
ALDOSTERONERachell E. Booth,¹ John P. Johnson,² and James D. Stockand¹

¹*Department of Physiology, University of Texas Health Science Center San Antonio, San Antonio, Texas 78229; and* ²*Department of Medicine, Renal-Electrolyte Division, University of Pittsburgh, Pittsburgh, Pennsylvania 15213*

Aldosterone plays a pivotal role in electrolyte and fluid homeostasis and thus control of blood pressure. The “classical” view of aldosterone action is that it targets epithelia of the distal colon and renal nephron to stimulate Na⁺ (re)absorption and K⁺ secretion. In these cells, aldosterone binds steroid receptors, promoting translocation to the nucleus, where they modulate gene expression with the induced proteins stimulating transport. This “genomic” action is dependent on transcription and translation and has a latency of 0.5–1.0 h. Recently, more rapid actions of aldosterone that are independent of transcription and translation have been described. These “non-genomic” actions are mediated by a distinct receptor that is insensitive to inhibitors of the classical mineralocorticoid receptor, such as spironolactone. The present review describes advances in our understanding of the classical model of aldosterone action as well as those that broaden this model to encompass nongenomic actions, nonepithelial targets, production of aldosterone outside of the adrenal gland, novel mechanisms of specificity, and novel mechanisms for mediating genomic actions.

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The steroid hormone aldosterone, synthesized in the zona glomerulosa of the adrenal cortex, plays a pivotal role in electrolyte and fluid balance. It has been the focus of much important investigation over the last half century, and many excellent contemporary review articles provide in-depth coverage of all aspects of aldosterone in physiology (14, 15, 17, 20, 31, 36, 43, 56, 57). The present review highlights recent discoveries that expand and challenge our current understanding of aldosterone actions.

Aldosterone, acting as a mineralocorticoid, is the final endocrine signal in the renin-angiotensin-aldosterone system that targets epithelia in the kidney and colon to regulate Na⁺ (re)absorption and K⁺ secretion. Water ultimately follows the movement of Na⁺ via osmosis, establishing chronic blood volume and thus blood pressure. Angiotensin II (ANG II) and K⁺ promote aldoste-

rone secretion. Blood pressure alters ANG II levels in a reciprocal fashion: decreased blood pressure increases ANG II production. Thus aldosterone secretion and its physiological actions are under negative feedback control, responding positively to decreases in blood pressure and increases in K⁺ and negatively to increases in blood pressure and decreases in K⁺. The importance of aldosterone in blood pressure homeostasis is particularly apparent considering that every known form of Mendelian hypertension in humans results from aberrant aldosterone signaling or hyperactivity of its final effectors (reviewed in Refs. 16, 17, 31).

CLASSICAL MODEL OF ALDOSTERONE ACTION

Figure 1 shows an idealized cell model of the classical aldosterone-sensitive epithelial cell, the principal cell

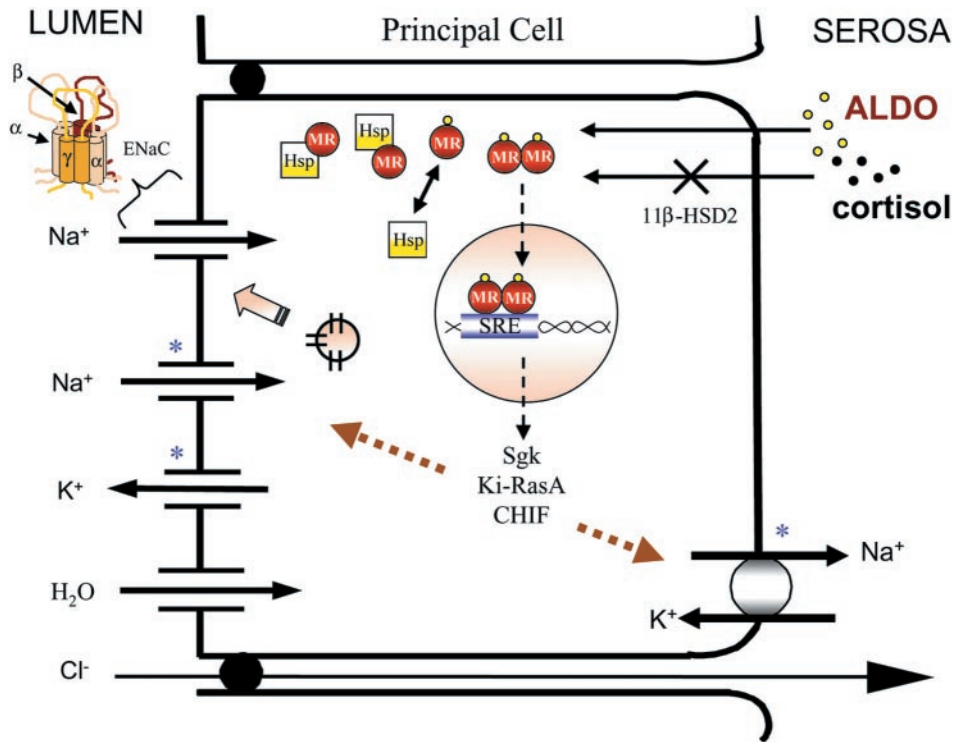


FIG. 1.

