Mechanisms of Mineralocorticoid Action

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Abstract—Sodium transport in epithelial tissues is regulated by the physiological mineralocorticoid aldosterone. The response to aldosterone is mediated by the mineralocorticoid receptor (MR), for which the crystal structure of the ligand-binding domain has recently been established. The classical mode of action for this receptor involves the regulation of gene transcription. Several genes have now been shown to be regulated by aldosterone in epithelial tissues. Of these, the best characterized is serum- and glucocorticoid-regulated kinase, which increases sodium influx through the epithelial sodium channel. Turnover of these channels in the cell membrane is mediated by Nedd4–2, a ubiquitin protein ligase; serum- and glucocorticoid-regulated kinase interacts with and phosphorylates Nedd4–2, thereby rendering it unable to bind the sodium channels. In nonepithelial tissues, particularly the cardiovascular system, aldosterone also has direct effects, activating an inflammatory cascade, leading to cardiac fibrosis. A critical role for the MR in cardiovascular disease has now been demonstrated by the beneficial response to MR blockade in 2 large clinical trials in patients with cardiac failure. It is these nonepithelial actions of MR activation that need to be exploited for the development of antagonists that target the cardiovascular system while avoiding the undesirable side effects of renal MR blockade. (Hypertension. 2005;46:1227-1235.)

Key Words: aldosterone • sodium channels • fibrosis

The isolation of aldosterone just over 50 years ago established it as the primary physiological mineralocorticoid.1 Sodium transport, and hence salt balance, is regulated by a number of mechanisms; however, aldosterone has a critical role. Aldosterone exerts its effects on the distal nephron (and colon) as the last point of sodium reabsorption; it is thus the final arbiter.2,3 The importance of aldosterone in the maintenance of sodium homeostasis is seen in a series of monogenetic causes of hypertension,2 in Conn’s syndrome, and in disorders in which mineralocorticoid action is compromised with consequent, life-threatening salt losses.3 Although other pathophysiological consequences of aldosterone excess were identified by Selye4 over 60 years ago, their significance has only recently been appreciated. Evidence from the Randomized ALdactone Evaluation Study (RALES) and EPlerenone neuroHormonal Efficacy and SurvivAL (EPHESUS) trials of beneficial effects of mineralocorticoid antagonist therapy on morbidity and mortality in cardiac failure has focused attention beyond the kidney to effects of mineralocorticoids more broadly in the cardiovascular system.5

Although an understanding of the molecular basis of aldosterone action has tended to lag behind advances in the biology of other steroid hormones, the last decade has seen significant advances toward an understanding of the mechanisms of mineralocorticoid action. The primary mediator of the response to aldosterone is the mineralocorticoid receptor (MR), the ligand-binding domain (LBD) of which has been recently crystallized.6-8 Although the MR primarily acts as a transcription factor, recent evidence suggests that it may also mediate nongenomic (or nonnuclear) activation of second messenger pathways. In addition, there is a growing body of evidence that some actions of aldosterone may involve a receptor other than the MR. The cellular and molecular mediators, including proteins induced by aldosterone, have been characterized in sodium transporting epithelia; however, the critical molecular events in the vasculature remain to be determined. In this brief review, we explore these issues and consider their implications for pathophysiology and for future therapies.

Mineralocorticoid Receptor

The MR is the principal effector of the cellular response to mineralocorticoids. The pivotal role of the MR in the mineralocorticoid response is demonstrated in transgenic mice rendered null for the MR.9 These mice exhibit profound mineralocorticoid-unresponsive salt-wasting, which is inevitably fatal in the early neonatal period.

