and to permit frequent monitoring of plasma potassium levels to guide doses of oral KCl replacement. Under these conditions of close monitoring, the FST has been very safe in our hands. The FST is considered diagnostic of PA if upright (1,000 h) plasma aldosterone fails to suppress to <6 ng/100 ml (165 pM) at the conclusion of 4 days administration of a high-sodium diet and slow-release sodium chloride (Slow Na, 30 mmol given 3 times daily with meals) and of fludrocortisone acetate (0.1 mg every 6 h), provided that 1) upright renin is suppressed (PRA to <1·day

3) Plasma cortisol concentrations on day 4 are lower at 1000 than at 0700 h, consistent with ACTH falling at this time of day as part of its normal circadian rhythm. This observation excludes the occurrence of an acute (for example, stress-induced) rise in ACTH which may have prevented suppression of aldosterone.

Several highly reputable groups, including that of Young et al. (589), measure urinary aldosterone concentrations following oral salt loading to diagnose PA. For this protocol, patients are encouraged to consume enough dietary salt to achieve a urine sodium excretion of over 200 mmol/day and are given sufficient potassium supplementation to maintain normokalemia. A 24-h urinary aldosterone concentration of over 12 μg/day on the third day is regarded as diagnostic. Our application of Young’s criterion to urinary aldosterone concentrations measured in collections obtained on the fourth day of the FST permitted detection of 50 of 80 patients with a positive FST and 8 of 10 subsequently cured by surgery (209).

A commonly used approach has been to administer intravenous 0.9% saline (usually 2 l over 4 h) with measurement of plasma aldosterone at the end of the infusion. Concentrations regarded as diagnostic for PA have varied from >5 to >10 ng/100 ml (>140 to >280 pM) (234, 261, 291). This approach has the advantage of requiring only a brief outpatient visit. In the authors’ experience, saline infusion [using a diagnostic post-saline plasma aldosterone of >8 ng/100 ml (>220 pM)] detected only 17 of 97 patients confirmed as having PA by FST, and only 3 of 10 patients who were subsequently cured of PA following unilateral adrenalectomy, but these results may have been at least partly influenced by the use of a more rapid infusion protocol (2 l over 2 h) (209). Willenberg et al. (562) similarly found SST to lead to greater suppression of plasma aldosterone than FST (562). Because aldosterone levels can be higher upright (e.g., seated) than recumbent in patients with PA and upright levels are used during FST, we hypothesized that seated SST (SSST) is more sensitive than recumbent SST (RSST), especially for posture-responsive PA. In a pilot study of 31 patients who underwent FST (upright plasma aldosterone levels measured at 10 a.m. basally and after 4 days fludrocortisone 0.1 mg every 6 h and oral salt loading) and SST (aldosterone levels measured basally at 8 a.m. and after infusion of 2 l normal saline over 4 h) both recumbent
and seated in randomized order, FST confirmed PA in 23 patients, excluded PA in 3, and was originally “inconclusive” in 5 (8). However, one with “inconclusive” FST had PA confirmed by lateralizing AVS and was reclassified “unilateral PA.” Of the 24 with confirmed PA (8 unilateral, 11 bilateral, and 5 undetermined subtype), 23 (96%;) tested positive by SSST compared with only 8 (33%) by RSST (P < 0.001). RSST missed 1 unilateral, all 11 bilateral, and 4 with as yet undetermined subtype. RSST was positive in 7 of 10 (70%) posture-unresponsive versus 1 of 14 (7.1%) posture-responsive patients (P < 0.005). Clearly larger studies are required, but these preliminary results suggest that SSST may be superior to RSST in terms of sensitivity for detecting PA, especially posture-responsive forms, and may represent a reliable, yet much simpler, cheaper, and quicker alternative to FST (FIGURE 7).

The captopril challenge test differs from the above approaches in that it does not involve salt loading as a means of achieving suppression of renin, but instead measures aldosterone and renin responses to captopril, an ACE inhibitor. Patients receive 25–50 mg captopril orally after sitting or standing for at least 1 h (3, 80, 221, 247). Blood samples are drawn for measurement of PRA, plasma aldosterone, and cortisol at time 0 and at 1 or 2 h after challenge, with the patient remaining seated during this period. In subjects without PA, plasma aldosterone is suppressed (>30%) and PRA stimulated by captopril because the reduction in ANG II formation leads to both a reduction in direct stimulation of aldosterone production via the AT1 receptor on ZG cells and a loss of negative feedback of renin production by the renal juxtaglomerular cells. As a result, the ARR falls. In patients with PA aldosterone fails to suppress, PRA remains suppressed and the ARR remains elevated. In one commonly used protocol, failure of plasma aldosterone to fall to below 8.5 ng/dl (240 pM) and the ARR to <30 (with aldosterone expressed as ng/dl and PRA as ng·ml⁻¹·h⁻¹) 2 h after receiving 50 mg captopril is considered diagnostic of PA. Advantages of captopril challenge testing include its relative simplicity and the fact that it does not involve salt loading, and therefore avoids the potential for inducing both fluid overload (in patients at risk because of compromised cardiac or renal function) and hypokalemia. However, captopril challenge testing has been reported to have poor discriminatory power for PA in hypertensive patients with high basal ARR (346, 556) and may cause hypotension in these seated or standing patients.

XI. DIAGNOSING UNILATERAL FORMS OF PA: A HIGHLY CONTROVERSIAL TOPIC

A. New Concepts in the Definition of PA Subtypes

Once the diagnosis of PA has been confirmed, the final step in the diagnostic workup is aimed at differentiating the various subtypes. Traditionally, PA has been divided into four main subtypes, three of them based on adrenocortical histopathology (APA, BAH, and aldosterone-producing carcinoma) and one rare subtype distinctive for its familial occurrence and for being glucocorticoid-repressible (FH-I). With increasing examination of adrenals removed from subjects with PA, it has become ap-

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<tr>
<th>FST</th>
<th>Day 0 Aldo (pmol/L)</th>
<th>Day 4 Aldo (pmol/L)</th>
<th>Day 4 DRC (mU/L)</th>
<th>Day 4 Cort (nmol/L)</th>
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<tr>
<td>Rec (7am)</td>
<td>67</td>
<td>597</td>
<td>88</td>
<td>270</td>
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<td>Up (10am)</td>
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<th>Criteria for positivity</th>
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<td>Day 4 Up DRC ≤ 8mU/L</td>
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<td>Day 4 Up Cort ≤ Rec</td>
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<td>Aldo at 4h &gt; 165 pmol/L</td>
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<td>Day 4 Up DRC ≤ 8mU/L</td>
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<td>Cort at 4h ≤ basally</td>
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<th>Recumbent SST</th>
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<td>Aldo at 4h &gt; 140 pmol/L</td>
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<td>Day 4 Up DRC ≤ 8mU/L</td>
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<td>Cort at 4h ≤ basally</td>
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FIGURE 7. Results of fludrocortisone suppression testing (FST), seated saline suppression testing (Seated SST), and recumbent saline suppression testing (Recumbent SST) in a 50-yr-old female who was diagnosed having primary aldosteronism (PA) by FST and subsequently found to have bilateral PA by adrenal venous sampling. The diagnosis of PA was confirmed by seated SST and missed by recumbent SST. As shown on the basal day of her FST, she had a brisk aldosterone (Aldo) response to upright posture, DRC, direct renin concentration; Cort, cortisol; Rec, recumbent; Up, upright. Criteria for positivity of each test are shown in the pink-shaded box.
Parent that this classification is a gross simplification and the morphology associated with PA is far more diverse (206, 496). Although APAs are usually unilateral, they may be bilateral, and their cellular composition varies from mostly ZF-like cells to mostly non-ZF (with cells resembling ZG or “hybrid” cells having characteristics of both ZF and ZG) (366, 530). Hyperplasia, though usually bilateral, may be unilateral (241, 313, 399, 467), and can be diffuse, micronodular, or macronodular and again may involve primarily either ZF or ZG. Furthermore, rather than being solitary lesions, APAs are more commonly associated with some degree of hyperplasia (diffuse or nodular) within the remaining ipsilateral adrenal cortex, usually of ZG but also of ZF (366).

Recent studies have reported on the use of immunohistochemical staining of resected adrenals in an attempt to identify the true source of aldosterone excess (360, 362, 386, 547). Volpe et al. (547) found APAs to show strong immunoreactivity for aldosterone synthase (547). In adrenals from a significant minority of patients, they found either “adenomas” (presumably defined as adrenocortical tumors of at least 1 cm in diameter) that were not CYP11B2 positive or no “adenoma” at all, but smaller nodules with strong CYP11B2 immunoreactivity. Nanba et al. (362), in a study of 32 patients with PA who underwent unilateral adrenalectomy, found 22 to show positive CYP11B2 immunostaining in their tumors, which they designated as APAs. Another eight with either CYP11B2-negative adenomas (n = 4) or without tumors on CT showed clusters of cells with CYP11B2 immunostaining in ZG, termed “aldosterone-producing cell clusters” (APCCs) (362). The clinical significance of APCCs in PA is unclear, however, as they also occur in adrenals from subjects without PA (193, 371). The findings of these and other studies employing immunohistochemical stains for steroidogenic enzymes highlight limitations in traditional approaches to histopathological examination using haematoxylin and eosin (H&E) staining in PA, and further broaden the diversity of the histopathological subtypes responsible for this condition (183, 362). Clearly it cannot be assumed that a dominant or even an isolated nodule within a removed adrenal gland is the sole source of aldosterone excess, or is even producing aldosterone at all. Several studies have reported that in excised glands containing multiple nodules, it is not always the largest that is responsible for aldosterone excess (113, 335). These findings may help to explain a previous report of low CYP11B2 expression in 4 of 16 tumors thought to be APAs (283). Variegated staining within a nodule may suggest that some, but not all, tumor cells are producing aldosterone, and may reveal mismatching between cell type (e.g., ZF- versus ZG-like) and aldosterone synthase positivity (183).