Model of the genomic actions of aldosterone (ALDO) in epithelial cells. Asterisks denote final effectors of aldosterone involved in Na^+ and K^+ reabsorption and secretion, respectively. The aldosterone-induced proteins serum and glucocorticoid-inducible kinase (Sgk), corticosteroid hormone-induced factor (CHIF), and Kirsten Ras (Ki-Ras) increase the activity and/or no. of these transport proteins during the early phase of action. During the late phase of aldosterone action, expression levels of transport proteins themselves increase. ENaC, epithelial Na^+ channel; Hsp, heat shock protein; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; SRE, steroid response element; $11\beta\text{-HSD2}$, 11β -hydroxysteroid dehydrogenase type 2.

of the renal collecting duct. Monolayers of these cells serve two primary and related functions: acting as barriers separating the internal and external environments and allowing (re)absorption of Na^+ followed by water. The barrier function is primarily dependent on the lipid composition of the apical membrane and the formation of high-resistance, electrically tight monolayers, an emergent property of the apical plasma membrane and the junctional (tight junctions) complexes coupling these cells. The transport function is regulated by aldosterone.

Transcellular transport across these cells is electrogenic and dependent on serosal Na^+/K^+ -ATPases that establish the electrochemical driving forces necessary for luminal entry and exit of Na^+ and K^+ , respec-

tively. The limiting step in Na^+ (re)absorption is the activity of the luminal amiloride-sensitive epithelial Na^+ channel (ENaC). The limiting step in K^+ secretion is the activity of the luminal K^+ channel (likely rat outer medullary K^+ channel). Water follows the transcellular and paracellular movements of Na^+ and Cl^- , respectively, across the monolayer.

Final effectors of aldosterone action in epithelia have traditionally been considered to be the luminal ENaC and luminal K^+ channel and the serosal Na^+/K^+ -ATPase. However, other intrinsic membrane proteins localized to the apical membrane of epithelia in gut and kidney are now also recognized as final effectors (6, 28). Aldosterone increases activity of the luminal Na^+/H^+ -exchanger (NHE3) in the proximal portion of

the colon and the luminal thiazide-sensitive Na^+/Cl^- cotransporter (NCC) in the distal renal tubule. Increases in NHE3 and NCC translate into the sustained elevation of electroneutral Na^+ (re)absorption in the colon and kidney in response to volume contraction.

Because apical channel activity is limiting for transcellular ion movement, there are ultimately only two ways aldosterone can enhance transport: 1) by increasing the open probability of apical ion channels, and 2) by promoting channel insertion to increase the number of functional channels. There is considerable and often conflicting evidence supporting both mechanisms of action (23, 26), suggesting that aldosterone utilizes both. Aldosterone also increases Na^+/K^+ -ATPase activity to stabilize transport capacity. Figure 2 shows an idealized time course and possible primary mediators of aldosterone action on Na^+ transport.

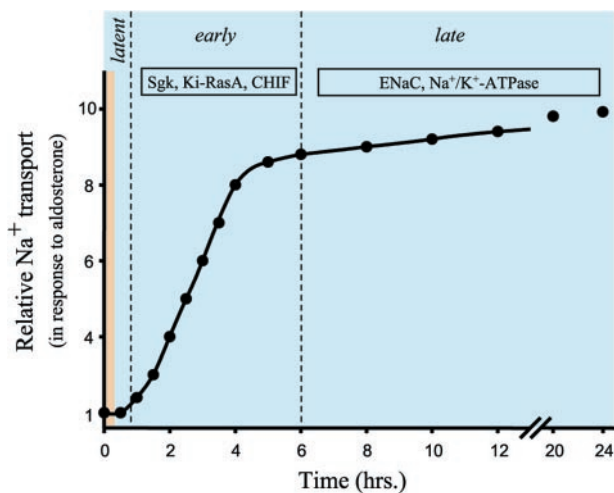


FIG. 2.

Time course of aldosterone action. This idealized graph shows the typical, time-dependent increase in Na^+ (re)absorption across epithelia in response to the genomic actions of aldosterone. Genomic actions are divided into 3 phases (latent, early, and late), demarcated by dashed lines. Proteins that are regulated at the level of transcription by aldosterone during each phase, which may mediate steroid actions during these phases, are indicated in the boxes. The blue box, spanning time (0–24 h) shows the time course of the genomic action. The time course of a nongenomic response, such as that initiated by aldosterone in smooth muscle, is identified for comparison by the tan box (spanning 0–5 min).

In epithelia, aldosterone initially affects transcellular electrolyte transport prior to affecting expression levels of channels and transport proteins (56). This has led to a somewhat arbitrary separation of aldosterone action into early (1–6 h) and late (>6 h) phases. The early actions of aldosterone in classical epithelia targets are mediated exclusively by primary effects on gene expression. In contrast, the later phase results from both primary and secondary effects on gene expression.

Cellular Mechanism of the Classical Model

The classical actions of aldosterone are mediated by intracellular receptors that translocate to the nucleus upon ligand binding. The activated steroid receptor modulates gene expression by functioning as a transcription factor. Two distinct molecular mechanisms, depicted in Fig. 3, define the actions of nuclear receptors on gene expression. The classical mechanisms involve activation and repression via direct interaction with DNA binding sites [referred to as steroid response elements (SREs) and negative response elements (nSREs)]. More recent studies of glucocorticoid:receptor function have established a novel complementary mechanism by which steroids affect transcription (reviewed in Refs. 25, 40). In this process, transcription interference or synergy are mediated by protein-protein interactions between activated steroid receptors and other factors. In this second mechanism, the steroid receptor does not physically bind DNA, even though the mechanism does impinge upon gene expression.