MR function is in part regulated at a prereceptor level. At least in epithelial tissues, the MR is coexpressed with the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2),10 which metabolizes cortisol to cortisone (corticosterone to 11-dehydrocorticosterone in rodents). Cortisol binds and activates the MR, which, given that its concentration is at least an order of magnitude greater than that of aldosterone, would see it fully occupying the MR. 11β-HSD2, by con-
Converting cortisol to inactive cortisone, serves to “protect” the MR. The importance of 11β-HSD2 is seen when the enzyme is inactivated by mutations as observed in the syndrome of apparent mineralocorticoid excess (AME) or through excess consumption of licorice, the active ingredient of which, glycyrrhetinic acid, inhibits 11β-HSD2. In each of these situations, salt-sensitive hypertension with hypokalemia, alkalosis, and a suppressed plasma renin level is observed, the classic hallmarks of mineralocorticoid-induced hypertension. However, in this scenario, aldosterone levels are low. The principal MR agonist and antagonists are shown in Figure 1.

The MR is a member of the steroid/thyroid/ retinoid nuclear receptor family of ligand-dependent transcription factors. As for all members of this family, the receptor consists of 3 principal domains: the N-terminal domain, the DNA-binding domain, and the C-terminal domain or LBD. The DNA-binding domain of 66 amino acids is the region that essentially defines the superfamily. Two groups of 4 cysteines complex around 2 zinc atoms to stabilize the “zinc fingers,” which, in reality, form α-helices, 1 of which lies in the major groove of the DNA, where it makes sequence specific contacts.

The LBD exhibits strong structural conservation with the other receptors, although significant sequence variation occurs. The MR is held, in the absence of ligand, in a transcriptionally inactive conformation with the hsp90 complex in the cytoplasm. Ligand-binding results in a conformational change that renders the receptor, on translocation to the nucleus, transcriptionally active. The crystal structure of the human MR LBD closely resembles that of the glucocorticoid receptor (GR), androgen receptor, and progesterone receptor. The MR LBD consists of 11 α-helices, with 4 β-strands folded into a 3-layered helical sandwich. These structural studies define the determinants of binding and activation in the MR; it should be noted that in 1 study, the ligand used was corticosterone, not aldosterone. In 2000, Geller et al described a kindred with an activating mutation of the MR LBD (serine 810 leucine), which caused mineralocorticoid hypertension that was exacerbated in pregnancy. Subsequently, Rafestin-Oblin et al showed that cortisone was able to activate this mutant MR. The activation results in part from stabilization of an interaction between helix 3 and helix 5. In addition to direct interactions within the ligand-binding pocket, amino acids outside the pocket also contribute to binding. Aldosterone-binding specificity is conferred by a region including helices 6 and 7, which do not contribute to the pocket. Li et al analyzed the interactions of this region with residues that contribute to the pocket; it may also be that the interaction of such regions with the hsp90 complex may be important in determining the conformation of the unliganded pocket. Although none of these studies have crystallized the MR LBD with the antagonists spironolactone or eplerenone, the analysis of Bledsoe et al and indeed previous modeling suggest that its mechanism of inactivation differs from that of RU486 in the progesterone receptor and tamoxifen/raloxifene in the estrogen receptor, where displacement of helix 12 is a major component of the antagonism. Stabilization of helix 12, the most C-terminal region of the receptor, in a strong interaction with helix 10 appears critical for activation. This conformation allows binding of LXXLL motif-containing coactivator molecules to a surface groove bounded by helices 3, 4, 5, and 12.

The N-terminal domain, although highly conserved across different species of MR, is poorly conserved across the nuclear receptor superfamily. The N-terminus contains an activation function and also 4 sumoylation sites; beyond that, structural and functional characterization remains limited. Critical to defining the transcriptional response to an activated steroid receptor are interactions with coregulatory molecules, coactivators, and corepressors. Pascual-Le Talec and Lombe recently reviewed the interactions of the MR with general and potentially MR-specific coregulators. The relative importance of these molecules to MR function has not been defined in vivo; a salt-wasting phenotype has not,