In a fascinating recent report, APCCs were shown to be common among 42 normal adrenals removed from kidney donors, contained both ZG- and ZF-like cells on H&E staining but bore transcriptomes which more closely resembled those of adjacent ZG (but with enhanced aldosterone-producing capacity) than ZF, and, in 35% of cases, had somatic mutations in CACNA1D and ATP1A1 (but, interestingly, not in KCNJ3) which were not present in the adjacent “normal” cortex (372) (FIGURE 8). Whether APCCs, previously missed by traditional staining, are precursors for APAs or are sufficiently active in terms of aldosterone production to sustain PA in their own right requires further investigation.

From a practical clinical standpoint, subtype differentiation aims not to try to determine which of the above highly diverse morphological moieties is present, but to guide optimal management by separating unilateral from bilateral adrenal aldosterone overproduction, benign from malignant lesions, and glucocorticoid-suppressible from non-glucocorticoid-suppressible PA, as discussed in the next section.

FIGURE 8. Laser capture microdissection (LCM) of aldosterone-producing cell clusters (APCCs) for targeted next generation sequencing (NGS). APCCs were identified by CYP11B2 immunostaining on frozen tissue sections for NGS. A: low-magnification view with the regions captured for APCC (red box) and paired normal adrenal tissue (yellow box) shown. B–E: photomicrographs showing pre- (B and D) and post-LCM images (C and E) that confirm isolation of desired cell populations. A somatic CACNA1D F747L mutation was identified exclusively in the APCC component. [From Nishimoto et al. (372).]
B. Importance of Differentiating Subtypes of PA

Subtype differentiation in PA is designed to subdivide patients according to whether 1) the patient has FH-I, in which low-dose glucocorticoid treatment would be expected to bring about excellent hypertension control and family members should be screened for the presence of this subtype of PA; 2) autonomous aldosterone production, if not due to FH-I, is unilateral (confined to one adrenal, as with APA) or bilateral (consistent with BAH). Whereas unilateral adrenalectomy leads to either cure of hypertension or at least substantial improvement in control in the great majority of patients with unilateral PA, most patients with bilateral PA are currently managed medically with drugs (such as spironolactone, eplerenone, and amiloride) that antagonize aldosterone action. 3) The patient has a large (for example, >2.5 cm) mass lesion in the adrenal gland on computed tomography (CT) scanning or magnetic resonance imaging which would certainly warrant follow-up and possibly consideration of removal based on its potential to be malignant.

While genetic testing quickly deals with the first issue, and CT scanning the third, it is the differentiation of unilateral from (non-FH-I) bilateral PA that poses the greatest challenge. It has been argued that, given the effort and expense involved in the diagnostic workup for PA (and especially subtype differentiation), consideration should be given to using spironolactone or eplerenone as first line agents in every patient thought to have PA (255, 257) and even in all new hypertensives (160, 162). However, this approach would mean that the 30% or so patients with PA who have unilateral forms would be denied the opportunity to undergo surgery and thereby experience a surgical cure and, in terms of both cardiovascular outcomes (81, 504) and quality of life (9), superior benefits to those observed with medical therapy in bilateral PA. The superiority of surgical treatment is perhaps not surprising as the removal of an APA which is the entire source of aldosterone excess would be expected to result in a more complete correction of the adverse effects of excessive aldosterone production than that attempted by medical MR blockade (which may not be sufficient, in clinically tolerated doses, to reverse these effects in their entirety). Most patients who undergo unilateral adrenalectomy for unilateral PA enthusiastically report a marked improvement in general well-being (even if treated with spironolactone up until the time of surgery) that is much less apparent in patients with bilateral disease treated medically. Furthermore, because it has interactions with testosterone and progesterone receptors (albeit weaker than with MR), treatment with spironolactone can be accompanied by side effects such as gynaecomastia, sexual dysfunction, and menstrual irregularities even in doses as low as 12.5–50 mg daily (497). Eplerenone, a more selective MR antagonist which minimizes sex steroid-related side effects, is not available as a cost-subsidized agent for treatment of hypertension in many countries (including Australia) making the cost prohibitive for most patients with PA. Cure of hypertension (with cessation of all antihypertensive medications) occurs in 40–80% of operated patients, and the great majority of the rest are on fewer antihypertensives than preoperatively (84, 447, 497, 509). In contrast, the mean number of medications taken by PA patients to control hypertension after commencing an aldosterone antagonist (if the antagonist is counted as one) surprisingly does not fall (497).

C. Genetic Testing for Familial Forms

As described in section VIC, genetic testing (either by Southern blotting or by a faster and less expensive PCR-based method developed in our laboratory) of peripheral blood DNA for the hybrid CYP11B1/CYP11B2 mutation has revolutionized the detection of FH-I and effectively supplanted dexamethasone suppression testing for this familial form of PA. Within our own center (where genetic testing by long-PCR is readily available), all patients with PA undergo hybrid gene testing to exclude FH-I before proceeding (in those who test negative) to AVS. This is because 1) patients with FH-I can be assumed to have bilateral PA and are not surgical candidates and therefore do not require AVS; 2) patients with FH-I demonstrate excellent clinical responses to small doses of glucocorticoids, which is the treatment of choice for this (but no other) subtype of PA; and 3) detection of an individual with FH-I heralds the need for family screening (again by genetic testing) which may yield potentially large numbers of additional patients with FH-I who are also at risk of hypertension and early death from hemorrhagic stroke, but who will similarly benefit greatly from specific glucocorticoid treatment. It is acknowledged, however, that this subtype of PA is rare (<1% of patients) and the yield from such “unselected” testing is therefore low. Because of this, the recommendation by the Endocrine Society is to test patients with onset of confirmed PA earlier than at 20 yr of age and those who have a family history of PA or of strokes at young age (<40 yr) (163).

The case for screening for germline mutations in KCNJ5 (causing FH-III) and CACNA1H (also associated with familial PA) is not as strong as that for FH-I given that these are even rarer than hybrid gene mutations and, as yet (unlike FH-I), no specific form of treatment exists for patients with PA due to KCNJ5 and CACNA1H mutations. This, of course, may change with time. It could be argued that detection of the T158A, I151S, and G151R mutated forms of KCNJ5 would identify patients more likely to require bilateral adrenalectomy (because of more florid PA and marked adrenal enlargement) than those with G151E mutations, in whom hypertension control can usually be achieved with an MR antagonist. Possibly the strongest case for KCNJ5 and CACNA1H genetic testing at the moment is to identify individuals in whom genetic screening of family members
should be undertaken to facilitate timely institution of medical or surgical treatment specifically directed against aldosterone excess in those who test positive. Because virtually all patients with mutations in these genes have shared early-onset hypertension as a common phenotype, it would be reasonable to restrict KCNJ5 and CACNA1H genetic testing to very young patients with PA.

D. Biochemical Approaches

As a group, patients with APA tend to show more severely disturbed biochemistry than those with BAH. Because of this, attempts have been made to develop multi-parameter biochemical models to distinguish the two subtypes (14, 148). Parameters typically included in such models have included plasma sodium, potassium, total carbon dioxide, aldosterone, and renin. Others have claimed reliable discrimination of APA from BAH by demonstrating greater suppressibility of aldosterone levels in the latter subtype following extracellular volume expansion (539). However, the degree of suppression varies within the subtype in the individual patient (185, 248, 544, 554). A clinical prediction score developed by Küpers et al. (271), who reported presence of a unilateral mass lesion on CT “typical” for APA plus a serum potassium of <3.5 mM or an estimated glomerular filtration rate of at least 100 ml·min⁻¹·1.73 m⁻² to have 100% specificity (but only 53% sensitivity) for unilateral PA, was found to be much less reliable when applied to patients within the German Conn’s Registry (420), except in those under 40 yr of age. Candy et al. (68) also found the clinical prediction score to have a lower specificity (88%) among a United Kingdom cohort of patients with PA, and were unable to show superiority over an imaging-based strategy.

In the 1980s, patients with APA or FH-I were found to differ from those with BAH by demonstrating elevated levels of “hybrid” steroids, 18-hydroxy-cortisol and 18-oxo-cortisol (191, 202, 487, 533, 534, 579). The demonstration of normal levels of “hybrid” steroids in patients with PA, although requiring assays not easy to perform and not widely available, thus potentially represented a means by which BAH could be diagnosed and differentiated from other subtypes. Also of considerable significance was the finding that plasma aldosterone levels in patients with APA failed to rise in response to a change in posture from recumbency to an upright position (48, 177), or to infusions of ANG II (568), as was also the case in patients with FH-I (138, 175, 201, 486), whereas in patients with BAH, responsiveness of plasma aldosterone to these maneuvers was retained, and perhaps even enhanced (177, 568). From such observations arose the proposition that patients with PA who demonstrated significant (for example, >50%) responsiveness of plasma aldosterone to posture or ANG II infusion could be presumed to have BAH, and thus commenced on spironolactone or amiloride treatment without the need to undergo more intensive and invasive investigations to exclude APA.