Many aldosterone-induced genes have been described. Evidence that gene repression is, in part, needed for aldosterone action, however, has been more difficult to demonstrate, primarily arising from the fact that genes negatively influenced by aldosterone have not been well described and the aldosterone-sensitive nSREs involved in gene repression are yet to be identified. Recently, a number of aldosterone-repressed transcripts have been identified (41, 50), and it is now apparent that aldosterone represses almost as many genes as it induces. It follows, then, that aldosterone-sensitive gene repression plays an important but yet-undefined role in the final cellular response. Repressed genes likely encode factors that

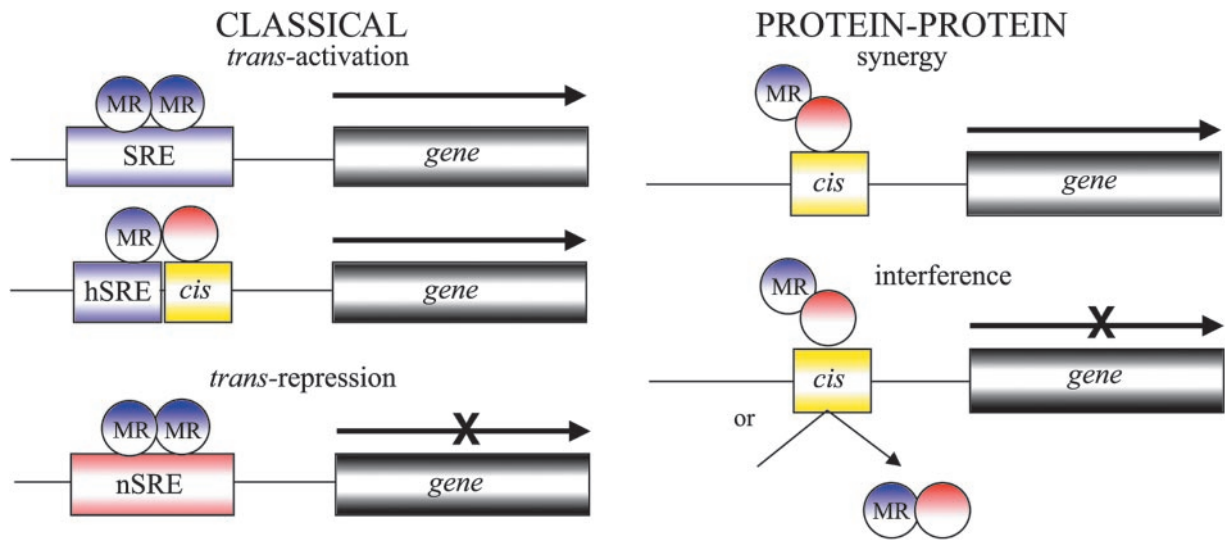


FIG. 3.

Molecular mechanism of aldosterone action. Aldosterone may utilize 2 types of molecular mechanisms to influence transcription: a classical mechanism (*left*) and a more novel mechanism employing protein-protein interactions (*right*). For the classical mechanism, the steroid:receptor complex interacts directly with genomic DNA at its cognate binding site steroid response element (SRE) to *trans-activate* or *trans-repress* gene expression. hSRE, half-site of SRE. For the latter mechanisms involving protein-protein interactions, which include transcription synergy and interference, ligand-activated steroid receptors are tethered to affected genes by distinct [non-glucocorticoid receptor (GR) or mineralocorticoid (MR)] *trans-acting* factors, which themselves bind DNA. Such a coupling enables gene modulation in response to steroid without steroid receptors actually interacting directly with DNA. Transcription interference may also involve mutual antagonism of DNA binding between the steroid receptor and other *trans-acting* factors involved in this process. Blue and red circles represent ligand-activated steroid receptor and active forms of distinct *trans-acting* factors, respectively. Blue and red boxes indicate SRE and negative SRE (nSRE) positively and negatively, respectively, affected by activated steroid receptors. The yellow box indicates a *cis*-element other than SRE and its permutations. Normal and crossed arrows indicate activation and repression of transcription, respectively.

suppress transport or that are involved in feedback regulation of transport.

Mechanism of specificity in epithelia. Specificity of aldosterone action may be regulated at several sites along the response pathway, including tissue expression of the receptor, ligand binding to receptor, DNA binding sites, or factors affecting transcription (24). Early studies of specificity demonstrated the presence of receptors with high affinity for aldosterone localized to known aldosterone target tissues such as colon, parotid glands, and distal nephron of kidney. These were called type 1, or mineralocorticoid, receptors (MR) to distinguish them from steroid receptors with higher affinity for glucocorticoids, called type 2, or glucocorticoid, receptors (GR). Specificity of steroid action was thus a function of tissue localization and innate specificity of ligand binding of

these receptors. In support of this paradigm, MR were found to be largely confined to aldosterone target tissues whereas GR were ubiquitous.

Difficulties with this paradigm emerged from studies of whole animal physiology. In most animals, plasma levels of glucocorticoids are more than 100-fold greater than plasma aldosterone levels. This is a greater difference than the difference in binding affinity between aldosterone and glucocorticoids described for MR. Some of this difference may be accounted for by different degrees of protein binding by the two classes of steroids, since glucocorticoids are highly protein bound and aldosterone largely unbound in the circulation. Nonetheless, circulating levels of glucocorticoids should be sufficient to bind type 1 receptors most of the time. The model of intrinsic selectivity was further confounded when MR

was cloned (1) and found to have striking homology in the DNA-binding domain (94%) and the ligand-binding domain (57%) to GR. Although cloned MR expressed in cells had high affinity for aldosterone, it demonstrated little intrinsic steroid selectivity. Clearly, a new paradigm for mineralocorticoid specificity was required.