![Figure 1. Structures of the MR agonists aldosterone, deoxycorticosterone, and cortisol; the product of cortisol dehydrogenation, cortisone, and the antagonists spironolactone and eplerenone.](image-url)
for instance, been reported for any of the various transgenic mice that are null for steroid receptor coactivators. The majority of the reported interactions are observed for GR and MR; however, 2 recent reports identify molecules exhibiting varying degrees of specificity.22,23 Of perhaps more relevance to the subsequent discussion is evidence for mechanisms that provide ligand specificity. In tissues in which the MR is expressed in the absence of 11β-HSD2, differential effects of cortisol and aldosterone may be the result of interactions with ligand-specific coactivators. Kitagawa et al 24 found that the coactivation of the MR by an RNA helicase, which interacts with the N terminus, occurred in the presence of aldosterone but not cortisol. Similarly, the N/C interaction is seen only weakly with cortisol, which antagonizes the response to aldosterone.25 Specific mutations in the MR-LBD can also dissociate the effects of aldosterone and cortisol on coactivator interactions.25 These observations together with studies of other steroid receptors indicate an unexpected degree of plasticity in the receptor allowing the conformation to be in part dictated by the ligand.

Regulation of gene expression by an activated nuclear receptor classically involves binding to a hormone response element (HRE) in the promoter region of the target gene (Figure 2). However, many “genomic” actions of nuclear receptors are HRE independent. This is best characterized by the GR, in which many of its properties, particularly its anti-inflammatory actions, involve mutual transrepression of other transcription factors through a direct protein:protein interaction.26 This interaction may sequester both partners away from their respective response elements. Although the MR has been reported to interact with nuclear factor κB,27 the relevance of this or other interactions to MR physiology remains to be determined. It is tempting to speculate that the aldosterone resistance observed in the context of nephritis, graft rejection, etc,7 may be mediated via such a mechanism.

Epithelial Aldosterone-Induced Proteins
Aldosterone-responsive vectorial sodium transport occurs principally in the distal nephron and the distal colon. Entry of sodium into the cell at the apical membrane is mediated by the amiloride-sensitive epithelial sodium channel (ENaC), which represents the rate-limiting step and the principal point of control for the sodium flux (Figure 3). The eflux of sodium from the epithelial cells is an energy-dependent process that is mediated by sodium-potassium ATPase (Na,K-ATPase) in the basolateral membrane.11,28,29 Although the expression of the α-, β-, and γ-subunits of ENaC is regulated by corticosteroids in a tissue-specific manner,11 this is not the principal mechanism by which aldosterone regulates ENaC activity.30 Regulation of ENaC activity could occur through increasing the number of channels inserted in the plasma membrane or by increasing their open-probability, current evidence points to the former as being the primary mechanism of aldosterone-mediated regulation. The turnover of ENaC is mediated by the ubiquitin–protein ligase Nedd4–2.28,29 In Liddles syndrome (pseudoadosteronism), mutations in the C termini of the β or γ ENaC subunits impairs their interactions with Nedd4–2, thereby slowing ENaC removal from the plasma membrane.2 As in AME, features of mineralocorticoid excess are present, but again, aldosterone levels are low. In contrast to AME, Liddles syndrome does not respond to blockade at the MR; however, both syndromes will respond to amiloride, which inhibits ENaC.3 The Nedd4–2 gene is not regulated by aldosterone;31 rather, Nedd4–2 activity is modulated via phosphorylation by serum- and glucocorticoid-regulated kinase 1 (sgk1).28

Expression of sgk1 is rapidly upregulated in vivo in kidney and colon by aldosterone.32,33 The role of sgk1 in the renal response has been reviewed by McCormick et al.28 In addition to interactions with Nedd4–2, there is recent evidence that sgk1 may phosphorylate the C terminus of the ENaC α-subunit and alter the activity of the channel.34 It has also been suggested that sgk1 may regulate ENaC subunit gene expression.35 Sgk1 has also been reported to phosphorylate the Kir1.1 (ROMK) subtype of inward rectifier K+ channel, thereby increasing channel density;36 such an effect may contribute to the kaliuretic action of aldosterone. The mild aldosterone-resistant phenotype of the sgk1 null mice is in contrast to that of MR or ENaC-subunit null mice;37,38 this may indicate that sgk1 is not the exclusive effector of aldosterone action or that other isoforms of sgk, sgk2, and sgk3, although not normally regulated by corticosteroids, may be able to compensate in this model.