In 1985, the Greenslopes Hospital Hypertension Unit recognized unilaterality of aldosterone production on AVS in a patient in whom aldosterone was responsive to both posture and ANG II infusion, and whose hypertension was subsequently cured by unilateral adrenalectomy. By 1987, the unit could report six such cases (201, 203). Subsequently, others (144, 243, 374) described similar patients with APA in whom aldosterone demonstrated responsiveness to upright posture and ANG II infusion. Furthermore, urinary levels of 18-hydroxy-cortisol and 18-oxo-cortisol were usually normal (201, 203, 488). These patients thus masqueraded biochemically as BAH, yet, like those with classic ANG II-unresponsive APA, could be cured of hypertension following removal of their tumors by unilateral adrenalectomy (201, 203, 501). The discovery of this new subtype of ANG II-responsive APA meant that APA cannot be excluded on the basis of retained aldosterone responsiveness to posture or ANG II infusion, or normal “hybrid” steroid levels.

Can BAH be excluded on the basis of posture unresponsiveness? It cannot. Importantly, it is not easy to convince patients to remain recumbent overnight from midnight until the first sample is collected, and to remain out of bed until the second (upright) sample is collected 2 h later. In 2003 we reported no difference in aldosterone responsiveness to posture between 14 patients with unilateral PA and 34 patients with with bilateral PA (71 vs. 75%) (497). This followed detection of significant numbers of patients with APA whose aldosterone was responsive to posture, but did confirm the accepted high level of responsiveness in BAH. Nevertheless, lack of posture responsiveness (and indeed other suggestive “markers” of APA, including the presence of hypokalemia, markedly elevated plasma aldosterone levels and ARR, and a unilateral adrenal mass on CT, especially in individuals of less than 40 yr of age) can serve as useful ancillary information to aid in clinical decision making when results of AVS, currently considered to be the “gold standard” method for differentiating unilateral from bilateral PA, are either unavailable or inconclusive (for example, because of failure to successfully cannulate both adrenal veins).

E. Imaging Approaches

Early attempts at imaging APAs involved relatively insensitive and invasive procedures such as abdominal X-ray/tomography with presacral gas insufflation (86) and adrenal venography (99), which carried with it a significant risk of contrast extravasation and adrenal hemorrhage (254, 369).

The advent of adrenal CT represented a significant step forward, having much better resolution than earlier ap-
proaches and being relatively noninvasive. However, CT lacks reliability for differentiating unilateral from bilateral PA as it fails to detect at least one-half of APAs (which are often small, with an average diameter of ~1 cm), and yet may demonstrate nonfunctioning nodules in the contralateral gland and apparently unilateral lesions in patients with BAH (198, 445, 449, 497, 588). Similar limitations apply to adrenal magnetic resonance imaging (92, 290, 439). Radioisotope scanning of the adrenals with radiolabeled selenocholesterol has demonstrated low sensitivity and specificity for APA (198, 543), and is not routinely performed in most centers.

Adrenal CT is essential for the detection of larger mass lesions (for example, >2.5 cm in diameter) which have greater potential for malignant behavior and may warrant consideration for removal on that basis alone, or at the very least deserve careful follow-up.

F. Adrenal Venous Sampling

For reasons discussed in section XI, C and D, AVS has proven to be the only reliable way to differentiate unilateral from bilateral forms of PA. As recommended by the Endocrine Society guideline (163), all patients diagnosed with PA (other than those who are found to have FH-I by genetic testing) within our center undergo AVS, regardless of biochemical or CT findings. Prior to AVS, all undergo CT scanning because it can assist in localizing the adrenal veins (108, 499). Performing AVS in the morning following overnight recumbency avoids the confounding effects of changes in posture on aldosterone concentrations in patients with ANG II-responsive varieties of PA. Furthermore, because endogenous ACTH levels are higher in the early morning than later in the day, morning AVS ensures that samples are collected during maximal ACTH-induced stimulation of aldosterone production (unless, of course, stimulation with exogenous ACTH is employed) (260). A radiologist highly skilled in this technique collects samples from each of the presumed right and left adrenal veins and, simultaneously with each adrenal vein sample, from a “peripheral” vein (either the antecubital fossa vein, inferior vena cava well below the adrenal veins, or iliac vein). At least two samples collected from each side to maximize the chances of successful sampling and as a safeguard should sample handling issues or dilutional errors in the laboratory occur. The risk of adrenal hemorrhage associated with AVS is low (<2% of all procedures) in experienced hands provided adrenal venography is avoided (108, 433).

Examination of the ratio or “gradient” between adrenal and peripheral venous cortisol concentrations permits an assessment of the adequacy of AVS. Within our center, gradients of at least three are taken to indicate adequate sampling. Samples demonstrating gradients of between two and three may provide useful information, but those with gradients of less than two are always excluded from further consideration. Poor reproducibility of AVS subtype diagnosis has been observed when samples with lower cortisol gradients (e.g., less than 2–3) have been included for analysis (85, 347).

Because the adrenal veins are small, successful cannulation during AVS requires a high level of skill and considerable experience. The right adrenal vein tends to be harder to cannulate than the left because it is usually smaller and shorter and, unlike the left adrenal vein, usually empties into the inferior vena cava rather than the renal vein (122). In highly experienced units, success rates can reach 90% or higher (108, 587). Aids to successful cannulation include the use of CT scanning to localize the right adrenal vein prior to AVS (108, 499) and “on-the-spot” plasma cortisol measurement which permits determination of adrenal venous cortisol levels within minutes of collection (326, 575).

Adrenal venous samples can differ considerably in the degree to which they are “diluted” with nonadrenal venous blood. Because of this, direct comparison of aldosterone concentrations in these samples will frequently give misleading results. Furthermore, in a patient with APA, aldosterone concentrations on the side of the contralateral suppressed, “normal” gland, while lower than on the side of the APA, are quite often higher than peripheral, and can thereby give the mistaken impression of bilateral adrenal autonomous aldosterone production. Presumably, the normal gland can still produce small quantities of aldosterone (despite suppression of renin/ANG II) in response to secretagogues such as potassium and ACTH, and, because adrenal venous blood is very slow flowing, only very small amounts of secreted aldosterone are required for concentrations to become substantially higher than peripheral.

Calculating the aldosterone/cortisol ratio for each adrenal and peripheral venous sample corrects for differences in dilution with nonadrenal venous blood. Within our center, if the average aldosterone-to-cortisol ratio on one side is at least two times higher than the simultaneous peripheral venous ratio, with a ratio no higher than peripheral on the other side, the study is considered to show lateralization, indicating that unilateral adrenalectomy should cure or improve the hypertension (198, 499). Criteria for lateralization vary widely from one institution to another, however (432). For example, many centers rely only on the comparison of aldosterone-to-cortisol ratios on one side versus the other (the so-called “lateralization index,” with lateralization defined as the ratio on the higher side being at least 2–4 times, depending on the center, that on the lower side) and do not require contralateral suppression (which involves comparison with peripheral ratios) to meet lateralization criteria. We recently reported contralateral suppression to be an important predictor of clinical outcomes in patients with PA undergoing unilateral adrenalectomy within our
center (573). As reported by Monticone et al. (339), however, the presence of contralateral suppression may be less predictive of outcomes in patients with lateralization indices that are >4.0 (339). Because of the nature of our lateralization criteria, a significant minority of our operated patients have lateralization indexes that fall into the range of 2.0–4.0, and it is possible that the presence of contralateral suppression is of particular importance for predicting hypertensive cure or improvement in this subgroup.

Several groups employ exogenous ACTH stimulation during AVS to 1) maximize adrenal/peripheral venous cortisol gradients, 2) reduce fluctuations in steroid secretion during nonsimultaneous AVS due to changing endogenous ACTH levels, and 3) stimulate aldosterone production by APAs and thus avoid sampling during a period of secretion “quiescence” (163, 338, 587). Concerns have been raised that ACTH stimulation could potentially lead to loss of lateralization by stimulating the contralateral gland in a patient with APA. We have found ACTH stimulation to be helpful in allowing subtype differentiation in patients whose basal AVS results were inconclusive because of apparent quiescence in terms of aldosterone production at the time of AVS (evidenced by adrenal venous aldosterone/cortisol ratios being lower on both sides compared with peripheral) (FIGURE 9). This has contributed to our recent decision to incorporate ACTH stimulation, administered as an intravenous infusion (50 μg/h) so as to ensure adequate and stable concentrations at the time of sampling (587), as a routine part of our AVS protocol. While the majority of studies performed in this way have so far appeared to yield satisfactory results, this has been counterbalanced by some that were less helpful and even confusing. Hence, the utility of ACTH stimulation, at least in our center’s hands, remains an open question.

Measurement of adrenal venous aldosterone and cortisol concentrations requires great care as concentrations of these hormones and their precursors and metabolites (which may have significant cross-reactivity on currently used immunoassays) may be very high, and small errors in sample dilution and assay technique may have a major effect on results. The use of new generation, highly reliable mass spectrometric techniques, which do not require sample dilution, is a major advance in this regard (517).