Part of the answer has emerged from unusual beginnings. Researchers studying the clinical syndrome of apparent mineralocorticoid excess associated with high intake of licorice discovered that the active component of licorice acted primarily to inhibit the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2). Similarly, congenital apparent mineralocorticoid excess results from defects within the 11 β -HSD2 gene. In a landmark paper, Funder and colleagues (18) advanced the hypothesis that 11 β -HSD2 served to protect the MR from glucocorticoid occupancy by metabolizing glucocorticoids to products with markedly reduced affinity for MR. In this paradigm, tissue specificity of MR action is mediated by enzyme action of 11 β -HSD2. Strong support for this model emerged from studies demonstrating colocalization of 11 β -HSD2 with MR in target tissues. Although this mechanism adequately explains aldosterone specificity in epithelia, the mechanism defining specificity in nonepithelial targets, such as neurons and cardiac cells, which lack 11 β -HSD2, is not well understood and likely is determined at the receptor and/or postreceptor levels.

There are, therefore, at least two components of specificity of aldosterone action: tissue localization of receptors and protection of occupancy by 11 β -HSD2. Although the action of 11 β -HSD2 is clearly important as a determinant of MR selectivity, it is not clear that it is sufficient by itself to explain the absence of glucocorticoid activation of MR with glucocorticoid concentrations two to three orders of magnitude greater than aldosterone levels. Factors specific to the interaction of aldosterone and MR have been implicated (2). Initial studies with the cloned MR, which showed equivalent binding of MR by aldosterone and glucocorticoids, also showed greater transactivation by aldosterone (1, 24). The mechanisms by which different ligands cause varying degrees of gene activation via the same receptor are not entirely known. Studies have suggested that conformational changes

in MR consequent to ligand binding vary depending on the structure of the ligand and that aldosterone binds in a manner that results in a more stabilized active conformation than that achieved by glucocorticoid binding (15). It is also possible that the ligand binding to MR may influence interactions with co-activators after binding to response elements. In any case, the issue of specificity remains an intriguing problem and clearly involves regulation at multiple sites, including tissue expression, protection of occupancy by 11 β -HSD2, and ligand-receptor interactions, with response elements leading to transactivation.

Early-phase aldosterone-regulated proteins. One distinction between the early and late phases of aldosterone action is that, during the early phase, aldosterone induces signaling proteins, which results in post-translational activation of existing ion channels and other proteins involved in transport. These early signaling factors also potentially promote a second round of gene expression. Exciting recent investigation has begun to identify such signaling factors and their associated transduction cascades. Leading candidates in this regard are the serum- and glucocorticoid-inducible kinase (Sgk) and the small, monomeric GTP-binding protein Kirsten Ras (Ki-Ras). The mechanisms of action by which these signaling factors and their dependent effector cascades ultimately influence transport proteins also are becoming clearer.

FUNCTION OF SGK. Of the recently identified aldosterone-induced genes, *Sgk* has received the most attention (reviewed in Refs. 35, 37). *Sgk* is a primary aldosterone-induced gene in renal epithelia. Aldosterone increases *Sgk* levels within 15–30 min, peaking after 1–2 h and subsequently tending toward pretreatment values soon after. Aldosterone induction of *Sgk* protein follows a similar time course.

Overexpression of *Sgk* with ENaC in the heterologous *Xenopus laevis* oocyte expression system leads to activation of the Na⁺ channel (see Refs. 35, 37 for further references). Enthusiasm for these experiments must be tempered, however, for this heterologous system is obviously quite different from the in vivo situation of aldosterone-stimulated Na⁺ transport across polarized, differentiated epithelial cells. Effects of *Sgk* on ENaC in oocytes may reflect nonspecific actions. Overexpression of *Sgk* with the cystic fibrosis

transmembrane conductance regulator Cl^- channel (58), as well as certain voltage-gated K^+ channels (29), also leads to activation of these latter channels.

In addition to regulating ion channels, preliminary evidence from oocytes suggests that Sgk also activates the Na^+/K^+ -ATPase and $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (BSC-1) via promoting insertion into the plasma membrane when these proteins are chronically co-overexpressed with the kinase (65). It is not immediately clear how a “specific” mediator of a mineralocorticoid response could activate such diverse channel types and transporters, which localize to distinct membranes in polarized epithelia. One possible explanation is that Sgk affects membrane trafficking via a general mechanism. Indeed, this idea is currently the most favored explanation of Sgk action on transport (37), but it raises the specter of how specificity is determined with such a general mechanism of action.

An exciting preliminary study from the laboratory of F. Lang (personal communication) demonstrating linkage between the *Sgk* locus and hypertension in humans has begun to demonstrate that Sgk-to-ENaC signaling is extremely important for proper fluid and electrolyte balance. Moreover, preliminary findings from the Kuhl laboratory (62) appear to demonstrate conclusively for the first time that Sgk plays a pivotal role in the *in vivo* mineralocorticoid response. These investigators generated a mouse model containing *Sgk* lacking a functional kinase domain. These mice show no gross functional abnormalities, and their histology is normal in all organs assayed, including gut and kidney. However, these mice, which have appropriate Na^+ and K^+ metabolism when maintained on a normal diet, show inappropriate Na^+ wasting and hyperkalemia when maintained on a low- Na^+ diet.

