Aldosterone: Direct Effects on and Production by the Heart

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The main effects of aldosterone, the most physiologically important mineralocorticoid, are on electrolyte transport across epithelia, particularly in the kidney, but also in other tissues, such as salivary glands and colon. Aldosterone acts to increase sodium (and consequently water) resorption and potassium excretion by directly or indirectly increasing the activity of epithelial sodium channels and sodium, potassium-adenosine triphosphatase (1). Aldosterone excess, whether from genetic causes or primary aldosteronism (hypertension or aldosterone-secreting adenomas), is well documented to cause hypertension. Hypertension, in turn, has significant adverse effects on the cardiovascular system, including left ventricular hypertrophy and cardiac fibrosis.

Clinical evidence has been accumulating at an accelerating rate suggesting that aldosterone has direct adverse effects on the heart that are independent of its effects on blood pressure. Patients with primary aldosteronism are more likely to have left ventricular hypertrophy and stroke than patients with essential hypertension of comparable severity. More importantly, patients with severe heart failure have a 30% reduction in morbidity and mortality when given a mineralocorticoid receptor antagonist, spironolactone, in addition to conventional therapy of an angiotensin-converting enzyme (ACE) inhibitor, digoxin, and furosemide. The dose of spironolactone used in this study (25 mg) had no incremental effect on blood pressure, suggesting a direct cardioprotective effect (2). Remarkably, spironolactone had a significant beneficial effect only in patients with above median baseline levels of a marker of collagen synthesis, procollagen type III amino-terminal peptide. Elevated baseline levels of this peptide were associated with an increased risk of death and hospitalization, and circulating levels were decreased by spironolactone treatment. These results suggest that the limitation of cardiac fibrosis may be one of mechanisms by which spironolactone benefits patients with congestive heart failure (3).

The present article reviews recent evidence for direct cardiac effects of aldosterone and synthesis of aldosterone within the heart. Details of the role of aldosterone in congestive heart failure and the use of aldosterone antagonists in treating heart disease can be obtained from other reviews (4).

Direct cardiac effects of mineralocorticoids

For aldosterone (or other mineralocorticoids such as deoxycorticosterone) to have direct, specific effects in the heart, it is necessary that the mineralocorticoid receptor be expressed. Moreover, glucocorticoids are potential ligands for the mineralocorticoid receptor and are normally prevented from occupying it by 11β-hydroxysteroid dehydrogenase-2 (11-HSD2), an enzyme in mineralocorticoid target tissues that converts cortisol and corticosterone to inactive cortisone and 11-dehydrocorticosterone, respectively (5). In addition, aldosterone may have rapid nongenomic effects on cardiac function through pathways that have yet to be completely defined (6–9). However, most of the pathological processes discussed in this review have a time course measured in weeks, consistent with effects mediated by classic mineralocorticoid receptors.

In fact, both the mineralocorticoid receptor and 11-HSD2 are expressed in the human heart (10–12), consistent with the idea that aldosterone could affect the heart directly and not just through its pressor effects. However, the heart is comprised of several types of cells, including cardiomyocytes, fibroblasts, endothelial cells, neurons, and vascular smooth muscle cells. Moreover, these cell types are not functionally equivalent throughout the heart due to (for example) the dramatically different pressure loads experienced by the various chambers of the heart. The potential cardiac effects that aldosterone might have obviously depend on the cell types and regions in which the mineralocorticoid receptor and 11-HSD2 are expressed.

Effects of aldosterone on cardiac fibrosis. The most widely used animal model for studying cardiac effects of aldosterone is the uninephrectomized rat given 0.9% saline to drink. Aldosterone may be continuously administered by osmotic minipump, in which case severe cardiac hypertrophy and fibrosis develop within weeks. Although severe hypertension typically develops in this model, cardiac hypertrophy and fibrosis are blocked by spironolactone at doses that do not ameliorate the hypertension (13, 14).