**G. Recent Innovations**

Traditional AVS provides information about which adrenal, or both, is overproducing aldosterone, but not which part of the gland. Japanese workers have recently reported on the use of “super-selective” AVS, in which different branches of the adrenal vein are selectively cannulated, permitting comparison of aldosterone production by different regions within a gland (59, 60). Although requiring a considerable increase in the duration of the procedure, the potential advantage is that small aldosterone-producing lesions can be localized with sufficient resolution to permit removal by partial, rather than total unilateral, adrenalectomy

<table>
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<th>Aldo (ng/dL)</th>
<th>Cort (μg/dL)</th>
<th>Cort AV/PV</th>
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<tr>
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<td>15</td>
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<td>09:22</td>
<td>42</td>
<td>11</td>
<td>3.8</td>
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</tr>
</tbody>
</table>

**FIGURE 9.** Results of adrenal venous sampling performed both pre- (top rows) and post- (bottom rows) stimulation with intravenous synacthen (ACTH, 250 μg) in a 44-yr-old hypertensive, hypokalemic female who had raised aldosterone-to-renin ratios, positive fludrocortisone suppression testing, and a 15-mm nodule in her right adrenal on computed tomography scanning. Pre ACTH, adrenal venous aldosterone-to-cortisol ratios were lower than peripheral on both sides, suggesting that both adrenals were quiescent in terms of aldosterone production at the time of sampling. Post-ACTH results show unequivocal lateralization of aldosterone production to the right adrenal, consistent with a right aldosterone-producing adenoma. Laparoscopic right adrenalectomy led to cure of hypertension and hypokalemia and normalization of the aldosterone-to-renin ratio.
tomy (385, 452). This is particularly relevant for patients suspected of having bilateral APAs or those who have only one functioning adrenal that may contain an APA, for whom partial adrenalectomy may correct PA and yet leave sufficient normal adrenal in situ to avoid the need for steroid replacement therapy post-operatively. Such patients are relatively uncommon however.

Promising new approaches for differentiating APA from BAH include the use of semiquantification of 6β-[131I]iodomethyl-norcholesterol (NP-59) single photon emission computed tomography (SPECT)/CT (299) and [11C]metomidate positron emission tomography (PET)-CT (63). These scans, which rely on the excessive uptake by cells overproducing aldosterone of a labeled steroid precursor analog or 11β-hydroxylase inhibitor, respectively, capitalize on the advances in radioisotope and CT imaging technology that have occurred in recent years. Whether this will render them sufficiently sensitive and specific to be suitable for routine clinical use, and to be considered as a substitute for AVS, will require validation in different centers and with larger numbers. One disadvantage of the [11C]metomidate approach is the very short (20 min) half-life of this isotope, restricting its use to facilities possessing a cyclotron, but the development of other (for example 18F) radiotracers should overcome this issue.

As discussed in section XI D, measurement of “hybrid steroids” (18 hydroxy- and 18-oxo-cortisol) as a means of differentiating APA from BAH was first described in the 1980s (191, 202). The subsequent two decades saw a decline in its use, resulting in part from the demonstration that normal levels did not exclude the ANG II-responsive variety of APA (which therefore could not be distinguished from BAH by this method) (201, 203, 488), and the lack of ready availability of the reagents needed for these radioimmunoassays. With the recent development of rapid and highly reliable mass spectrometric methods of measuring steroids, there has been a resurgence in interest in hybrid steroid measurement over the past few years. Nakamura et al. (361) reported good differentiation of APA from BAH using lateralization indexes derived from adrenal venous 18 oxo-cortisol/cortisol (measured by LC-MS/MS) ratios. However, these did not appear to be superior to those derived from aldosterone-to-cortisol ratios. Furthermore, it is likely that the majority of the APAs were of the ANG II-unresponsive variety as they were primarily comprised of “clear” (ZF-like) cells, and posture studies were not performed to exclude this possibility. Mulatero et al. (348) reported mean peripheral levels of hybrid steroids, measured by enzyme-linked immunoassay, to be higher in APA than BAH, but with considerable overlap. Satoh et al. (451), using LC-MS/MS, reported a lesser, but still substantial, degree of overlap and found 18-oxo-cortisol to be superior to 18-hydroxy-cortisol in this respect. Even more recently, LC-MS/MS has been used to determine both peripheral and adrenal venous “profiles” of multiple steroids in patients with APA and BAH (394, 566). While such analyses are yet to find a place in the routine workup of this condition, already they have yielded important insights, including the apparent superiority of several steroids over cortisol in defining cannulation success during AVS (129), and the much higher levels of both peripheral and adrenal venous 18-oxo-cortisol levels in patients with APAs bearing somatic KCNJ5 mutations compared with APAs without (566). The latter finding is particularly interesting as it supports the concept that somatic KCNJ5 mutations cause (and are probably the predominant basis for) a phenotype that is synonymous with the ANG II-unresponsive form of APA, with excessive production of hybrid steroids now contributing to the list of other similarities (which includes predominant female gender, younger age, larger tumors, more florid PA, lack of aldosterone responsiveness to upright posture and ZF-like morphology, as discussed in section VIF).

A. Factors Potentially Affecting Aldosterone and Renin Levels and the Aldosterone-to-Renin Ratio

1. Gender and the menstrual cycle

Exogenous estrogen administration stimulates production of the renin substrate angiotensinogen (115, 190). As a result, angiotensin levels rise, and this in turn leads to falls in renin release and in DRC via negative feedback on the juxtaglomerular apparatus (150). Because renin substrate rises but enzyme concentration falls, the production of angiotensin I (and therefore PRA) is stable or only minimally increased (150). Hence, the ARR tends to rise when DRC is
used to measure renin but remains relatively stable when using PRA. Progesterone, secreted during the luteal (second) phase of an ovulatory menstrual cycle, is an MR antagonist (378) and can cause natriuresis. The ensuing reduction in plasma volume brings about compensatory increases in plasma renin and aldosterone. Fluctuations in estrogen and progesterone during the menstrual cycle thus have the potential to influence the ARR.

In a study of healthy, normotensive subjects, Pizzolo et al. (412) reported a higher proportion of women [8 of 51 (14%)] than men [1 of 43 (2%)] to have elevated ARR (using DRC to measure renin). In a separate analysis involving hypertensive subjects with raised ARR, intravenous saline suppression testing confirmed PA by demonstrating nonsuppressible aldosterone in only 39% of the 54 women compared with 85% of the 27 men. These findings suggest that female gender, probably via effects of sex steroids, predispose to false-positive ARR testing.

Among 26 mildly hypertensive women with low renin levels, Fommei et al. (151) reported the proportion with elevated ARR (renin as PRA) combined with an aldosterone concentration of >15 ng/dl (a criterion required by some investigators for positive screening testing) to rise from 27% on day 7 to 68% at day 21 (151). Which of these ARR test results represented false positives or negatives could not be ascertained as confirmatory testing was not performed. Pizzolo et al. (412) reported both aldosterone and DRC concentrations to be higher, but with no change in mean ARR, in the luteal versus follicular phase in 33 healthy, normotensives females.

We reported luteal concentrations of plasma aldosterone, DRC, and PRA to be higher than follicular in 19 healthy, normotensive females (12). The ARR was also higher but only when calculated using DRC and not when using PRA. In two subjects, luteal ARR was elevated to above the reference range when calculated using DRC (but normal using PRA), and aldosterone demonstrated normal suppression during saline infusion, thereby excluding PA and confirming the raised aldosterone-to-DRC ratios as being false positives. Hence, when screening premenopausal women for PA by ARR testing, it may be preferable to use PRA if available, or if not, to screen during the follicular phase or the menses when estrogen and progesterone concentrations are at their lowest rather than the luteal phase.

2. Medications capable of causing false-positive ratios

Because renin production by juxtaglomerular cells is mediated by β-adrenoceptor stimulation, blockade of these receptors brings about profound suppression of renin production and hence plasma levels (196, 387). The resulting fall in aldosterone levels is not as marked, possibly because of ongoing stimulation by plasma potassium and ACTH. Consequently, the ARR rises (10). Methyldopa (387) and clonidine (304) can also raise the ARR as they both reduce central sympathetic outflow. Renin levels are also sup-
pressed in patients receiving nonsteroidal anti-inflammatory agents due to their tendency to induce renal sodium and water retention and to suppress renal prostaglandins which normally enhance release of renin. They also promote retention of potassium leading to stimulation of aldosterone production and further elevation of the ARR (331). While some investigators have questioned whether the potential for these agents to elevate the ARR is sufficient to cause false-positive test results (465, 585), our own experience has been that falsely elevated ratios are not uncommon in patients receiving these drugs. Hence, if the ARR is elevated in this situation, our approach is to withdraw the potentially confounding agent where possible and to repeat the ratio before deciding whether to proceed to confirmatory suppression testing.

Progesterone and some progestogenic agents used in contraceptive preparations can induce natriuresis, probably as they act as MR antagonists, and consequently stimulate renin and aldosterone. Pizzolo et al. (411) recently reported a falsely elevated ARR value (with renin measured as DRC) in a normotensive 34-yr-old female patient taking a combined preparation of drospirenone (a progestogenic agent and potent MR antagonist) and ethinyl estradiol. They subsequently reported the mean ARR to rise in a cohort of 27 women after commencement of a combined preparation of gestodene and estradiol, and the number of positive tests increased from 4 to 9 (412). We recently reported the ARR to rise significantly in 17 normotensive healthy women following commencement of the combined oral contraceptive preparation ethinyl estradiol plus drospirenone (EE+D), but only when renin was measured as DRC and not with PRA (11). Aldosterone/DRC ARR values were elevated above the normal range in three subjects after 3-wk treatment. Rises in aldosterone and PRA occurred as expected, given drospirenone’s MR antagonist properties, but DRC fell. This presumably reflected an effect of the estrogenic component of EE+D, stimulating renin substrate (angiotensinogen) and thus angiotensin production, resulting in greater negative feedback on renin production (and hence DRC) as explained above. In a separate study of 15 other normotensive healthy females, treatment with subdermal etonorgestrel (a progestin-only contraceptive preparation) did not result in significant changes of aldosterone, DRC, PRA, or in ARR calculated by DRC or PRA (11). Hence, at least for women receiving estrogen-containing oral contraceptive agents, the use of PRA may be preferable to DRC when screening for PA.