FUNCTION OF KI-RAS. Overexpression of constitutive-active Ki-Ras with ENaC in *X. laevis* oocytes increased channel open probability (34). We (51) demonstrated that induction of Ki-Ras during the early phase is necessary and sufficient for some part of aldosterone’s action on Na^+ transport in polarized, renal epithelial cells. In this study, Ki-Ras was shown to be critical for stabilization of ENaC in the open state. The molecular mechanism of this action remains elusive.

FUNCTION OF PHOSPHATIDYLINOSITOL 3-KINASE. Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase important to both aldosterone- and insulin-dependent actions on epithelia (3, 12). PI3K is not, however, an aldosterone-induced protein, but its activity is increased by both aldosterone and insulin in renal epithelial cells. In addition to aldosterone and insulin, antidiuretic hormone stimulation of Na^+ transport in epithelial cells has been reported to be dependent, in part, on PI3K. Thus PI3K may be a focal point where insulin, vasopressin, and aldosterone signal transduction converge to activate a common cascade directed toward ENaC and/or the Na^+/K^+ -ATPase.

Blockade of PI3K impedes both the early and late phases of aldosterone actions with PI3K, apparently promoting/protecting ENaC levels in the apical membrane. Similar effects are observed when PI3K is inhibited during insulin induction of Na^+ transport. Thus PI3K is either permissive for Na^+ transport or it is common to both the aldosterone- and insulin-signaling pathways that culminate in increased Na^+ transport (see Fig. 4). If the latter scenario is true, then PI3K activity must be directly and continuously linked to ENaC activity, for when this kinase is inhibited, sustained Na^+ transport is quickly diminished, even in the continued presence of aldosterone and insulin.

FUNCTION OF CORTICOSTEROID HORMONE-INDUCED FACTOR. A fourth candidate signal molecule for aldosterone action is corticosteroid hormone-induced factor (CHIF), expressed in epithelia of the distal colon and nephron (2, 5, 48). CHIF is a member of the newly identified FXYD protein family (52) and, similar to other FXYD proteins, is a transmembrane regulator of ion channels and other transport proteins. The γ -subunit of the Na^+/K^+ -ATPase is also an FXYD protein. It is unclear whether family members can substitute functionally; however, this remains a distinct possibility, which may readily explain the role played by CHIF during a mineralocorticoid response. Aldosterone in the kidney promotes translation of CHIF through an as-yet-unidentified mechanism. CHIF is localized primarily to the basolateral membrane, where it presumably interacts with its final effector to stimulate transport.

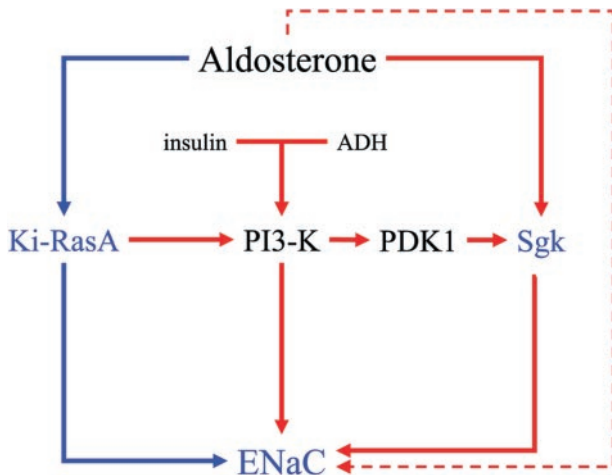


FIG. 4.

Integrated model of aldosterone signaling to ENaC, which includes many of the known aldosterone-regulated proteins. ADH, antidiuretic hormone; PI3K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase-1. Red and blue arrows indicate pathways involved in increasing ENaC number and open probability, respectively. Solid and dashed arrows indicate events important for the early and late phases of aldosterone action, respectively. Blue font indicates proteins regulated directly by aldosterone at the level of transcription.

Integrated model of aldosterone signaling to ENaC. It is hard to overlook the fact that there is a possible linear signaling relation between aldosterone-induced Ki-Ras and Sgk with PI3K positioned between these factors. Considering the common nature of these factors to signaling cascades that control cellular growth, apoptosis, and differentiation, one generalized view of aldosterone action on epithelia is that the steroid programs cells to “differentiate” more toward a Na^+ -reabsorbing phenotype and that Ki-Ras and Sgk are merely the early messengers of this signal. Adding further support for such a generalized mechanism is evidence that corticosteroids initiating a complex interaction between Ras and PI3K signaling are known to induce functional polarity and promote the formation of tight junctions and transepithelial resistances in mammary epithelia (61). Moreover, signaling through MR promotes the differentiation of brown adipocytes (38), and PI3K is a central switch directing tubulogenesis of epithelial cells (27). This generalized cascade would contain multiple converging and diverging pathways exerting pleiotropic ef-

fects on epithelia. Shown in Fig. 4 is one possible signaling cascade that includes many of the known aldosterone regulated proteins. What is clear in this idealized cascade is that Ki-Ras via PI3K impinges upon Sgk activity.