These effects of aldosterone may involve direct stimulation of proliferation of cardiac myocytes and fibroblasts as well as a reparative response to inflammation and cell death (15).

There are conflicting data concerning the presence of mineralocorticoid receptors in cultured rat cardiac fibroblasts (16, 17) and similarly inconsistent results concerning the effects of aldosterone on collagen synthesis in such cells (17–20). However, in a recent detailed study aldosterone increased the proliferation of adult rat cardiac myofibroblasts by activating Kirsten Ras (Ki-RasA) and its effector, the MAPK1/2 cascade. It increased absolute and active (GTP-
inflammatory lesions, which were characterized by mono-
tophological analysis of the heart revealed severe coronary
phenotype at least in part by increasing oxidative stress (23).

Some of the discrepancies between these studies may re-
fect technical differences that result in important phenotypic
differences in the cultured cells. Moreover, it is difficult to
e extrapolate the results of in vitro studies to an intact tissue
consisting of several different cell types. Thus, it is uncertain
whether aldosterone affects cardiac fibrosis in vivo by a direct
effect on fibroblasts or an indirect mechanism.

The effects of aldosterone on the proliferation of noncar-
diac fibrous tissue have been studied using an sc croton
oil-induced pouch model. Spironolactone significantly in-
hits pouch growth and hydroxyproline concentration. Ad-
renalectomy did not ameliorate, and infused aldosterone did
not exacerbate, pouch formation. Mineralocorticoid receptor
expression in pouch tissue was demonstrated by RT-PCR as
well as both isozymes of 11-HSD. These data are consistent
with extraadrenal synthesis of mineralocorticoids, whose ef-
fects on the proliferation of fibrous tissue are blocked by
spironolactone (22).

Effects of aldosterone on perivascular inflammation in the heart.

After 4–5 wk of treating rats with aldosterone and salt, mac-
rphages, lymphocytes, and proliferating endothelial and
vascular smooth muscle cells and fibroblast-like cells are
seen in the perivascular space of intramural coronary arteries
and at sites of lost cardiomyocytes in both ventricles. At the
same time, fibrosis is induced, as evidenced by a significant
increase in ventricular collagen volume fraction. These
changes are accompanied by increases in mRNA for proin-
flammatory mediators, including intracellular adhesion mol-
ecule 1, monocyte chemoattactant protein 1, and TNFα (23).

Chronic aldosterone and salt treatment increases the ex-
pression of NADPH oxidase, as evidenced by increases in its
heme-containing subunit, gp91phox. NADPH oxidase cata-
lyzes the formation of superoxide anion, which can react
with nitric oxide to form peroxynitrite. This, in turn, modifies
tyrosine residues on proteins to 3-nitrotyrosine, which can be
detected immunologically and used as a marker for super-
oxide formation. As measured by this method, NADPH ox-
idase activity in myocardium is also increased by aldoste-
rone. These changes are concentrated in inflammatory and
endothelial cells in the perivascular space. Nuclear factor-κB,
a transcription factor that is a key regulator of host defense
responses, is activated in the same sites, as measured by
immunohistochemistry for its RelA subunit. Spironolactone
and two antioxidants, pyrrolidine dithiocarbamate and
N-acetylcysteine, each attenuate all of these responses, sug-
gesting that aldosterone and salt induce a proinflammatory
phenotype at least in part by increasing oxidative stress (23).

In a very similar study by different researchers (24), his-
topathological analysis of the heart revealed severe coronary
inflammatory lesions, which were characterized by mono-
cyte/macrophage infiltration and which resulted in focal
ischemic and necrotic changes. The histological evidence of
coronary lesions was preceded by and associated with the
elevation of cyclooxygenase-2, macrophage chemoattractant
protein-1, and osteopontin mRNA expression, measured by
quantitative RT-PCR. Eplerenone, a highly selective miner-
alo corticoid antagonist, attenuated proinflammatory mole-
cule expression in the rat heart and subsequent vascular and
myocardial damage. In contrast to other studies, no signif-
cient increases in myocardial interstitial collagen fraction or
hydroxyproline concentration were detected.