3. Medications capable of causing false-negative ratios

Medications that stimulate the production of renin are associated with false-negative ratios. Both potassium-wasting (including thiazides) and potassium-sparing diuretics (such as spironolactone, eplerenone, and amiloride), by inducing volume contraction and sympathetic nervous system stimulation, stimulate renin (198, 589). In addition, non-potassium-sparing diuretics increase renal potassium losses, thereby lowering plasma potassium which in turn leads to reduced aldosterone secretion. Renin rises during treatment with dihydropyridine calcium channel antagonists (60, 350) probably because of reflex sympathetic stimulation as blood pressure falls, natriuretic effects, and direct stimulation of calcium-dependent renin regulatory pathways. These agents can also interfere with aldosterone production by blocking intracellular, calcium-dependent steps in biosynthesis (20). Verapamil, in contrast, appears to have minimal if any effects on aldosterone levels (344, 475). Although verapamil has been reported to demonstrate an inhibitory effect on some of the mutated forms of KCNJ5 in vitro (272, 334), intravenous infusion of verapamil to patients with APA (of whom presumably some had somatic KCNJ5 mutations) was reported by Opocher et al. (388) not to have any effect on plasma aldosterone levels in vivo. By blocking production of ANG II or its receptor respectively, ACE inhibitors (350) and ARBs (350) can have profound stimulatory effects on renin by interfering with negative feedback of ANG II on renin production. These agents also inhibit aldosterone production by reducing ANG II stimulation of ZG cells, and this would be expected to lower aldosterone concentrations in patients with ANG II-resistant forms of PA (27).

We recently explored the question as to whether antidepressant medications, being used with increasing frequency in clinical practice, could affect the ARR. Aldosterone, PRA, and DRC levels all rose in normotensive, depressed male patients following commencement of treatment with either of the selective serotonin uptake inhibitors (SSRIs) sertraline (n = 12) or escitalopram (n = 14) (7). Because renin rose to a greater degree, the ARR (calculated by either DRC or PRA) fell significantly in both treatment groups. Further studies involving hypertensive patients are required to address the question as to whether this could lead to false-negative ARR levels in patients with PA.

4. Other medications with the potential to affect the ARR

The newly introduced renin inhibitors, by inhibiting renin’s enzymatic activity, reduce endogenous production of angiotensin I (and therefore lower PRA) and ANG II. Reduced ANG II leads to a reduction in negative feedback on the juxtaglomerular apparatus, causing a rise in production of renin (and hence higher DRC). Therefore, their effects on the ARR will depend on how renin is measured, raising it (and causing false positives) if renin is measured as PRA, and lowering it (causing false negatives) if measured as DRC (66).

5. Posture

With the assumption of upright posture, translocation of blood into the lower limbs causes a fall in renal perfusion
pressure and an increase in sympathetic output and β-adrenergic receptor stimulation, resulting in a rise in renin production by the juxtaglomerular cells and consequently a rise in plasma aldosterone (198, 528). Contributing to the rise in aldosterone levels is a reduction in its metabolic clearance due to reduced hepatic blood flow. Because this effect is more rapid than that of increased renin, close correlation between the rise in aldosterone and that of renin from before to shortly (<1 h) after assuming upright posture may be lost. A longer (at least 2 h) period of ambulation would be expected to permit better correlation in this respect (198, 491).

Among patients with PA, aldosterone demonstrates normal responsiveness to upright posture (defined as a rise in plasma concentrations of at least 50% above basal) in those with ANG II-responsive forms, which includes all with ANG II-responsive APA and most with BAH (201, 203). ACTH assumes a dominant role over ANG II in regulating aldosterone in ANG II-unresponsive forms including ANG II-unresponsive APA or FH-I, and, because ACTH falls during the early morning hours when these posture studies are carried out, patients with those subtypes of PA demonstrate a lack of responsiveness of aldosterone to upright posture or even a fall (201, 203).

It could be anticipated that samples collected for ARR measurement during recumbency might be more sensitive for detecting the ANG II-unresponsive forms, whereas samples collected during upright posture may be more sensitive for detecting the ANG II-responsive forms. In practice, however, upright samples seem to be adequately sensitive for both varieties of PA. This is because upright levels in patients with ANG II-unresponsive forms of PA are similar to those with ANG II-responsive forms (whose recumbent levels are usually much lower). Most centers use a mid-morning upright sample, usually after sitting for 5–15 min. These are more convenient than having to provide recumbent conditions for a period such as 1 h, and appear to be more sensitive overall (401), which is not unexpected as the majority (~70% of patients diagnosed by our center) of patients with PA are ANG II-responsive.

6. Time of day

Because circulating renin and ANG II levels are chronically suppressed in PA, plasma aldosterone concentrations are strongly influenced by ACTH. Demonstrating a striking circadian rhythm, ACTH levels are highest around 0800 h and fall rapidly thereafter (211). Patients with PA are therefore more likely to demonstrate elevated ARR levels in blood collected during the morning rather than in the afternoon (197), when false negative results are likely.

7. Dietary sodium intake

Habitual dietary salt restriction stimulates renin production and may lead to a lowering of the ARR (198, 499). Sensitivity of the ratio is therefore improved if patients maintain a liberal dietary salt intake prior to testing. Conversely, it is likely that in patients with particularly high salt intakes, profound suppression of renin would have the potential to result in false-positive ratios.

8. Plasma potassium concentration

Because potassium stimulates aldosterone secretion, hypokalemia may be associated with false-negative ratios in patients with PA (198, 499). This can be avoided by correcting hypokalemia with supplemental slow-release potassium chloride tablets before ratio measurement. The presence of hypokalemia can be obscured, however, if care is not taken during sample collection to avoid false elevations of potassium concentrations (59). Common contributors to this phenomenon include 1) fist clenching, which drives potassium out of the muscles and into the blood; 2) failure to release the tourniquet while blood is being collected; 3) “difficult” sampling which can cause hemolysis; 4) the use of Vacutainers (which can also cause hemolysis) rather than syringes; 5) failure to separate plasma from cells within 30 min of blood collection, which allows potassium to leave the red blood cells as their metabolism slows down; and 6) measurement of potassium in serum rather than plasma, which results in higher levels due to release of potassium from the cells during clotting (59, 121).

9. Renal dysfunction and ageing

In patients with renal impairment (324), renin levels tend to fall as a result of reduced renin secretory mass and also salt and water retention, while any associated hyperkalemia tends to elevate aldosterone. This can result in false-positive ARR values. False-positive ratios are also encountered in the elderly, in whom falling renin levels accompany gradually reducing renal function (105), while the fall in aldosterone levels is less marked (301).

10. Effects of coexisting conditions

Conditions that may coexist with PA and lead to previously suppressed renin being released from suppression (and thereby resulting in a false-negative ARR) include renal artery stenosis (498), malignant hypertension (356), and pregnancy (210). As mentioned in section IXB, false-positive ratios occur in familial hyperkalemic hypertension (200, 377, 542, 546), a disorder of renal tubular function characterized by excessive resorption of both sodium (with consequent hypertension and suppression of renin) and potassium (causing chronic hyperkalemia, which prevents suppression of aldosterone). In contrast, both renin and aldosterone are suppressed and the ratio therefore normal in Liddle’s syndrome (469), the syndrome of apparent mineralocorticoid excess (481), and hypertensive forms of congenital adrenal hyperplasia due to 11β-hydroxylase deficiency (559) or 17α-hydroxylase deficiency (258).
B. Approaches to the Application of Aldosterone-to-Renin Ratio Testing

Given the complex array of factors and conditions that affect aldosterone and renin levels, it is perhaps not surprising that approaches used to control for factors potentially confounding the ARR have varied considerably from one center to the next. The following approach is currently used by our center (491) and has allowed us to detect large numbers of patients with PA, including those with surgically correctable forms (which make up around a third of patients in our experience) who have been either cured or had hypertension markedly improved following laparoscopic adrenalectomy, and those with bilateral forms who have usually responded well to medical treatment with drugs that antagonize aldosterone action (494) with unilateral adrenalectomy only rarely indicated (510).

1. Preparation for ARR measurement

Patients are encouraged to liberalize (rather than restrict) sodium intake.

Hypokalemia is corrected, after measuring plasma potassium in blood collected slowly using a syringe and needle, avoiding fist clenching during collection, waiting at least 10 s after tourniquet released if it was used to achieve insertion of needle, and ensuring separation of plasma from cells within 30 min of collection. Vacutainer tubes are avoided.

Medications that have the potential to significantly affect the ARR are withdrawn 1) at least 4 wk before testing for diuretics (including spironolactone); 2) at least 2 wk before testing for β-blockers, clonidine, methyldopa, nonsteroidal anti-inflammatory drugs, ACE inhibitors, ARBs, and dihydropyridine calcium blockers.

Other antihypertensive medications that have lesser effects on the ARR, such as verapamil slow-release (with or without hydralazine) and/or prazosin or doxazosin, are commenced where necessary to ensure ongoing control of hypertension.

Estrogen-containing or drospirenone-containing oral contraceptive agents are not withdrawn unless confident that they can safely be replaced with effective, alternative methods of contraception.

Conditions for collection of blood: 1) blood is collected mid-morning, after at least 2 h of ambulation and after being seated for 5–15 min, and 2) blood is collected carefully to avoid stasis and hemolysis (see sect. XIIA1).

Factors to take into account when interpreting results: 1) time of day, recent diet, posture, and length of time in that posture; 2) all medications; 3) method of blood collection including any difficulty; 4) concentration of potassium; 5) renal function; 6) advanced age (over 65 yr); and 7) phase of menstrual cycle in women.