CHALLENGES TO THE CURRENT MODEL OF ALDOSTERONE ACTION

Recent challenges to the classical model of aldosterone action have arisen in primarily two areas: molecular mechanism of action and target tissues. The two most provocative challenges have been recent descriptions of the nongenomic mechanism of action and local aldosterone systems targeting novel tissues (reviewed in Refs. 14 and 36).

Nongenomic Actions

The genomic actions of aldosterone mandate changes in gene expression, which results in a substantial latent period (from 0.5 to 1.0 h) prior to overt changes in cellular activity. Inhibitors of transcription, translation, and steroid receptor translocation and steroid receptor antagonists abrogate genomic actions. Recent studies, mostly of nonepithelial cells, identified early effects of aldosterone (<15 min) that are not sensitive to these inhibitors (14, 20). These rapid responses are referred to as the nongenomic action of aldosterone and are considered to be independent of direct effects on gene expression.

Much remains to be learned about nongenomic actions, particularly with regard to the systemic importance of such acute and transient responses to aldosterone. Insight, however, has been gained recently with a study of humans that showed that administration of aldosterone significantly changed systemic vascular resistance and cardiac output within 3 min. The cardiovascular response dissipated after 10 min (46). This time course is thought to be too rapid for genomic actions. The systemic significance associated with the capability of a rapid response mediated by nongenomic actions should become even clearer as our understanding of local aldosterone systems increase.

Nongenomic actions of aldosterone were first definitively identified in erythrocytes, which clearly lack nuclei (49). Subsequently, many laboratories have

confirmed this initial observation, and recently major advances have been made in understanding this mechanism (14, 20, 36). Studies on the nongenomic actions of aldosterone have been in three areas: 1) description of the putative receptor, 2) identification of second messenger systems involved in this action, and 3) identification of final effectors.

Nongenomic actions result from interaction with a cytosolic and/or plasma membrane receptor that is clearly distinct from the classical steroid receptor. However, definitive identification of this nongenomic receptor has remained elusive. Nongenomic actions may actually represent a group of responses mediated by several different, nonclassical aldosterone receptors and/or responses to a diverging signaling cascade. Figure 5 shows a general comparison between genomic and nongenomic actions.

The nongenomic receptor. Because aldosterone is both a receptor ligand and a steroid, nongenomic actions potentially could result from activation of either the classical steroid receptor (MR) or a novel receptor and steroid actions on phospholipid mem-

branes. Most data are consistent with nongenomic actions being mediated by a novel aldosterone receptor that is not sensitive to traditional inhibitors of MR, such as spironolactone (14, 36). The most convincing study ruling out MR as the mediator of nongenomic actions was performed in MR knockout mice, which lack this receptor (21). In fibroblasts from these mice, both Ca^{2+} and cAMP were increased within minutes (<3 min) of aldosterone treatment. The converse experiment of overexpressing MR in cells normally lacking both this receptor and a rapid aldosterone response failed to reconstitute the nongenomic response (14).

The nongenomic receptor is most likely a membrane resident protein or one that closely associates with membranes. This property is distinct from that of MR, which is found in its inactive and active forms in the cytosol and nucleus, respectively. Moreover, aldosterone binding to the nongenomic receptor differs from binding to MR (see Ref. 14 for additional references). One of the most striking findings concerning the nongenomic response is that, in contrast to aldosterone, glucocorticoids at physiological concentrations,

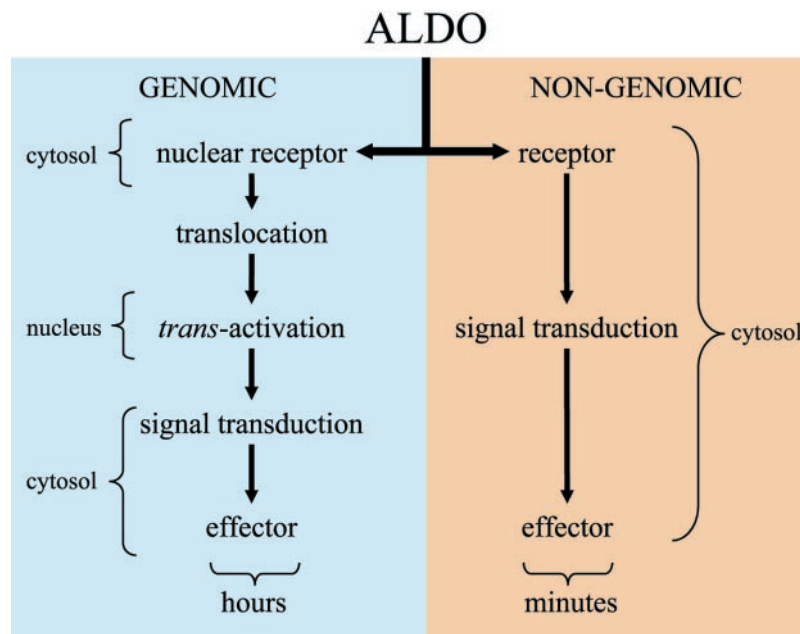


FIG. 5.
Comparison of the genomic and nongenomic actions of aldosterone. This flow chart highlights key points distinguishing between the genomic (left, blue box) and nongenomic (right, tan box) actions of aldosterone.

which are capable of binding MR and eliciting a mineralocorticoid response in the presence of inhibited 11 β -HSD2 (see above), do not appear to elicit nongenomic actions. An ~50-kDa membrane protein that binds aldosterone but not glucocorticoids has been isolated by Eisen and colleagues (13). Further characterization of this isolated, putative nongenomic aldosterone receptor is excitedly awaited.