Similar effects can be induced by other mineralocorticoids.
A single injection of deoxycorticosterone in oil is a potent
inducer of cardiac fibrosis in this model and increases cy-
clooxygenase 2 and osteopontin expression. Moreover, in-
hibition of 11-HSD2 with carbenoxolone allows glucocorti-
coids to occupy the mineralocorticoid receptor, and this has
the same effect (25).

Thus, treatment with mineralocorticoids (either deoxycor-
ticosterone or aldosterone) and salt in uninephrectomized
rats lead to severe hypertension and the development of a
vascular inflammatory phenotype in the heart, which may
represent one mechanism by which aldosterone contributes
to myocardial disease.

The development of perivascular inflammation is likely
to be due in part to endothelial dysfunction permitting
adhesion of inflammatory cells to the vascular wall and
egress into the perivascular space. Indeed, aldosterone
causes endothelial dysfunction in humans in vivo (26),
possibly by decreasing nitric oxide through superoxide
generation, as discussed above. Eplerenone improves en-
dothelial dysfunction (as measured by the vasodilator re-
response to acetylcholine) and decreases superoxide forma-
tion in aortas from rabbits fed 1% cholesterol (27). It is also
noteworthy that endothelin receptor antagonists are able
to ameliorate inflammatory markers and fibrosis in rats
 treated with deoxycorticosterone and salt (28, 29).

Effects on cardiomyocytes. The fact that primary aldosteronism
is associated with greater left ventricular hypertrophy than
is seen in other cases of hypertension of similar severity
implies that aldosterone might directly affect the growth
of cardiomyocytes. Aldosterone exposure for 24 h increases
calcium currents in adult rat ventricular cardiomyocytes, as
measured by patch clamp. This effect is inhibited by cyclo-
heximide, suggesting that it requires protein synthesis, and
it is blocked by spironolactone, indicating that it is mediated
through the mineralocorticoid receptor (30). Increased in-
tra cellular calcium might ultimately cause cardiac hypertrophy
by increasing the expression of calcineurin, a calcium/cal-
modulin-dependent protein phosphatase that is well docu-
mented to cause cardiac hypertrophy by dephosphorylating
the transcription factor NFAT3 (nuclear factor of activated T
cells 3), enabling it to translocate to the nucleus. Nuclear
factor of activated T cells 3 interacts with the cardiac zinc
finger transcription factor GATA4, activating cardiac tran-
scription of the genes normally required for growth of the
fetal heart (31). Indeed, aldosterone increases calcineurin
mRNA and activity, and by inhibiting calcineurin with
FK506 or cyclosporin blocks the cardiac hypertrophy and fibrosis induced by aldosterone and salt (32).

In rat neonatal cardiomyocytes, aldosterone stimulates ACE, as measured by both quantitative RT-PCR and enzymatic activity. These effects are inhibited by spironolactone (33). Increased ACE activity should increase local levels of angiotensin II, which is known to cause cardiac hypertrophy and fibrosis. Aldosterone may also increase cardiac expression of the AT1 angiotensin II receptor (34). Consistent with this, inhibition of the AT1 receptor with losartan blocks aldosterone-induced cardiac hypertrophy and fibrosis (32, 34).

Additional signaling pathways have been implicated in cardiac hypertrophy (35), but a detailed review is beyond the scope of this article. The effects of aldosterone on many of these pathways are unknown. Interpretation of the effects of aldosterone on myocytes is complicated by uncertainty as to whether myocytes express 11-HSD2; without it, glucocorticoids can interact with the mineralocorticoid receptor. Conversely, it has been reported that aldosterone can up-regulate serum and glucocorticoid-induced kinase (SGK, a known aldosterone target gene in mineralocorticoid target tissues) via the glucocorticoid receptor in myocytes (36).

