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Key Words

mineralocorticoid receptor, electrolyte transport, hypertension, polycystic kidney disease

Abstract

The steroid hormone aldosterone is a key regulator of electrolyte transport in the kidney and contributes to both homeostatic whole-body electrolyte balance and the development of renal and cardiovascular pathologies. Aldosterone exerts its action principally through the mineralocorticoid receptor (MR), which acts as a ligand-dependent transcription factor in target tissues. Aldosterone also stimulates the activation of protein kinases and secondary messenger signaling cascades that act independently on specific molecular targets in the cell membrane and also modulate the transcriptional action of aldosterone through MR. This review describes current knowledge regarding the mechanisms and targets of rapid aldosterone action in the nephron and how aldosterone integrates these responses into the regulation of renal physiology.

INTRODUCTION

MR: mineralocorticoid receptor, i.e., nuclear receptor NR3C2

RAAS: renin-angiotensin-aldosterone system

Almost 60 years ago, the corticosteroid hormone aldosterone was isolated, and its physiological effects on renal function were identified (1). Since then, there have been many important milestones in the evolution of our understanding of the mechanisms underpinning aldosterone action: the identification of the mineralocorticoid receptor (MR) as the principal aldosterone receptor (2); the characterization of aldosterone functional targets such as the epithelial Na^+ channel (ENaC), the renal outer medullary K^+ channel (ROMK), and the serum- and glucocorticoid-regulated kinase (SGK); and the elucidation of many of the processes that couple the receptor and effectors to achieve the physiological responses (3–6) (**Figure 1**). Aldosterone is released from the adrenal gland in response to two very distinct physiological stimuli: either (*a*) the production of angiotensin II through the renin-angiotensin-aldosterone system (RAAS), which occurs under conditions of hypotension, or (*b*) an elevated plasma K^+ concentration, which causes membrane depolarization of aldosterone-secreting cells (7–9). The nature of these stimuli defines the ultimate physiological effects of aldosterone release, which are either to elevate blood pressure through changes in vascular tone and electrolyte transport across absorptive epithelia or to promote

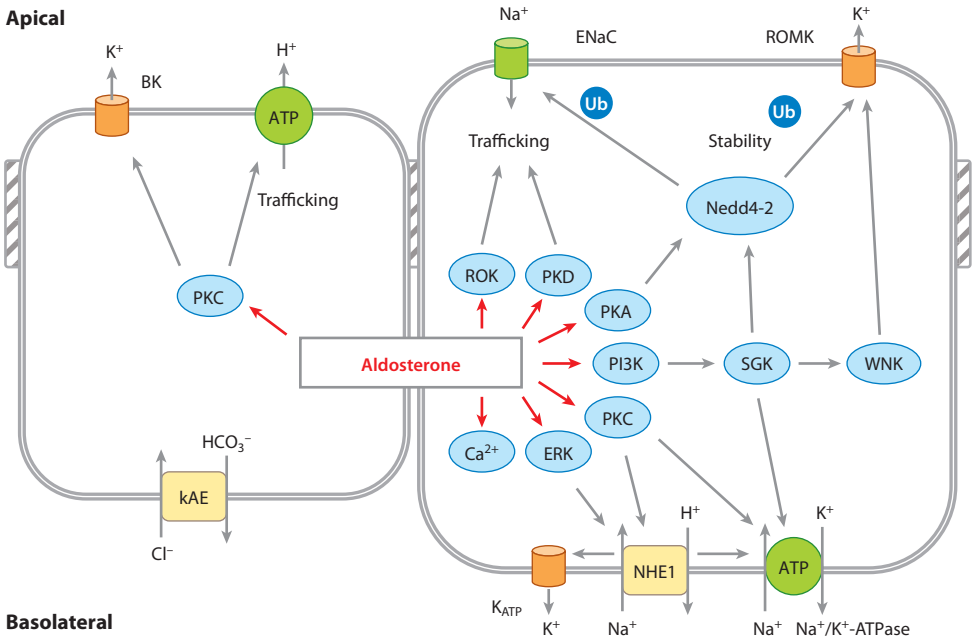

Figure 1

The modulation of membrane transporters by aldosterone-induced signaling intermediates. Aldosterone treatment modulates the activity of key signaling intermediates such as Rho-activated kinase (ROK), protein kinase D (PKD), protein kinase A (PKA), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC), extracellular stimulus–regulated kinase (ERK), serum- and glucocorticoid-activated kinase (SGK), and the with no lysine family kinases (WNKs) to modulate the activity of the epithelial Na^+ channel (ENaC), the renal outer medullary K^+ channel (ROMK), ATP-sensitive K^+ channels (K_{ATP}), Na^+/hydrogen exchanger 1 (NHE1), and the Na^+/K^+ pump (Na^+/K^+-ATPase) in the principal cells of the collecting duct. In the intercalated cells, aldosterone rapidly stimulates H^+ secretion via H^+-ATPase through PKC-dependent trafficking of the proton pump into the apical membrane. Aldosterone also modulates large-conductance K^+ (BK) channel activity and shifts kidney anion exchanger (kAE) activity from the basolateral membrane to the apical membrane.

the secretion of excess K^+ from the body under conditions of hyperkalemia.