Signal transduction of the nongenomic action.

The nongenomic actions of aldosterone are orchestrated by myriad second messenger systems. In vascular smooth muscle, aldosterone quickly increases phosphoinositide hydrolysis to elevate diacylglycerol (DAG) and inositol triphosphate (IP₃) levels (7). Also in these cells, aldosterone quickly promoted translocation of protein kinase (PKC), which is sensitive to both DAG- and IP₃-dependent Ca²⁺ signaling, from the cytosol to the membrane in a manner consistent with activation by these second messengers. Furthermore, inhibitors of PKC block rapid activation of the Na⁺/H⁺ antiporter by aldosterone (11). Others have shown that, in contrast to these findings, aldosterone rapidly inhibits PKC in myocytes (45). Nevertheless, PKC signaling appears central to nongenomic actions. Interestingly, Doolan et al. (9) report that, in colonic cells, aldosterone directly activates PKC independently of second messengers, suggesting that PKC might actually be the nongenomic receptor in this cell type.

In addition to Ca²⁺ and PKC signaling, aldosterone rapidly increases cAMP levels in smooth muscle. Normally, Ca²⁺ and PKC signaling are associated with contraction, whereas cAMP signaling is associated with relaxation, of smooth muscle. Thus the apparent conflict of aldosterone's activating both signaling pathways may actually represent the priming of these cells for rapid contraction-relaxation cycles. Indeed, a similar hypothesis has been presented to explain the apparently paradoxical finding that in colonic epithelia, aldosterone quickly activates ATP-sensitive K⁺ (K_{ATP}) channels but inhibits Ca²⁺-activated K⁺ channels (33).

Final effectors of the nongenomic action. Final effectors of the nongenomic actions of aldosterone include NHE3, K⁺ channels, and other proteins involved in cellular electrolyte and fluid balance and

Ca²⁺ metabolism (14, 20). The physiological consequences of these effects are clear at the cellular level, leading to transient changes in cellular pH and Na⁺, K⁺, and Ca⁺ levels. However, at the systemic level, the consequences of such transient activation are less clear. It is provocative to speculate that the rapid, nongenomic actions of aldosterone on epithelia enable appropriate transport responses during the latent period required for genomic actions. This possibility, however, currently remains unsubstantiated.

Emerging evidence suggests that nongenomic actions of aldosterone may be important for acute responses to local aldosterone production. Systems for local production and the ramifications of this production are just beginning to be described (36). Recent findings showing that aldosterone is synthesized in the heart (reviewed in Ref. 20) and that it affects extrarenal and colonic tissue have begun to redefine our perception of this important steroid. Aldosterone is now recognized to target diverse cell types including leukocytes, neurons and their support cells, both cardiac fibroblasts and myocytes, and vascular endothelial and smooth muscle, as well as adipose tissue. Indeed, direct action of aldosterone on cardiac cells plays a pathological role in inappropriate remodeling of the heart (63). Thus the "classical" target tissues of the distal colon and renal nephron and salivary and sweat glands no longer adequately define the systems affected by aldosterone.

Novel Target Tissues

The major physiological role of mineralocorticoids has long been thought to be the regulation of ion transport, so it was expected that MR, or type 1 receptors, would be found in epithelial tissues where Na⁺ transport was regulated. Consistent with this notion has been the localization of type 1 receptors and their protective enzyme 11 β -HSD2 in distal nephron of kidney, distal colon, sweat glands, and salivary gland epithelial cells (15, 17). In all of these cases, the role of aldosterone seems to be clearly related to regulation of transepithelial Na⁺ transport.

Beginning in the early 1980s, however, numerous investigators began to describe high-affinity aldosterone binding typical of type 1 receptors in a number of nonepithelial tissues. These tissues included pitu-

itary, hippocampus, cultured aortic cells, and myocytes (1, 15, 17). Interestingly, although these tissues showed the requisite high-affinity binding for aldosterone, they did not consistently demonstrate the decreased affinity for glucocorticoids then thought to be an intrinsic characteristic of MR. When MR was cloned, homologous mRNA for this protein was indeed localized to brain, pituitary, and heart (1), confirming that these high-affinity binding sites were indeed MR. Studies of nonclassical target tissues for distribution of 11-HSD partially explained the apparent lack of selectivity of nonepithelial MR. There are two distinct isoforms of 11-HSD. 11-HSD1, predominantly localized to liver and nonepithelial tissues such as adipose tissue and brain, is primarily a reversible reductase and may serve to amplify glucocorticoid action (47). 11 β -HSD2 is a dehydrogenase that inactivates glucocorticoids and protects MR occupancy (18). Most evidence suggests that 11 β -HSD2 is weakly expressed in adult brain and restricted to areas related to putative central actions of aldosterone on blood pressure and salt appetite (42) (see next section). In contrast to brain, 11 β -HSD2 has been demonstrated in cardiac myocytes (32) and vascular cells (53), suggesting that MR actions in these tissues may be more specific.

ALDOSTERONE AND CARDIOVASCULAR WELLNESS: AN INTEGRATED SYSTEMIC MODEL

It has long been known that aldosterone is associated with hypertension and is elevated in conditions of cardiac failure (59). Primary overproduction of aldosterone in Conn's syndrome has been demonstrated to result in sustained hypertension, and conditions of low cardiac output were known to stimulate adrenal synthesis of aldosterone through activation of the renin-angiotensin axis. These effects were thought to be mediated through MR located in classical epithelial target tissues and result from renal sodium retention. The discovery of MR in cardiac myocytes and fibroblasts, vascular endothelial cells and neurons of the paraventricular nuclei, along with the varying expression of the protective enzyme 11-HSD2, added a new complexity to the role of aldosterone in hypertension and cardiac disease.