The epithelial response to aldosterone is sensitive to phosphatidylinositol-3 kinase (PI3-kinase) inhibition; this reflects the requirement of sgk1 for phosphorylation for activation.38 Activation of sgk1 through the PI3-kinase pathway may serve to link aldosterone-activated pathways to other modulators of sodium transport such as insulin.28 Aldosterone increases PI3-kinase activity, perhaps through increased expression of the monomeric G-protein k-ras.39,40
Several other genes have been identified that are regulated by aldosterone in transporting epithelia. In the distal colon, corticosteroid hormone-induced factor (CHIF) is rapidly induced via a primary transcriptional mechanism. The phenotype of the CHIF knockout mice, as with the sgk1 null mice, is only induced by severe sodium depletion. CHIF is a member of the FXYD family of small transmembrane proteins that modulate the activity of pumps and channels. CHIF enhances the affinity of Na,K-ATPase for sodium, which may serve at least in part to explain the increase in Na,K-ATPase activity observed in response to aldosterone. An increase that precedes any increase in Na,K-ATPase subunit synthesis. Increased expression of the k-ras gene in epithelial tissues is a well-characterized component of the response in amphibian systems; increased expression is also observed in the rat in vivo, although the levels are low and the response modest. A series of screens using renal cell lines has been conducted to identify aldosterone-induced genes; sgk1 remains the most robust response. The roles of genes, such as glucocorticoid-induced-leucine-zipper protein and N-myc downstream regulated gene 2, identified through these screens, remain to be fully characterized in vitro and in vivo.

In addition to the regulation of sodium influx, aldosterone regulates the efflux of hydrogen and potassium ions. In part, this may reflect a passive electroneutral response to the sodium flux, but there is also evidence of direct effects and indeed spatial separation of the response. The role of mineralocorticoids in potassium homeostasis is important not only in mineralocorticoid excess, in which hypokalemia may be a presenting feature, but hyperkalemia may limit the use of mineralocorticoid antagonist therapy in cardiac disease. Although the potassium transport may in part reflect the effects of Na,K-ATPase, active mineralocorticoid-induced transport may also occur through ROMK and the K⁺/H⁺ exchanger isoform 1 (NHE-1) in human arteries; cortisol was also without effect, and spironolactone did not block the aldosterone effect, and spironolactone did not block the aldosterone

**Rapid Nongenomic Mechanisms: Signaling Through the MR**

In addition to steroid receptor modulation of transcription through either HRE-dependent or -independent processes, there is increasing evidence of rapid so-called nongenomic effects that involve activation of second messenger pathways (Figure 2). These have recently been reviewed in extenso, although whether aldosterone acts via the classical MR to mediate these effects or via a novel receptor, as is the case for progesterone and estrogen, remains controversial. More than a decade ago, Wehling et al demonstrated novel, rapid effects of aldosterone that were neither mimicked by cortisol nor blocked by spironolactone. It was suggested that these nongenomic effects were mediated via a membrane receptor distinct from the classical MR. Rapid “nongenomic” effects differ from traditional “genomic effects” in that they do not occur as a result of gene transcription after activation of cytosolic nuclear receptors; responses appear within 1 to 3 minutes and dissipate within 5 to 10 minutes, a time course that is too rapid to be explained by genomic effects. In addition, they are not blocked by actinomycin D.

That rapid aldosterone signaling is mediated by a novel receptor rather than the classic cytosolic MR has been a moot point since the first rapid signaling effects were described. Rapid, nongenomic responses have now been described for other steroid hormones, including estrogen and progesterone. Moreover, the recent cloning of G-protein–coupled receptors for progesterone and estrogen support the hypothesis that membrane receptors might also be found for aldosterone. Although little evidence exists for the regulation of rapid MR signaling via a novel MR, the presence of a pool of classical estrogen receptors associated with caveolae in the plasma membrane, which mediate nongenomic signaling, suggests that similar mechanisms may exist for signaling via other steroid receptors including the MR, although this has yet to be formally demonstrated.