Direct electrophysiological effects of aldosterone in the cardiovascular system. Aldosterone rapidly (within minutes) increases free intracellular calcium in vascular smooth muscle cells as determined by fura-2 spectrofluorometry in single cultured cells from rat aorta. This effect is specific for mineralocorticoids and is blocked by neomycin and short-term treatment with phorbol esters, but is augmented by staurosporine, indicating an involvement of phospholipase C and protein kinase C. The Ca\(^{2+}\) effect appears to involve the release of intracellular Ca\(^{2+}\), as shown by an inhibitory effect of thapsigargin (7). Similarly, aldosterone induces sodium influx in vascular smooth muscle cells within minutes by activating Na\(^+\)-H\(^+\) exchange. Although these effects are too rapid to be genomic in nature, they are blocked by mineralocorticoid receptor antagonists and activated by glucocorticoids if 11-HSD2 is inhibited (6, 8). These characteristics suggest that these nongenomic effects are mediated by classic mineralocorticoid receptors. The implications for myocardial function are as yet uncertain.

Aldosterone also affects the electrophysiology of the mycardium. Although aldosterone up-regulates Na\(^+\),K\(^+\)-adenosine triphosphatase in kidney and colon, the Na\(^+\)-K\(^+\) pump current is decreased as measured by patch clamp in cardiac myocytes isolated from rabbits given aldosterone compared with controls. This is apparently due to a decrease in the apparent Na\(^+\) affinity of the pump rather than an effect on maximal pump capacity. The effect is blocked by spironolactone (37). An iv bolus of aldosterone prolongs monophasic action potentials (thus indicating an inhibitory effect on myocardial repolarization) in patients with supraventricular arrhythmias, with the maximal effect seen 4–6 min after injection. This effect may increase the risk of arrhythmia in congestive heart failure and other types of hyperaldosteronism (9).

The synthetic mineralocorticoid fludrocortisone exacerbates (38), and spironolactone improves (39), the electrophysiological behavior of the heart with regard to QT dispersion, which is increased or decreased, respectively, by these interventions. This, in turn, affects left ventricular function, as measured echocardiographically (38). Intravenous aldosterone also rapidly affects left ventricular function, as measured by cardiac catheterization (40). Spironolactone’s beneficial effects on QT dispersion are mediated at least in part by blocking the rapid component of the delayed rectifier K\(^+\) current mediated by the product of the human ether-a-go-go-related gene (39). This appears to be a direct molecular interaction between spironolactone and human ether-a-go-go-related gene channels, independent of mineralocorticoid receptor.

Genetic modification of mineralocorticoid receptor expression. Taken together, the above studies suggest that aldosterone has several direct effects on the heart, but the exact nature and relative importance of these effects remain somewhat uncertain. One way to resolve this dilemma might be to modulate mineralocorticoid receptor expression in the heart using genetically modified mice. The mineralocorticoid receptor can be overexpressed in target organs (including both heart and kidney) by making a mouse transgenic for extra copies of a mineralocorticoid receptor minigene under the control of the mineralocorticoid receptor promoter. Such mice have mild dilated cardiomyopathy (increased left ventricle diameter, decreased shortening fraction, increased heart rate) with normal blood pressure, consistent with the idea that aldosterone-induced cardiac hypertrophy is mediated through the mineralocorticoid receptor. Moreover, there were increases in cardiac expression of atrial natriuretic peptide, serum- and glucocorticoid-induced kinase, and early growth response gene 1 detected by microarray studies (41). It might be predicted that a mouse lacking the mineralocorticoid receptor would be resistant to the cardiac effects of aldosterone. However, such mice usually die within the first 2 wk of life of pseudohypoadosteronism. When examined at 8 d of age, there were no changes in cardiac expression of genes of the renin-angiotensin system, although circulating levels of renin, angiotensin, and aldosterone were all very high (42). This suggests that cardiac expression of these genes are not normally regulated through negative feedback mediated through the mineralocorticoid receptor.