It is important to emphasize the potential danger in ceasing medications in nonhospitalized patients to achieve washout. Although this can be achieved safely in mildly hypertensive patients who are seen frequently, it is more often necessary to commence a relatively renin-neutral drug such as verapamil slow-release (with or without hydralazine) and/or prazosin or doxazosin. Where a potentially interfering medication cannot be withdrawn, useful information can still be obtained by taking into account its known effects when interpreting the ARR result. For example, a normal ARR in the presence of β-blocker treatment would make PA very unlikely, whereas a raised ratio in patients receiving a diuretic, ACE inhibitor, angiotensin receptor blocker, or dihydropyridine calcium blocker would make the diagnosis very likely.

C. Factors Potentially Affecting Results of Confirmatory Testing

For each of the methods of confirmatory testing described in section X, it is necessary to avoid drugs (such as diuretics, ACE inhibitors, angiotensin receptor blockers, and dihydropyridine calcium blockers) which stimulate renin production. This is because each test depends on achieving (or maintaining) “complete” suppression of renin/ANG II to ascertain whether aldosterone synthesis is at least partially autonomous. Measuring renin during the testing protocol serves to determine whether this goal has been achieved. Hence, it is best to maintain patients off renin-stimulating medications after ARR testing has been completed and throughout the remainder of the diagnostic workup for PA, using alternative agents (see sect. XII B) where necessary to maintain hypertension control. Fortunately, for most patients undergoing FST, the bed-rest obtained in the hospital helps to lower blood pressure levels and the ability to closely monitor blood pressures reduces associated risk.

Because plasma potassium is a powerful regulator of aldosterone production, failure to correct hypokalemia during confirmatory testing by adequate supplementation with slow-release KCl tablets has the potential to lead to false-negative test results. Conversely, acute rises in ACTH (as indicated by a rise in plasma cortisol) induced by physical or psychological stress may prevent suppression of aldosterone in patients without PA, thereby leading to false positives, and should be avoided by undertaking testing in a quiet, calm environment.

We recently reported aldosterone levels to be higher in nine premenopausal women with hypertension and raised ARR undergoing FST during the luteal phase than in 12 studied during the follicular phase of the menstrual cycle (13). All
studied during the luteal phase had positive FST, and all three with negative FST were studied during the follicular phase. As expected, progesterone was markedly higher (consistent with ovulation) during the luteal phase. Aldosterone was higher ($P = 0.01$) in women studied in the luteal (but not follicular) phase compared with men. These findings raise the possibility that higher progesterone levels, by inducing natriuresis and stimulation of renin/ANG II, could prevent suppression of aldosterone during FST and thereby lead to false-positive results in patients undergoing FST during the luteal phase. Further studies, preferably comparing results in women undergoing suppression testing during each of the two phases, are required to answer this question.

D. Factors Potentially Affecting Results of Adrenal Venous Sampling

Because ACTH stimulates both aldosterone (acutely) and cortisol (acutely and chronically), stress-induced rises in ACTH may impact on AVS results. Within our center, patients receive prior relaxation training provided by a clinical psychologist and are permitted to listen to music during the procedure in an attempt to minimize stress-induced steroid fluctuations. To avoid the potential for changes in posture to confound AVS results, patients are admitted to the hospital so that they can be maintained in a recumbent position overnight before and throughout the test. Performing AVS in the morning reduces the chance of sampling during a relative period of quiescence in terms of steroid production, which falls rapidly towards the afternoon as part of its circadian rhythm. Despite these measures, as described in section XII above, we have encountered occasional patients in whom neither adrenal appeared to be producing significant amounts of aldosterone at the time of AVS, despite undergoing the procedure during early morning hours, and assume this reflects pulsatile secretion. It is possible that performing AVS under stimulation by exogenously administered ACTH overcomes many or all of the above potential confounders of result interpretation.

Lateralization of aldosterone production during AVS in patients with unilateral forms of PA is reliant on the contralateral gland being suppressed due to chronic suppression of renin/ANG II. Because of this, medications which stimulate renin production are best avoided leading up to AVS as this could result in stimulation of aldosterone production by an otherwise suppressed contralateral adrenal and hence loss of lateralization, giving a mistaken impression of bilateral PA.

XIII. SURGICAL MANAGEMENT

Although treatment decisions rely substantially on the results of AVS and other diagnostic procedures, they should be tailored to the particular characteristics and wishes of the individual patient. Surgical treatment may therefore be inappropriate for some patients who lateralize on AVS and, conversely, may be a reasonable option in rare patients who do not, as is discussed in the following sections. Careful discussion with the patient (and family, if appropriate) is a critical component of treatment. All management options and their possible outcomes should be fully explored and explained before a treatment is chosen.

A. Patient Selection

Approximately 30% of patients with PA who undergo AVS demonstrate clear lateralization, with definite aldosterone production by one adrenal and contralateral suppression of the other (198, 351, 494, 497). These patients are candidates for unilateral adrenalectomy, which results in cure of hypertension in 50–60% and significant improvement in the remainder (84, 207, 447, 494, 501) (FIGURE 10). To obtain optimal results from surgery, an essential prerequisite is to have performed suppression testing and successful AVS, that is, all appropriate criteria have been met. Importantly, cure or improvement is universally seen regardless of whether patients are hypokalemic or normokalemic preoperatively (501).

In patients with bilateral forms of PA, rarely it may be appropriate to consider and carefully discuss with the patient the option of unilateral adrenalectomy. For example, both spironolactone and, less commonly, amiloride have...
sometimes been tolerated poorly even at low doses, or the dose of spironolactone required to control hypertension has produced adverse effects, such as sexual dysfunction, painful gynecomastia in males, and mastalgia and menstrual disturbance in females. The appropriate action in this situation is to remove the gland that has the higher aldosterone-to-cortisol ratio on AVS, or if both are equally affected, the larger gland. As expected, the likelihood of benefit is less than with clearly lateralizing PA (507).

If AVS demonstrates BAH but one adrenal contains a mass 2.5 cm or larger (some centers use higher cutoffs of 3.0 cm or even 4.0 cm), the surgical option should also be considered and discussed because of the risk of developing a malignancy (499).

For rare patients with marked, bilateral adrenal hyperplasia and severe, bilateral PA (including those with severe forms of FH-III), bilateral adrenalectomy (often in 2 stages, to gauge the effect of unilateral adrenalectomy first) may be required to control hypertension and biochemical manifestations of PA (90, 94, 484).

B. Pre- and Peri-operative Management

Patients who have been treated for several months preoperatively with aldosterone antagonist agents such as spironolactone, eplerenone, or amiloride (see below) usually demonstrate a “smoother” peri-operative course, with blood pressure levels that are easier to control and being less likely to develop hypokalemia once medications are withdrawn postoperatively. In some patients with lateralizing PA whose hypertension has been particularly severe and caused substantial hypertensive heart disease, it may be advisable to defer surgery for several months so as to allow a period of preoperative treatment with an aldosterone antagonist, the aim being to reduce operative risk by improving cardiovascular function through optimization of hypertension control, repletion of body potassium stores, and blockade of other adverse effects of aldosterone excess.

Laparoscopic adrenalectomy is now the favored surgical approach as it is associated with less pain and a shorter time to recovery than with the previously used open technique (166, 446). Conversion to an open operation is rarely required, the usual prompts being unusual anatomy or significant obesity (446).

Because AVS only indicates which adrenal is at fault in patients with unilateral PA, it cannot confirm whether a nodule visualized on CT scanning is actually an APA rather than a nonsecreting nodule which happens to be situated in the same gland as a smaller, nonvisualized autonomously secreting lesion. For this reason, the entire adrenal is almost always removed even when an apparent adenoma is seen on CT. Exceptions include patients in whom 1) AVS is compli- cated by adrenal hemorrhage (with potential loss of function of the involved gland) and lateralizes to the contralateral adrenal which contains a definite nodule on CT, 2) PA recurs in association with a definite nodule on CT in the remaining adrenal some years after unilateral adrenalectomy for APA, and 3) bilateral APA is suspected (for example, on the basis of particularly florid PA, lack of responsiveness of aldosterone to upright posture, and bilateral discreet adrenal mass lesions on CT) in a patient in whom AVS is nonlateralizing. In these situations, consideration could be given to remove the nodule (or nodules if bilateral) and preserve the residual adrenal tissue, provided it appears morphologically normal and the blood supply can be preserved, and thereby potentially avoid the need for replacement steroid therapy. This approach does, however, carry a risk of persistent PA or a further recurrence. As discussed in section XI.G, “super-selective” AVS has been used to provide greater confidence that visualized lesions in such circumstances are indeed the source of aldosterone excess (385, 452), but this procedure is only performed in a very small number of centers.