Further complications arose with the discovery of local aldosterone synthesis by cardiac and vascular cells (22, 55). This area of research was given great impetus by the Randomized Aldactone Evaluation Study (RALES) trial, which demonstrated that the mineralocorticoid antagonist spironolactone produced an astonishing reduction in morbidity and mortality in patients with severe congestive heart failure (39). This effect was far out of proportion to the modest diuretic effect of the agent and suggested a direct effect of MR action on cardiovascular tissues that was, in fact, pathological. These effects might be autocrine or paracrine in nature, given the potential for local production of the steroid. One of the ironies arising from this body of work is that physiological effects of MR in these tissues remain largely unknown compared with the detailed knowledge of physiological effects of MR in classical epithelial target tissues, whereas the pathophysiological effects of MR in nonclassical tissues are the subject of intense study.

A number of elegant studies have suggested that MR localized to the paraventricular nuclei and amygdala in the brain are associated with salt intake and the generation of secondary or salt-sensitive hypertension (44). Studies of injections of either MR antagonists or antisense oligonucleotides for MR into brain tissues of rats made hypertensive by daily infusion of mineralocorticoids such as deoxycorticosterone have suggested a direct role for brain MR in the pathogenesis of hypertension in this model. Blockage of MR in amygdala results in a decrease in salt intake by affected animals and substantial reduction in hypertension (44). Alternatively, it has been demonstrated that intraventricular injection of aldosterone can induce hypertension in salt-loaded rats (16), and these effects may be antagonized by infusion of either glucocorticoids or MR antagonists, suggesting differential responses of central nervous system (CNS) MR to glucocorticoids and aldosterone (19).

Taken together, these and other studies indicate that MR may mediate substantial changes in blood pressure via CNS-regulated functions that include control of sodium intake (44). An interesting feature of these studies, as well as many of those of direct cardiac and vascular effects of aldosterone mediated by MR, is the dependence on salt intake. For example, Takeda and colleagues (54) demonstrated an effect of high so-

dium intake to increase aldosterone synthesis from heart and vascular tissues of stroke-prone spontaneously hypertensive rats (SHRsp) at a time when circulating aldosterone levels were suppressed, and Dorrance and colleagues (10) demonstrated a reduction in cerebral infarct size in SHRsp rats by aldosterone antagonists possibly mediated by blockade of MR-mediated vascular remodeling. Conclusions of this type in cardiovascular and CNS tissue are tentative at present due to lack of knowledge of MR-regulated target proteins in these tissues.

Aldosterone has been shown by a number of studies to induce cardiac fibrosis in the hypertensive or failing heart (4, 59) and to alter cardiac remodeling after myocardial infarction (8). These effects appear to be mediated by cardiac and vascular MR and may be regulated, in part, by local synthesis of aldosterone, which is enhanced in the failing heart (64). Cardiac and interstitial fibrosis is enhanced by aldosterone and blocked by MR antagonists and results, in part, from increased synthesis and deposition of collagen and procollagen. The exact proteins induced by MR under these conditions have not been identified, although some of the known targets of aldosterone in classical epithelial tissues do appear to be upregulated by aldosterone in heart, including *ras* and *Sgk* (unpublished observations).

Studies with transgenic mice overexpressing MR have also revealed an increase in cardiac expression of a number of genes detected by microarray analysis. These include *Sgk*, atrial natriuretic peptide, and early growth response gene 1 (30). These animals develop a dilated cardiomyopathy, consistent with a direct effect of MR on cardiac remodeling. Taken together, studies of cardiac and vascular aldosterone actions demonstrate that the potential for both local aldosterone production and protected MR-mediated actions have substantial and direct effects on blood pressure and cardiac function and provide a basis for further exploration of the cellular events underlying the impressive clinical effects of MR antagonists on outcomes of patients with cardiac failure observed in the RALES trial.

Further complicating this evolving area is the demonstration of rapid, possibly nongenomic effects of aldosterone on cardiac function. Infusions of aldoste-

rone during cardiac catheterization resulted in significant changes in systemic vascular resistance and cardiac output, which were detectable within 3 min of infusion, suggesting that not all aldosterone effects on cardiovascular tissues are mediated by MR-regulated gene expression (60).

Clearly much has been learned about aldosterone and its action the last few years. These findings have challenged the existing dogma that aldosterone exclusively targets epithelia via a genomic mechanism to modulate electrolyte and water balance. Although alternative target tissues, molecular mechanisms of action, and final effectors have been discovered, it is striking that almost every action of aldosterone, be it signaled by a genomic or nongenomic signal at an epithelial cell or some other cell type, is ultimately tied to regulation of the cardiovascular system. Thus the contemporary description of aldosterone is that this hormone targets the entire cardiovascular system and not just the kidney to regulate blood pressure.

Address for reprint requests and other correspondence: J. D. Stockand, Univ. of Texas Health Science Center San Antonio, Dept. of Physiology - 7756, 7703 Floyd Curl Dr., San Antonio TX 78229-3900 (E-mail: stockand@uthscsa.edu).

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