It would be interesting to study a mouse in which the mineralocorticoid receptor was selectively inactivated in the heart using the cre-lox system, but such a study has not yet been performed to our knowledge. As an alternative, antisense mRNA for the mineralocorticoid receptor was expressed in the heart of a transgenic mouse strain using a tetracycline-suppressible promoter. Surprisingly, this mouse develops severe heart failure and fibrosis by 2–3 months without hypertension or hyperaldosteronism. Moreover, cardiac failure and fibrosis were fully reversible when antisense mRNA expression was suppressed by doxycycline (43). Given that these effects are precisely the opposite of what is seen with mineralocorticoid receptor blockade, it seems most likely that these results reflect a toxic effect of transgene expression, rather than being a consequence of decreased expression of the mineralocorticoid receptor.
Cardiac steroidogenesis

Because the evidence is now strong that aldosterone has effects on the heart independent of its salt-retaining effects on the kidney, it is reasonable to ask whether these effects are mediated solely by adrenal aldosterone, or whether aldosterone is synthesized within the cardiovascular system itself and has autocrine and/or paracrine effects. To answer the question of whether aldosterone is synthesized in the heart, it is necessary to determine whether the enzymes required for aldosterone biosynthesis are expressed in cardiac tissue, and whether, in fact, increased concentrations of aldosterone are present within the heart compared with the circulation.

To synthesize aldosterone, cholesterol is successively converted to pregnenolone, progesterone and deoxycorticosterone by the actions of cholesterol side-chain cleavage enzyme (CYP11A), 3β-HSD2, and 21-hydroxylase (CYP21), respectively. The final conversion of deoxycorticosterone to aldosterone involves three successive oxidations (11-hydroxylation, 18-hydroxylation, and 18-oxidation) mediated by the same enzyme, aldosterone synthase (CYP11B2). With the exception of 3β-HSD2, which is a short-chain dehydrogenase, all of these enzymes are cytochromes P450 (CYP) (1). The synthesis of glucocorticoids (corticosterone and cortisol) also requires 11-hydroxylation, which is mediated by a distinct isozyme closely related to aldosterone synthase, CYP11B1 (steroid 11β-hydroxylase).

The mRNAs for many of the enzymes involved in the formation of adrenal corticosteroids have been detected by quantitative RT-PCR using pooled RNA samples from normal human hearts (12). CYP11A, 3β-HSD2, CYP21, and CYP11B1 were expressed within all cardiac regions with the exception of the ventricles, which did not express CYP11B1. Most of the mRNAs were present at levels approximately 1000-fold lower than in the adrenals. Similar results were obtained in individual samples of normal ventricles (44).

Thus, all enzymes required for synthesis of deoxycorticosterone and (in the atria) corticosterone are expressed in the normal human heart. Because the heart is much larger than the adrenals, the total masses of mRNA for many of the steroidogenic enzymes in the entire heart are probably at least 1% of those in the adrenals. However, it has not yet been established that all of these enzymes are expressed in the same cells, and most of these enzymes have Km values in the micromolar range. Thus, it is not certain that precursors can be provided to each enzyme at a concentration that will permit the synthesis of appreciable amounts of product. Corticosteroid biosynthesis of 1%, or even 0.1%, of the quantity required to synthesize the adrenal gland seems potentially adequate to produce local (i.e. autocrine or paracrine) effects in cardiac tissue because locally produced steroids are not diluted into the entire circulation. The next sections address the question of whether and under what circumstances aldosterone synthase (CYP11B2) is also expressed in the heart.