In our center, following unilateral adrenalectomy for APA, plasma potassium levels are monitored at least twice daily for the first 1–2 days and at least daily thereafter until discharge from the hospital, which is most often by day 4 post-surgery by which time plasma potassium, volume status, and blood pressure levels have usually stabilized; many other institutions discharge earlier than this however. Provided that plasma potassium does not drop to <3.0 mM, all potassium replacement is withheld during the operation and in the first 24 h postoperatively. For concentrations <3.0 mM, potassium replacement is given only with extreme caution (at half the usual rate), since aldosterone production by the remaining adrenal gland may be markedly suppressed because of chronic suppression of renin/ANG II, causing plasma aldosterone levels to drop to very low values following removal of the APA. This is less likely if the patient has received aldosterone antagonist treatment in the weeks-months leading up to surgery. Postoperative fluids should be given as normal saline, 1 liter every 12 or 8 h, again on the assumption that aldosterone levels may be very low following surgery with therefore a tendency to urinary salt wasting. Potassium sparing diuretics (spironolactone, eplerenone, and amiloride) are withheld peri-operatively. Other antihypertensives can generally be withheld, and reintroduced over the next few days if required to keep blood pressure in the normal range. Although it is sometimes possible to discharge patients from the hospital off all antihypertensives, more commonly gradual withdrawal of antihypertensive medications occurs over the ensuing 3–12 mo. In our center, all operated patients have been cured of hypertension or have required substantially reduced medications following unilateral adrenalectomy.
C. Importance of Recognizing Concurrent Autonomous Cortisol Secretion

Simultaneous autonomous overproduction of cortisol and aldosterone by an APA is increasingly recognized although still apparently uncommon (1, 134, 155, 156, 230, 478). Because cortisol levels are used during AVS to judge success of adrenal venous cannulation and to correct for differences in dilution of adrenal with nonadrenal venous blood when assessing for lateralization, unilateral cortisol overproduction with contralateral suppression could confound the interpretation of AVS results (213). Furthermore, if a patient with unilateral PA is suspected of having concomitant autonomous adrenal overproduction of cortisol, the need for steroid cover peri-operatively and for a variable period following surgery should be anticipated. For these reasons, overnight dexamethasone suppression testing with basal (predexamethasone) ACTH measurement is performed on all patients undergoing diagnostic workup for PA in our center. Failure of serum cortisol to suppress to <2 μg/100 ml (<50 nM), accompanied by a low basal ACTH, suggests concomitant autonomous adrenal overproduction of cortisol.

D. Post-surgical Assessment

Always invariably, hypokalemia (when present preoperatively) resolves following unilateral adrenalectomy for unilateral PA. Normalization of the ARR is another reassuring sign that PA has been biochemically “cured,” even if blood pressure has not returned to normal (which it may with time, unless the patient has reasons other than PA which are contributing to persisting hypertension). Commonly, serum creatinine rises after surgery, but this reflects correction of the volume expanded state and glomerular hyperfiltration (caused by excessive sodium retention) that existed prior to adrenalectomy rather than a deterioration in renal function per se (464). Within our center, patients are encouraged to undergo FST 1–3 mo post-operatively to detect any autonomous aldosterone production by the remaining adrenal. Preoperative lateralization on AVS has been associated with either biochemical cure (70%) of PA or significant improvement (30%) on post-operative FST (447). By 3–6 mo post-operatively, repeat assessments of left ventricular mass index on echocardiography and urinary protein and albumin excretion commonly show improvements in these parameters and eventually even resolution where abnormalities existed prior to surgery.

XIV. MEDICAL MANAGEMENT

In patients with PA who do not lateralize aldosterone production to one side, or who lateralize but prefer medical to surgical treatment or are unfit for surgery, treatment with specific drugs that block aldosterone action is indicated.

These medications correct hypokalemia easily and swiftly in all but the severest cases of PA, and potassium supplements should therefore usually be ceased when they are commenced. Overtreatment with these agents can cause volume contraction with prerenal failure, raising creatinine levels, and potentially life-threatening hyperkalemia (198, 207). They should therefore be used with great caution in patients with existing renal impairment. In patients with reduced renal glomerular function, concurrent administration of a potassium-wasting diuretic in low dosage can be helpful to avoid hyperkalemia, but potassium and creatinine levels should still be carefully monitored (207, 499). Hyperkalemia is more likely in patients who are taking other potassium-retaining agents such as ACE inhibitors, ARBs, or nonsteroidal anti-inflammatory drugs (NSAIDs).

For drugs that inhibit or antagonize aldosterone action, the onset of action on blood pressure is slow. Weeks or even months should be allowed to elapse before their full effect on blood pressure is assessed at each dose. Very small doses (for example, spironolactone 12.5 mg once or twice daily, amiloride 2.5 mg once or twice daily, and eplerenone 25 mg once or twice daily) should be initiated (207, 499). At these doses, these drugs are usually well tolerated. If necessary, other antihypertensive agents with more rapid onset of action could be employed during this period, and then later reduced or withdrawn.

A. Spironolactone

Spironolactone has a steroidal structure and competitively inhibits aldosterone at its receptor. As spironolactone shows inhibitory activity at the testosterone receptor and agonist activity at the progesterone receptor, its use can be associated with sex-steroid-related adverse effects, including gynecomastia and loss of libido, menstrual irregularities, and aggravation of breast fibrocystic change (289). The incidence is dose-related. At 12.5–50 mg daily, the incidence of gynecomastia is ~10–15% (409, 497) while at doses of 150 mg daily or more it exceeds 50% (250). Gynecomastia and other side effects can often be avoided by utilizing a combination of spironolactone (in lower dosage) and amiloride. Where available, canrenone (an active metabolite of spironolactone) or potassium canrenoate (the open E-ring water soluble congener of canrenone) might be considered. As they do not require CYP450 activation in the liver, they may be advantageous in some patients (for example, those with hepatic dysfunction).

B. Eplerenone

Eplerenone is another option for patients in whom spironolactone is poorly tolerated and where amiloride is unable to achieve sufficient aldosterone blockade. Eplerenone is an MR antagonist that appears to be more selective for the
ACE inhibitors to lower blood pressure would seem to be ANG II in PA are suppressed, and hence the capability for patients with PA. Although circulating levels of renin and (75, 237, 358) have been shown to lower blood pressure in ACE inhibitors (215, 309) and calcium channel antagonists favor one class of agents over another in this situation. Both to induce or worsen hypokalemia, there is little evidence to wasting diuretics (other than in occasional patients with aldosterone antagonists) because of the propensity of aldosterone exerts its effects. Because of its less potent aldosterone antagonist action and its much lower propensity to induce adverse effects, amiloride may be the drug of first choice in many patients, particularly those with milder degrees of hypertension, biochemical disturbance, and target organ effects (for example, no echocardiographically demonstrated left ventricular hypertrophy). It could be argued that because amiloride does not block the MR, it may not have equivalent benefits to MR antagonists in preventing or reversing non-blood pressure-dependent adverse cardiovascular and renal effects of aldosterone excess. However, because both experimental and clinical data suggest that these adverse effects are profoundly salt-dependent (83, 405), it is possible that they can be ameliorated by amiloride’s natriuretic action, and perhaps to a sufficient degree to completely prevent or reverse them in patients with relatively mild PA. Studies comparing the efficacy of amiloride to that of MR antagonists in this context are needed to definitively answer this question.

C. Amiloride

Amiloride (and triamterene) acts directly on the epithelial sodium channel where aldosterone exerts its effects. Because of its less potent aldosterone antagonist action and its much lower propensity to induce adverse effects, amiloride may be the drug of first choice in many patients, particularly those with milder degrees of hypertension, biochemical disturbance, and target organ effects (for example, no echocardiographically demonstrated left ventricular hypertrophy). It could be argued that because amiloride does not block the MR, it may not have equivalent benefits to MR antagonists in preventing or reversing non-blood pressure-dependent adverse cardiovascular and renal effects of aldosterone excess. However, because both experimental and clinical data suggest that these adverse effects are profoundly salt-dependent (83, 405), it is possible that they can be ameliorated by amiloride’s natriuretic action, and perhaps to a sufficient degree to completely prevent or reverse them in patients with relatively mild PA. Studies comparing the efficacy of amiloride to that of MR antagonists in this context are needed to definitively answer this question.

D. Other Antihypertensives

In many patients with PA being treated medically with agents that antagonize aldosterone action, achievement of hypertension control requires the addition of other antihypertensive medications. Apart from avoiding potassium-wasting diuretics (other than in occasional patients with reduced glomerular function who are prone to hyperkalemia on aldosterone antagonists) because of the propensity to induce or worsen hypokalemia, there is little evidence to favor one class of agents over another in this situation. Both ACE inhibitors (215, 309) and calcium channel antagonists (75, 237, 358) have been shown to lower blood pressure in patients with PA. Although circulating levels of renin and ANG II in PA are suppressed, and hence the capability for ACE inhibitors to lower blood pressure would seem to be limited, it is possible that the effectiveness of these agents results from blockade of the tissue renin-angiotensin system (which is not suppressed in PA). Furthermore, circulating renin levels may become unsuppressed in patients receiving sufficient doses of aldosterone antagonist agents, thereby potentially increasing the efficacy of medications (like ACE inhibitors and ARBs) which block the renin-angiotensin system. Synthesis of aldosterone in ZG cells is dependent on rises in intracellular calcium, and falls in aldosterone levels induced by calcium channel blockers in patients with PA have been reported (75, 358, 541). However, it is unclear whether this effect is sufficient to lower blood pressure to a significantly greater degree than by other, less-specific actions of calcium channel blockade.

E. Treatment of Familial Hyperaldosteronism

Glucocorticoids are highly effective for the long-term treatment of FH-I. In our experience, most patients are able to maintain control of hypertension on doses of dexamethasone as low as 0.125–0.25 mg/day (485). Previously recommended higher daily doses (0.5–2.0 mg; Refs. 172, 557), in most patients, induce complete suppression of circadian cortisol levels (490), and therefore of ACTH and presumably hybrid gene expression. In contrast, the lower doses that we have used in our center are associated with only partial suppression of cortisol, consistent with a lower total glucocorticoid level and therefore a lower risk of side effects. As expected, hybrid gene expression is not completely suppressed at these doses, as evidenced by suppressed PRA, elevated ARR, and elevated urinary 18-oxo-cortisol levels and tight correlation of circadian aldosterone with cortisol (rather than PRA) levels (485). It therefore appears that hypertension control can be achieved without having to normalize urinary 18-oxo-cortisol levels and completely abolish ACTH-regulated aldosterone overproduction. Our center’s approach is to use the lowest dose of glucocorticoid treatment required to maintain normal clinic, home, and ambulatory blood pressures and normal left ventricular mass index and diastolic function on periodic (for example, yearly) echocardiographic assessments. Patients also undergo dual energy X-ray absorptiometry every 2–3 yr to monitor for the development of glucocorticoid-induced osteoporosis.