Nongenomic aldosterone actions have been described for an increasing number of epithelial and nonepithelial cell types, including mononuclear leukocytes, endothelial cells, vascular smooth muscle cells (VSMCs), and cardiac myocytes, with several patterns of agonist and antagonist activity now emerging. The first studies by Wehling et al showed that whereas various mineralocorticoids had similar agonist effects, cortisol had no activity even at 1000-fold higher doses. More recently, Alzamora et al have similarly shown aldosterone-induced rapid increases in intracellular pH via the sodium–hydrogen exchanger Na⁺/H⁺ exchanger isoform 1 (NHE-1) in human arteries; cortisol was also without effect, and spironolactone did not block the aldosterone

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**Figure 3.** Representation of an aldosterone-responsive epithelial cell. The proteins encoded by aldosterone-induced genes are discussed in the text: ENAC, α, β, and γ CHIF, sgk, and ras are indicated as are their known or putative functions.
response. However, when 11βHSD2 activity was blocked, cortisol was indistinguishable from aldosterone, and responses to either corticosteroid were blocked by the water-soluble MR antagonist RU28318 (but not spironolactone as before). Also following this pattern of response are increases in cytosolic calcium that have also been ascribed to rapid aldosterone signaling by other investigators;55 again, neither dexamethasone nor the classic MR blockers can modulate this response. Of interest, a rapid intracellular Ca\(^{2+}\) flux in response to aldosterone was retained in the keratinocytes of MR knockout mice, leading to the possibility that there are distinct receptors for rapid signaling and indicating further levels of complexity in MR signaling.56

Rapid responses blocked by MR antagonist spironolactone or potassium canrenoate indicate signaling via the classic MR. Those thus far described include interactions with the epidermal growth factor signaling pathway, which results in rapid dose-dependent phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase 1/2 kinases.57,58 Similarly, in rabbit cardiomyocytes, aldosterone increases Na\(^{+} /K\(^{+}\) pump activity via direct activation of the Na\(^{+}/K\(^{+}\)/2Cl\(^{-}\) cotransporter. However, pump current is completely blocked by potassium canrenoate and stoichiometrically by cortisol, supporting a role for classic MR.59 These responses have been further characterized and shown to specifically involve protein kinase Cε (PKCε); agonist peptides for the PKCε isof orm mimic the effect of aldosterone, and isoform-specific antagonist peptides block the effect. Very recently, whole-cell patch-clamp studies in rabbit cardiac myocytes and VSMCs revealed that cortisol can mimic the effects of aldosterone when the intracellular redox state is altered by oxidized glutathione, paralleling the effect of carbenoxolone on vascular smooth cells.54

At present, the role of nongenomic MR signaling responses in the initiation and progression of vascular damage and cardiac fibrosis remains unclear and may involve subtle modulation of intracellular sodium levels or the redox state of the cell. Evidence surrounding this phenomenon is far from definitive. Although these effects have been demonstrated in a range of tissues, evidence supporting a physiological relevance for these rapid effects is limited.50,61 Studies have predominantly been in vitro; however, recently, rapid reductions in forearm blood flow in conscious subjects after aldosterone infusions have been reported in some but not all studies.49,60 These studies do not distinguish the cell types involved but demonstrate that physiologically measurable effects are possible in the same time frame.

**Nonepithelial Mechanisms of Aldosterone Action**

The action of mineralocorticoids has long been associated exclusively with controlling electrolyte homeostasis via MR activation in epithelial target tissues; however, it is now clear that this was an incomplete account of the physiological roles of mineralocorticoids.

High-affinity binding sites for mineralocorticoids in non-epithelial tissue have been identified that, in vitro, had high affinity for cortisol (corticosterone) and aldosterone equivalent to that of the renal MR; their structural identity has also been demonstrated.12 MR signaling in these nonepithelial tissues appears also to include rapid, “nongenomic”-type pathways.