CYP11B2 expression and aldosterone synthesis in normal hearts. CYP11B2 expression has been detected in normal rat heart using quantitative RT-PCR (45, 46). Cardiac CYP11B2 expression was increased by treating animals with angiotensin II, a stimulus that also increases CYP11B2 expression in the adrenals; one study demonstrated this in adrenalectomized animals (46). Moreover, aldosterone and its precursor, deoxycorticosterone, were detected in both the homogenate and perfusate of isolated rat hearts using RIA (45), and aldosterone was detected in similar experiments by HPLC and mass spectroscopy or by conversion of [14C]deoxycorticosterone to [14C]aldosterone (46). Perfusion with angiotensin II increased aldosterone production and decreased deoxycorticosterone, suggesting that aldosterone is formed within the isolated heart from locally available substrate (45). Increases in aldosterone synthesis could also be demonstrated in hearts taken from animals treated with angiotensin II; conversely, ACE inhibitors decreased cardiac aldosterone synthesis (46). Contradictory results have been reported for the effect of sodium on cardiac aldosterone synthesis. A week of a low sodium-high potassium diet increased cardiac CYP11B2 expression in one study, which is consistent with the known effects of such a diet on adrenal CYP11B2 expression (45). Paradoxically, other investigators found that cardiac aldosterone production and CYP11B2 expression were increased by 4 wk of sodium loading in both normal and stroke-prone spontaneously hypertensive rats, although plasma aldosterone levels fell (46–48). It was proposed that this paradoxical response might contribute to the development of cardiac hypertrophy from salt-loading that occurred independently of blood pressure (47). Cardiac expression of CYP11B2 was higher in stroke-prone spontaneously hypertensive rats than in normal WKY rats, and it was suggested that this might contribute to the pathological responses to salt seen in these animals (48). As yet, there are no data to suggest whether these divergent results might be explained by the different durations of the dietary interventions. CYP11B2 expression has also been detected in cultured neonatal rat cardiomyocytes, where its expression is regulated by the natriuretic peptides, atrial natriuretic peptide and bone natriuretic peptide (49). In contrast, CYP11B2 expression could not be documented in the mouse heart (44).

CYP11B2 mRNA could be detected in pooled RNA samples from fetal heart and adult aorta, but not in any chambers of the normal adult heart (12). Similar results were obtained in individual samples of normal ventricles (44); another study was able to detect low levels of CYP11B2 transcripts after 40–50 PCR cycles (50, 51). However, there is no evidence for the actual synthesis of aldosterone by the normal human heart (52).

Thus, the available evidence is relatively consistent in suggesting that aldosterone might be synthesized at low levels in the normal rat heart, but not normal human or mouse hearts. This might reflect species-specific differences. The role of sodium intake in regulating cardiac CYP11B2 expression is uncertain.

The significance of CYP11B2 expression in the human fetal heart requires further study (12). Levels of CYP11B2 transcripts in the fetal heart were at least 1000-fold lower than those in the adrenal. Considering that aldosterone affects cardiovascular remodeling in pathological states, it may play a role in regulating fetal cardiac development. This is consistent with the observation that many of the changes in gene expression seen during cardiac remodeling in adults mimic the pattern of cardiac gene expression in the fetus (53). However, individuals with genetic defects in aldosterone biosyn-
thesis, such as 21-hydroxylase or aldosterone synthase deficiencies, do not have obvious defects in cardiovascular anatomy (1).

CYP11B2 expression and cardiac production of aldosterone in pathological states. The main interest in the role of aldosterone in the cardiovascular system concerns its effects in pathological states such as myocardial infarction or congestive heart failure. The expression of CYP11B2 was studied in rats subjected to myocardial infarction by coronary artery occlusion. In the noninfarcted myocardium of the left ventricle, myocardial infarction increased CYP11B2 mRNA, aldosterone levels, and angiotensin II levels. All of these changes were blocked by treatment with losartan, suggesting that these increases are mediated primarily by cardiac angiotensin II via the AT1 subtype receptor. The collagen deposition induced by myocardial infarction in noninfarcted left ventricular myocardium was prevented by both spironolactone and losartan (54). In a similar study enalapril failed to inhibit cardiac aldosterone production, although losartan was effective. This may be related to the phenomenon of aldosterone escape, which refers to the tendency of circulating aldosterone levels to return to normal or supranormal levels during long-term ACE inhibitor therapy of chronic heart failure (55). Similarly, CYP11B2 expression increased in rat hearts that developed experimental autoimmune myocarditis. Cytokine, chemokine, and extracellular matrix genes also increased (56). It is likely that the increase in CYP11B2 expression was a consequence of myocardial dysfunction induced by the myocarditis.