Spironolactone, eplerenone, and amiloride are alternative options in the treatment of hypertension in FH-I. Amiloride or eplerenone may be preferable for affected children, since they avoid the potential problems of growth-retardation associated with glucocorticoids and androgen blockade with spironolactone.

F. Role of Dietary Salt Restriction

In both animals and humans, excessive endogenous aldosterone and dietary salt intake appear to interact to induce
target-organ deterioration. Pimenta et al. demonstrated that both left ventricular mass (403) and degree of proteinuria (404) in patients with PA were dependent on 24 h sodium excretion rate (as a marker of dietary salt intake). Increased dietary sodium and aldosterone were also shown by these workers to be related to severity of obstructive sleep apnea in patients with resistant hypertension (406). Incorporation of dietary salt restriction into the management of patients with PA may therefore help to limit and perhaps even reverse target organ damage and morbidity.

G. Use of Laboratory Testing to Guide Management

Provided other agents that affect renin levels are not co-administered, the extent to which renin levels become “unsuppressed” is a useful indication of the degree of blockade of aldosterone effect induced by any given treatment dose of spironolactone, eplerenone, or amiloride. Given that aldosterone excess is now known to induce adverse cardiovascular and renal effects independently of its effects on blood pressure, it could be argued that treatment should strive for “complete” reversal of excessive aldosterone action rather than just normalization of potassium and blood pressure levels. Normalization of renin levels may be more reassuring in this sense, since it indicates that the dose of mineralocorticoid antagonist is adequate to correct the sodium/volume expansion and is therefore providing better long-term protection against aldosterone-induced cardiovascular and renal injury (the development of which appears to be dependent on sodium balance). In some patients, tolerability will limit the degree to which the dose of spironolactone can be increased to achieve normalization of renin levels, since even at doses of 12.5–25 mg daily, side effects (such as gynecomastia in males and menstrual irregularity in females) are not uncommon. These are not seen during treatment with amiloride of eplerenone, which therefore represent options for alternative, or additive (spironolactone “dose-sparing”), treatment in such circumstances. The serious risk of developing hyperkalemia and uremia with any agent blocking aldosterone is dose-dependent so that initial doses should be low and only gradually increased.

Provided that other medications are not affecting renin levels, measurement of renin is also helpful in guiding changes in management when hypertension has not yet become optimally controlled following introduction of mineralocorticoid antagonist treatment. For example, persisting hypertension may be better treated by commencing or increasing the dose of other antihypertensive medications rather than increasing aldosterone blockade if renin levels have already become “unsuppressed.” However, just as renin suppression can persist for long periods after “cure” of unilateral PA by adrenalectomy if PA was longstanding, it may therefore also take some time for renin to become “unsuppressed” even with “complete” pharmacological blockade of the MR.

H. Emerging New Therapies

Aldosterone synthase inhibitors are in development and hold some promise as alternative treatment approaches for PA, but more information is required to confirm their efficacy and safety in treating PA. Remaining challenges include lack of specificity for aldosterone synthase (with evidence, for example, of reduced cortisol synthesizing capacity) and inferior treatment effect compared with MR antagonism (19). Nonsteroidal dihydropyridine-based MR antagonists (36, 132) are a new drug class that have displayed similar in vitro potency to spironolactone, and without apparent actions on androgen and progesterone receptors and therefore presumably without the same risk of sex-steroid-related side effects. Finerenone, the first-in-class of this new generation of drugs, is currently in development for treatment of heart failure (36), with early results showing considerable promise (408), and studies in PA are awaited with interest.

XV. LONG-TERM OUTCOMES IN PATIENTS TREATED SURGICALLY OR MEDICALLY: A VERY IMPORTANT BUT UNCHARTED AREA

While the short- to medium-term (mostly ranging from several months to several years) outcomes of treatment of PA have been extensively studied and reported, little is known about outcomes extending into the long term (for example, 10 or even 20 yr). Outcome studies have sometimes included patients followed for such long periods combined with much larger numbers followed for shorter durations (437), but studies dedicated to exclusively assessing large cohorts followed long term are lacking. Such information would be highly valuable, having the potential to answer such critical questions as the following: 1) In patients who have undergone unilateral adrenalectomy for unilateral PA, what are the rates over the long term of recurrence of hypertension (or progression if hypertension was improved but not cured), recurrence (or progression) of biochemical evidence of autonomous aldosterone production, development (or progression) of abnormal adrenal morphology on CT scanning, and cardiovascular events (such as heart attack and stroke) and mortality? 2) What are the predictors of these long term events? 3) How do these long-term outcomes compare with those of patients with PA treated medically? 4) How do outcomes in patients with familial forms of PA (including FH-I and FH-II) compare with those of patients with apparently sporadic PA? With over 1,100 patients with PA followed up for over 10 yr and over 300 for over 20 yr after institution of specific surgical or medical
treatment, our center is in a strong position to provide high-quality data on these issues, adding valuable new information on the natural history of various forms of PA, the comparative effects of different treatment modalities, and whether familial forms differ from nonfamilial forms and from each other in this respect. From a clinical perspective, the identification of predictors of outcome may help guide future management decisions.

XVI. RELEVANCE OF NEW DETAILED KNOWLEDGE OF RENAL SALT HANDLING AND BLOOD PRESSURE REGULATION

Disturbance of distal renal salt balance can involve multiple mechanisms as expertly reviewed by McDonough and Nguyen (322), and this may include dysregulation of the thiazide-sensitive NaCl cotransporter (NCC), an important contributor to hypertension. This is demonstrated in familial hyperkalemic hypertension (Gordon syndrome) (56, 188, 567) and also by the efficacy of thiazide diuretics. These are powerful antihypertensives but can cause significant problems (hypokalemia, gout, diabetes, and dyslipidemia). Other drugs that target this pathway are needed. Although the importance of the NCC has long been recognized, it is only with recent advances in identification and clarification of the many different mutations resulting in Gordon syndrome that some of the molecular mechanisms that control the NCC [including the With No Lysine (WNK) kinases type 1 and 4, acting via the STE-20 kinases SPAK and OSR1 and regulated by Cullin 3 (CUL3) and Kelch-like 3 (KLHL3)] have been elucidated (56, 186, 187, 253, 376, 567). Any role for the renin-angiotensin-aldosterone system in governing these pathways is still unclear, but a small body of animal/ex vivo cell evidence suggests that aldosterone not only influences ENaC expression, but also (surprisingly) moderates NCC expression and phosphorylation by uncertain mechanisms (79, 273).

Recent progress in the isolation of urinary exosomes has provided the opportunity to measure NCC in urine, allowing us to examine the effects of aldosterone on the NCC in vivo. Exosomes are cell-derived vesicles present in most biological fluids, including urine, which contain constituents of the cells of origin (448). This includes channels such as the NCC, which can thus be quantified (407). Preliminary analysis using a crude technique to isolate exosomes has suggested that NCC expression may be higher in patients with PA than essential hypertensives (536). Accurate quantification of urinary exosomes has been hampered by interference from Tamm Horsfall protein (THP), which is present in urine at high concentration and agglutinates particularly in frozen samples to trap exosomes. In our laboratory we have successfully quantified NCC in crude exosomes, but more importantly, after a number of experimental iterations have also successfully applied a recently described method to remove THP from previously frozen urine without degrading exosomes (232). This technique possibly had not been used previously to quantitate NCC and its phosphorylated isoforms (pNCC), and may represent a major step forward in test performance. We have so far been able to demonstrate increases in urinary exosomal NCC and pNCC excretion in patients during sustained “artificial” activation of the MR by fludrocortisone during aldosterone suppression testing for PA (572). Recent studies have demonstrated that low K+ stimulates phosphorylation of NCC in a manner that is independent of the renin-ANG II-aldosterone system (520). This appears to involve cell hyperpolarization, which leads to a reduction in cytosolic chloride concentration that in turn triggers phosphorylation of WNK4 and SPAK. In this way, hypokalemia may exacerbate hypertension in patients with PA by worsening the degree of sodium retention. Conversely, it is possible that potassium supplementation in PA may help alleviate hypertension by inhibiting the NCC. Hence, the interaction between the renin-ANG II-aldosterone system, K+, and NCC appears to be a complex one. Clarifying the role that MR activation plays in NCC regulation will help further elucidate mechanisms that drive hypertension, and quantification of these channels may translate into useful tests to guide individualized antihypertensive therapy. Importantly, this may also translate into a tool for monitoring treatment in PA, going beyond aldosterone measurement to measuring functional effects of aldosterone on the kidney.

XVII. CONCLUSIONS

The past few decades have witnessed major changes in thinking, especially in terms of the role of aldosterone excess as a much greater contributor to hypertension, cardiovascular disease including stroke and heart attack, renal failure, and reduced quality of life than was previously thought. With recent appreciation that specific treatment directed against aldosterone excess reverses these harmful effects to a much greater degree than nonspecific antihypertensive medication, rates of screening for PA have significantly increased. Advances in confirmatory tests, identification of subtypes (including potentially curable unilateral forms), and treatment have progressed rapidly and continue to do so. Major challenges, such as further elucidating the etiology of PA and improving assay methodology, approaches to screening, confirmatory testing, and subtype differentiation, remain and represent opportunities for further progress into the future.

ACKNOWLEDGMENTS

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GRANTS

Work described in this review was supported by funding from the National Health and Medical Research Council of Australia, the Heart Foundation of Australia, the Princess Alexandra Research Support Scheme, and the University of Queensland Irene Patricia Hunt Memorial Hypertension Research Fund.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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