During the last 15 years, a new understanding for the breadth of actions of aldosterone has emerged, and it is now well accepted that aldosterone has physiological and pathophysiological effects in nonepithelial tissues including the heart, vasculature, and brain.62,63 In addition to the epithelial sites of expression, MR are also present in nonepithelial tissues including cardiomyocytes, the hippocampus, the blood vessel wall (VSMC and endothelial cells), and circulating monocytes. The MR in these sites have been shown to play a unique role in regulating cardiovascular homeostasis. An understanding of the regulation of the MR in specific cell types is also emerging, and it is now clear that several patterns of regulation exist. For example, in cardiomyocytes and the hippocampus, which both express MR but not 11βHSD2, the receptor is effectively occupied by endogenous glucocorticoids, which have opposing effects to aldosterone.54,60 In contrast, VSMCs express the MR and show 11βHSD2 activity:54 enzyme blockade by carbenoxolone in these cells allows physiological levels of cortisol to activate MR and produce similar responses to aldosterone or deoxycorticosterone.

**Aldosterone Actions in the Heart**

A role for MR activation in the development of heart disease was suggested by the work of Selye,4 who described “the general adaptation syndrome,” inflammation, and increased connective tissue in systemic organs in response to deoxycorticosterone administration. Some 4 to 5 decades later, Brilla and Weber62 demonstrated that the administration of aldosterone in conjunction with a high-salt diet produces hypertension, cardiac hypertrophy, and cardiac fibrosis and thus reinitiated work in this field. Now it is well accepted that these pathological actions of aldosterone, although dependent on the salt status of the animal, are independent of systolic blood pressure, hypokalemia, and cardiac hypertrophy, demonstrating a direct cardiovascular effect of aldosterone rather than a hemodynamic etiology for the pathology.64 More recent investigations of early time points in the establishment of cardiac fibrosis have now identified oxidative stress and early vascular inflammatory events as key mediators of MR activation in the blood vessel wall and essential steps in the progression to cardiac fibrosis.67–69

Aldosterone is also known to have effects in the heart distinct from the aforementioned fibrotic pathways. Recent studies have reported increased cardiac myocyte contractile force and myocyte hypertrophy in response to aldosterone administration in perfused hearts and cultured cells respectively, whereas responses in cultured human tissue are less clear.70–72 Moreover, artificial overexpression of the human MR in mice produces a mild dilated cardiomyopathy, a significant increase in heart rate but no change in systolic blood pressure.73 The mechanisms underlying the generation of arrhythmias remain unclear, although it has been shown that in normal rat neonatal cardiomyocytes, aldosterone increases l-type calcium current amplitude in ventricular myocytes.74 Aldosterone exerts opposing effects on T channel
isoform expression, increasing α(1)H and decreasing α(1)G. Although the exact role of these channels remains to be defined, overexpression of α(1)H may be in part responsible for arrhythmias associated with hyperaldosteronism.

Sato and Funder\(^7\) demonstrated PKC-dependent, aldosterone-induced hypertrophy in neonatal myocytes that was enhanced by elevated serum glucose and opposed by corticosterone. More recent studies have shown that cardiac hypertrophic markers (eg, natriuretic peptide precursor type A and B) and α-actin 1 are clearly increased in rat cardiac myocytes in response to aldosterone and involve phosphorylation of protein kinase D.\(^7\) A number of other intracellular signaling pathways can also be upregulated by aldosterone, including PI3-kinase–p100δ, which promotes expression of the collagen genes COL1A1, COL1A2, and COL3A1, transforming growth factor-β1 (TGF-β1) in rat cardiac fibroblasts (a known profibrotic factor), connective tissue growth factor,\(^7\) and plasminogen activator inhibitor.\(^7\) Regulation of these pathways often has several levels of complexity; for example, upregulation of connective tissue growth factor is mediated by MR activation, the p38 MAPK pathway, and interactions between the 2.\(^7\) The mitogen-activated protein kinase kinase (MEK)/ERK pathway has also been implicated as a mediator of aldosterone effects in the heart. Matrix metalloproteases 2 and 9 are increased in cardiac myocytes in response to aldosterone in a process that involves reactive oxygen species–dependent activation of MEK/ERK,\(^7\) adding further candidates to the list of potential indicators of aldosterone action.