In failing human heart, CYP11B2 was detected in at least some tissue samples in most (44, 50, 51), but not all (12), studies. Levels of CYP11B2 mRNA were correlated with myocardial collagen levels and inversely correlated with left ventricular ejection fraction (i.e. the most dysfunctional hearts had the highest levels of expression). CYP11B2 mRNA expression and collagen levels were lower in patients treated with a combination of spironolactone and angiotensin-converting enzyme inhibitors than in patients not treated with these drugs (50).

Attempts to use cardiac catheterization to detect cardiac aldosterone synthesis in humans with heart failure have yielded contradictory results. Two studies measured plasma aldosterone in the aortic root (from whence blood is pumped through the myocardium via the coronary arteries) and the coronary sinus (through which blood returns from the myocardium into the circulation) in normal subjects and patients with congestive heart failure. In one, plasma levels of aldosterone were ~20% higher in the anterior interventricular vein (which drains the left ventricular myocardium) and coronary sinus than in the aortic root in patients with heart failure, suggesting that aldosterone was secreted into the coronary circulation. No differences were seen in controls without heart failure. The difference in aldosterone levels between the anterior interventricular vein and the aorta was inversely correlated with left ventricular ejection fraction (52). In contrast, a second larger study by different researchers found that plasma aldosterone was approximately 20% lower in the coronary sinus than in the aortic root in normal subjects and in patients with congestive heart failure who did not receive spironolactone. There was no significant gradient in plasma aldosterone between the aortic root and coronary sinus in patients who received spironolactone. The transcatheter gradient of plasma aldosterone was correlated with levels of plasma procollagen type III amino-terminal peptide in the coronary sinus and with the left ventricular end-diastolic volume index. These results suggested that rather than (or perhaps in addition to) being synthesized in the myocardium, plasma aldosterone is extracted and concentrated from the blood by the heart in normal subjects and in patients with congestive heart failure who do not receive spironolactone (57).

This idea is supported by biochemical studies demonstrating that aldosterone is acylated to a less polar, more potent derivative in the heart (58). This derivative behaves chromatographically like aldosterone-20-monooacetate, although the 18- and 21-acetates might also be present. The concentration of aldosterone within the myocardium was 10 times the concentration in plasma, and 68% of it was monoacylated. Because acetylated aldosterone is less polar than aldosterone, it is more readily concentrated in lipid-rich tissues. Such derivatives are hydrolyzed in the blood within minutes at body temperature, explaining the failure to detect them previously. These studies provide an alternative explanation to in situ biosynthesis for the high concentrations of aldosterone previously noted in the rat myocardium (45).

The physiological regulation of aldosterone production primarily serves the requirements of fluid and electrolyte homeostasis, particularly in the kidney. Nongenetic actions of aldosterone would be freed of these regulatory constraints if the formation of a more potent derivative of the parent compound to which it is almost immediately hydrolyzed in the circulation were regulated within the nonepithelial target tissues.

In summary, there is evidence, albeit not entirely consistent, that cardiac aldosterone synthesis is increased in certain pathological states. In any case, all regions of the heart express genes for all enzymes required to synthesize deoxycorticosterone (i.e. CYP11A, 3β-HSD2, and CYP21) (12), which has significant mineralocorticoid activity and is, in fact, able to cause cardiac inflammation and fibrosis faster than aldosterone (25). Thus, deoxycorticosterone is potentially able to have pathological autocrine or paracrine effects mediated by the mineralocorticoid receptor. Therefore, evidence of cardiac deoxycorticosterone biosynthesis should be sought in normal and diseased hearts in both animals and humans.