The consequences of dysregulation of the physiology of aldosterone release, MR function, and their physiological targets are manifested in the contribution of such defects to rare genetic disorders such as pseudohypoaldosteronism type I (PHAI), in which MR is mutated (10), or PHAII, in which the key transporter regulators, WNK (with no lysine kinase) types 1 and 4 (WNK1 and WNK4), are mutated (11). These disorders have provided important information about aldosterone action and have helped to identify targets within the RAAS cascade for therapeutic intervention in chronic hypertension and the control of its adverse cardiovascular effects. The renal and cardiovascular systems are the key targets of aldosterone action, with impacts on electrolyte balance and blood pressure, but aldosterone also exerts its effects in the respiratory and digestive tracts, where MR is expressed, and in the brain (12). The kidney is the principal site for Na^+ conservation and the maintenance of K^+ homeostasis in the body. The cells of the distal nephron adapt their responses to the two different stimuli that promote aldosterone release through the interaction of aldosterone-induced responses with other components of the RAAS, in particular coincident angiotensin II–induced responses that are activated only under conditions of hypotension (13, 14).

To date, MR is the only known specific receptor for aldosterone. However, aldosterone can bind to the glucocorticoid receptor (GR) with low affinity (15). Moreover, the existence of a high-affinity membrane-associated receptor that is insensitive to MR antagonism and is unable to bind glucocorticoids has been detected in the vascular endothelium (16). The identity of this putative aldosterone membrane receptor has not been established, although supportive evidence for its existence comes from several sources. A 50-kDa protein with high aldosterone-binding affinity was isolated from human lymphocytes; unlike MR, this protein does not bind cortisol (17). In addition, aldosterone-induced rapid cyclic AMP (cAMP) and intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ responses were detected in cells isolated from MR knockout mice (18). In common with other steroid nuclear receptors, MR is a ligand-activated transcription factor. The binding of aldosterone stabilizes the interaction of MR with specific DNA sequence elements in the promoter regions of aldosterone-responsive genes to promote or suppress their expression through the recruitment of specific coactivators or corepressors (2). Aldosterone-bound MR binds to DNA-regulatory elements that are also recognized by GR. Consequently, heterodimerization as well as homodimerization of these receptors can occur at complete palindromic glucocorticoid response element (GRE) sites, adding a further level of integration to the regulation of gene expression by aldosterone and glucocorticoids. The transcriptional effects stimulated by aldosterone through MR are augmented by the rapid activation of signal transduction cascades, which are most often initiated through the interaction of aldosterone with MR. Even though some of these rapid signaling responses have been known for some time, it is only recently that their relevance to the physiological effects of aldosterone has been characterized and their synergism with the transcriptional responses to aldosterone has been established.

The expression of MR identifies aldosterone-sensitive tissues, including the vascular endothelium, cardiac myocytes, and the epithelium of the colon and the renal tubule. The nephron is the principal site for Na^+ conservation and K^+ secretion, and these critical processes are under precise hormonal control to maintain electrolyte homeostasis. The aldosterone-sensitive distal nephron (ASDN), comprising the distal convoluted tubule, the connecting tubule, and the collecting duct, expresses MR, and within the collecting duct, the principal cells express significantly more MR than do the intercalated cells (19). The principal and intercalated cell types express distinct subsets of transporters and channels. The transporters expressed by the MR-expressing epithelial cells of the nephron,

PHA: pseudohypoaldosteronism

Rapid responses to steroid hormones: signaling or physiological responses initiated by steroid hormones in target tissues that do not require changes in gene expression mediated by the transcriptional effects of their nuclear receptors

ASDN: aldosterone-sensitive distal nephron

such as ENaC, ROMK, the Na^+/K^+ pump, and Na^+/H^+ exchanger (NHE) isoforms, are all subject to regulation by aldosterone. This regulation is achieved through the modulation of transporter subunit expression, as in the case of the ENaCα subunit, or through the transcriptional modulation of regulatory proteins such as SGK1 (3, 20, 21). SGK1 is constitutively active, and unlike the other SGK isoforms, its expression is induced by aldosterone to modulate the activities of ENaC and ROMK. This is achieved either through the inhibition of channel ubiquitination or through interaction with the WNK family (14, 22). Acutely activated protein kinases and secondary messengers are also key regulators of transporter and ion channel activity, either directly through the modulation of site-specific subunit phosphorylation or indirectly through the stimulation of subcellular trafficking, to affect the abundance of these proteins in the cell membrane. The modulation of key transport proteins located in the ASDN by the signaling cascades that are rapidly activated by aldosterone is the subject of this review.