Aldosterone can also suppress inducible NO synthase and NO from isolated rat neonatal cardiomyocytes in a post-transcriptional TGF-β1–dependent manner, an effect blocked by spironolactone.\(^7\) The decrease in NO synthesis may also account, in part, for the known cardiovascular effects of aldosterone. Studies in the cardiac troponin T–Q92 transgenic mouse model of human hypertrophic cardiomyopathy (HCM) suggest that aldosterone is a major link between sarcomeric mutations and cardiac phenotype in HCM and, if confirmed in additional models, signals the need for clinical studies to determine the potential beneficial effects of MR blockade in human HCM.\(^7\)

Whether or not aldosterone acts directly on cardiac fibroblasts is unclear, with some investigators showing direct anabolic effects (collagen production),\(^8\) whereas others have not.\(^8\) Recently, Pratt et al suggested that aldosterone can promote proliferation of cardiac fibroblasts by activating specific cellular signaling cascades such as k-Ras and the MAPK1/2 cascade. In these studies, physiological concentrations of aldosterone (10 nmol/L) induced significant increases in cardiac fibroblast proliferation, an effect that was blocked by spironolactone.\(^8\) Similar effects have now been demonstrated in rat renal fibroblasts,\(^8\) aldosterone clearly causes increases in collagen gene expression via ERK1/2 pathways, leading to the progression of tubulointerstitial fibrosis.

Aldosterone regulation of cardiac MR expression at the RNA and protein level has also been shown in the aldosterone/salt-treated rat as well as in other hypertensive rat models, suggesting a mechanism for potentiation of aldosterone signaling in the heart rather than a specific MR-mediated response.\(^8\) In contrast, a recent study by Beggah et al\(^8\) reported that expression of MR antisense, and thus knockdown of the MR in cardiac myocytes, produced severe cardiac hypertrophy and fibrosis that was made worse with spironolactone treatment. It has been suggested that this response is not a specific effect of loss of MR signaling but was in large part attributable to the overexpression of a foreign protein in the myocytes, given that very similar heart failure and pathology was seen in cardiac myocyte overexpression of green fluorescent protein\(^9\) or an inflammatory response to the use of antisense. It is also important also to note that these results are inconsistent with the heterozygous MR knockout mice, which have no cardiac phenotype,\(^9\) and to the 11βHSD2 cardiac–expressing mice,\(^6\) in which an appropriate response to spironolactone is observed.

### The Blood Vessel Wall

The MR and GR and both 11βHSD isoforms have been shown to be expressed in the aortic wall in the mouse, whereas only 11βHSD2 activity has been shown in human vessels and rabbit VSMCs.\(^3\) In the mouse, it is suggested that 11βHSD1 expression is predominantly in VSMCs, whereas 11βHSD2 is localized in endothelial cells.\(^5\) 11βHSD2 is thus appropriately situated to modulate endothelial cell function, even though endothelial dysfunction in 11βHSD2 knockout mice cannot be explained simply by the presence of increased access of corticosterone to endothelial cell corticosteroid receptors, indicating additional mechanisms. One such mechanism may be regulation of receptor availability for other vasoactive peptides. Indeed, angiotensin II type 1 receptors are upregulated in the heart and blood vessel walls of aldosterone treated rats, enabling potentiation of the angiotensin response.\(^8\) The signaling in endothelial cells is also involved in the response to MR activation. Callera et al\(^8\) have shown that endothelin A receptor blockade decreases vascular superoxide generation in mineralocorticoid/salt hypertension and also influences leukocyte–endothelial cell interaction. It appears that the inflammatory and fibrotic effects of elevated plasma aldosterone are mediated in part via the upregulation of endothelin rather than by increasing receptor number. Studies in the mineralocorticoid/salt model of vascular inflammation and cardiac fibrosis over very short time courses indicate that the primary response is the initiation of oxidative stress and inflammation as shown by infiltrating macrophages and expression of inflammatory cytokines (osteopontin and cyclooxygenase 2) in VSMCs and epithelial cells.\(^6\) This also suggests a vascular origin for cardiac fibrosis. NHE-1 controls intracellular pH in the cardiovascular system, providing protection from the acidification that follows ischemia and is thus a potential intermediate in the translation of MR signaling into vascular inflammation and perivascular fibrosis. Indeed, the specific NHE-1 antagonist cariporide can reverse postischemic changes such as cardiac hypertrophy, arrhythmia, and impaired contractility.\(^1\) Furthermore, the cardiac effects of mineralocorticoid/salt treatment are substantially blocked by cariporide (100 mg/kg per day), supporting the hypothesis that activation of the MR causes modulation of intracellular pH and thereby promotes vascular damage.\(^2\) If the primary effects of circulating
aldosterone are on the coronary vasculature or cardiac myocytes, such effects may be via either modulation of gene transcription or sustained activation of nongenomic pathways (ie, activation of NHE-1). In support of this, the nongenomic effects of aldosterone on Na+/K+/2Cl− activation in rabbit cardiomyocytes have been shown to persist for a week in vivo, evidence that such rapid actions can be long lasting.59