Possible autocrine or paracrine effects of mineralocorticoids in the vasculature. As noted previously, one of the most striking cardiac effects of aldosterone is perivascular inflammation. It is worth considering that there may be autocrine or paracrine effects of aldosterone on the vasculature instead of or in addition to potential effects on the myocardium. Functional mineralocorticoid receptors are present in vascular tissue (27, 59–63). Nongenomic effects of mineralocorticoids on vascular smooth muscle cells and effects of mineralocorticoids on endothelial function have been discussed previously.

In pooled RNA samples, mRNAs encoding all steroidogenic enzymes necessary to synthesize aldosterone
were detected in human aorta, supporting the idea of de novo production of mineralocorticoids by this particular vascular tissue (12). This is consistent with a report that CYP11B2 is expressed in human umbilical vein endothelial cells and that aldosterone is apparently synthesized (64). However, differences in steroidogenic enzyme gene expression may exist among other vascular sites and perhaps between species. For example, endothelial cells and smooth muscle cells derived from human pulmonary arteries apparently do not express CYP11A (cholesterol desmolase) mRNA (62), whereas rat mesenteric artery does (65, 66). Disparities between the studies may be due at least in part to differences in species or experimental preparations (cultured cells vs. fresh whole tissue), or, in fact, variations in steroidogenesis may exist within the vasculature. Supporting the idea that local steroidogenesis might be important only in certain vascular sites, the mineralocorticoid receptor was detected by immunohistochemical techniques in rabbit aorta and pulmonary artery, but not in smaller vessels (60).

CYP11B2 genetic polymorphisms. Indirect evidence for cardiac effects of aldosterone has been sought in genetic polymorphisms of the aldosterone synthase gene, CYP11B2. The most intensively studied is located in the 5'-flanking region of the gene, 344 nucleotides upstream from the start of translation within a binding site for the transcription factor, steroidogenic factor-1; this position may be either a C or a T nucleotide (67). The C allele binds steroidogenic factor-1 approximately 4 times as strongly as the T allele (68). However, the functional significance of this polymorphism is obscure, because the entire DNA element can be deleted without affecting the expression of reporter constructs in cultured human adrenocortical carcinoma cells (69). As yet, there have been no attempts to correlate this polymorphism with CYP11B2 expression outside the adrenal.

Whereas these alleles have inconsistent associations with aldosterone secretion and blood pressure (70–75), the −344C allele is associated with increased left ventricular size and mass in young Finnish (72) and German (76) adults, with increased sensitivity of left ventricular mass to dietary sodium (72), and with an inability to decrease aldosterone secretion in response to dietary sodium (76). This allele is also associated with decreased baroreflex sensitivity (77). Moreover, the C allele interacted statistically with the classic risk factors, smoking and low levels of high density lipoprotein, to increase the risk of myocardial infarction in middle-aged dyslipidemic Finnish males (78).

Although these results seem consistent with a cardiac effect of CYP11B2 expression independent of blood pressure or aldosterone levels, they have been difficult to replicate in other populations and thus remain of uncertain significance (79, 80).

Conclusions

In summary, there is convincing evidence that mineralocorticoids have adverse effects on the heart in congestive heart failure in humans and in experimental animal models of mineralocorticoid excess. These effects can be blocked by mineralocorticoid receptor antagonists at doses that do not affect blood pressure, suggesting direct cardiac actions of mineralocorticoid independent of their pressor effects. Mineralocorticoids may have distinct pathological effects on cardiomyocytes, myofibroblasts, and vascular smooth muscle, but the exact contribution of each cell type to the pathological phenotype is uncertain. In addition to being synthesized in the adrenal cortex, mineralocorticoids may be concentrated or synthesized in the heart. In normal adult humans, the enzymes required to synthesize aldosterone are probably expressed in the vasculature, but not the heart; however, their expression may increase in pathological states such as congestive heart failure or myocardial infarction. Because the levels of expression are low, the pathophysiological significance of these observations remains to be established. The role of deoxycorticosterone as a locally produced cardiac mineralocorticoid deserves further investigation.

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