As in the heart, intracellular signaling pathways are now being characterized for vascular cell responses to aldosterone. It is now clear that processes such as VSMC proliferation, in response to aldosterone binding, involve ERK1/2 and BMK1 (by MAP kinase 1), at least in rapid signaling situations.93 Moreover, some of the responses to aldosterone may involve a synergistic effect when angiotensin II is also present,43 implying that blockade of both hormones would be more beneficial in the prevention of vascular remodeling.

The notion that MR activation, per se, rather than plasma aldosterone levels, determines the onset of vascular inflammation and cardiac fibrosis has been considered recently.95,96 Activation of MR in the vessel wall by nonspecific damage was clearly shown in a pig model of coronary angioplasty.97 These animals were fed a normal salt diet and had normal plasma aldosterone concentrations; however, lumen occlusion was significantly less when the selective MR antagonist eplerenone was administered. These data suggest that the MR may be activated by the presence of cellular damage and thus altered redox potential in the cell. It has been hypothesized that under normal circumstances, MRs are largely occupied by glucocorticoids and that this ligand is responsible for activation of MRs in the presence of tissue damage.95,96 If this is true, then the use of MR blockers in cardiovascular disease may be of broader benefit than thought previously.

Clinical Implications

Blockade of MR in moderate to severe heart failure reduces morbidity and mortality. This result from the RALES trial was interpreted as demonstrating a major role for aldosterone in the progression of heart failure.5 Specifically, patients with moderately severe heart failure given low-dose spironolactone in addition to best practice therapy (angiotensinconverting enzyme inhibitors, diuretics, etc) showed a 30% reduction in mortality and a 35% reduction in morbidity. More recently, the EPHESUS trial used the new selective MR blocker eplerenone similarly to show improved survival and reduced hospitalization in patients with heart failure after myocardial infarction. The patients in these trials did not have elevated plasma aldosterone levels, suggesting that it is the MR blockade per se that is critical to the response. However, it should be noted that a subsequent study of spironolactone use post-RALES in Ontario suggested that the rate of clinically significant hyperkalemia was much higher than in the original trial.97 Therefore, at this stage, the epithelial response to MR antagonism, hyperkalemia, may limit its therapeutic utility.

Conclusion

In epithelial tissues, the regulation of vectorial electrolyte transport by aldosterone involves a pathway that is now relatively well characterized, from the prereceptor regulation of steroid access by 11β-HSD2 through the MR and its induced-proteins, particularly sgk1, to the apical channels and basolateral sodium pump. Less well characterized are the responses in the nonepitHELial tissues of the cardiovascular system, in which pathophysiology has preceded physiology, and the mechanisms of both remain to be fully characterized. However, that the MR plays a central role in both tissues is clearly established. The therapeutic potential of MR antagonists in cardiovascular disease is tempered by the consequent hyperkalemia mediated in epithelial tissues. A more complete understanding of the molecular interactions of the MR in the cardiovascular system may provide the basis for the development of tissue-specific antagonists of the MR.

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