THE COUPLING OF MINERALOCORTICOID RECEPTOR TO GROWTH FACTOR RECEPTORS

The interaction between aldosterone and MR initiates rapidly activated signaling cascades that can be blocked using specific MR antagonists such as spironolactone and eplerenone. The capacity to display rapid aldosterone actions can be conferred on MR-null cells by exogenous expression of the receptor (23), and these signaling events can be initiated by expressing recombinant MR that lacks the coactivator-binding and DNA-binding domains (24). Some actions of aldosterone in renal cells cannot be conferred through MR expression or antagonized by spironolactone pretreatment; these include the transient rise in $[Ca^{2+}]_i$ (23). These data have been interpreted in different ways as evidence either that a completely novel, structurally distinct receptor initiates the nongenomic signal or that MR assumes different tertiary conformations that reconfigures the structure of its ligand-binding domain. The ligand-receptor conformation adopted may depend on the identity of the MR-interacting proteins, which in turn affect MR sensitivity to a particular antagonist. Evidence also points to aldosterone-binding sites on the membranes of renal cells (25, 26), which again either suggests that a small subset of expressed MR is associated with the cell membrane or points to the existence of a distinct membrane receptor for aldosterone. There is no physicochemical evidence for a transmembrane domain in any steroid nuclear receptor, and because MR lacks the palmitoylation site proposed for the membrane estrogen receptor, MR most likely associates transiently with the membrane through its interaction with another protein (as yet unidentified) that is more stably tethered at the cell membrane. Such an interaction may result in a MR conformation that is less spironolactone sensitive. It has further been proposed that spironolactone-insensitive effects of aldosterone can be effectively blocked using the MR antagonist eplerenone due to the more flexible structure of this compound (27).

The intermediate steps coupling the aldosterone-MR interaction to the rapid nongenomic activation of specific protein kinases are not fully characterized. However, the transactivation of the epidermal growth factor receptor (EGFR) is a crucial step in transducing this activating signal to the various downstream signaling intermediates that are responsive to aldosterone (28). The molecular mechanism by which EGFR activation is coupled to MR is still unclear and appears to be EGFR ligand independent. An activation model that is akin to the transactivation of EGFR by the estrogen receptor and is based on the matrix metalloproteinase cascade and the liberation of heparin-bound EGF cannot be ruled out (29, 30). c-Src phosphorylates EGFR within 5 min of aldosterone treatment. The activation of c-Src is one of the earliest signaling responses promoted by aldosterone in renal cells and identifies this small tyrosine kinase as

a key intermediate signal (31). MR resides in a complex with other proteins such as heat shock protein 90 (Hsp90) prior to ligand binding and nuclear shuttling. Antagonism of Hsp90 protein interactions using the geldanamycin analog 17-AAG blocks the c-Src-dependent phosphorylation of EGFR at Tyr845 and also suppresses EGFR-dependent downstream signaling events following aldosterone treatment (31, 32). Aldosterone-induced signaling events that are coupled to EGFR include the activation of extracellular stimulus–regulated kinase (ERK)1/2, MAP kinase (28), and protein kinase D (PKD) (32).

Phosphoinositide 3-kinase (PI3K) is a key regulator of aldosterone action and was believed to be the point of signaling convergence with insulin-induced effects in the nephron (33). SGK1 is activated in HEK 293 cells following insulin treatment through phosphorylation of SGK1 at residues Thr256 and Ser422, and insulin-induced SGK1 activation is stimulated within 2 min through PI3K-dependent activation of 3-phosphoinositide-dependent kinase 1 (PDK1) (34). Aldosterone treatment stimulates PI3K activity to promote Akt activation within 10 min of treatment in A6 cells through a pathway dependent on transactivation of insulin-like growth factor receptor-1β (IGF-R1β) (35). In this experimental system, aldosterone stimulated, within 1 h of treatment, a two- to three-fold increase in the transepithelial, amiloride-sensitive current, which was blocked by PI3K antagonism. PDK1 can activate PKD via the activation of novel protein kinase C (PKC) isoforms such as PKCδ or PKCε (36); such activation may point to further synergy between aldosterone and insulin-induced signaling responses.

REGULATION OF ENaC STABILITY BY ALDOSTERONE

The epithelial Na^+ channel ENaC is a major effector of aldosterone. Fully active ENaC—a heterotrimeric channel complex composed of one α subunit, one β subunit, and one γ subunit—localizes to the apical membrane of the principal cells of the distal nephron and other absorptive epithelia (37, 38). ENaC surface abundance and open probability determine the rate of Na^+ reabsorption from the renal ultrafiltrate across the epithelium. Consequently, ENaC activity is a key regulator of aldosterone-mediated Na^+ conservation by the kidney. ENaC continually cycles between the cell membrane and a subapical, intracellular pool (39). ENaC can also be ubiquitinated by Nedd4-2 ubiquitin ligase and directed for degradation by the proteasome (40). Thus, equilibrium exists between the various processes that contribute to ENaC subunit transcriptional regulation, subcellular trafficking, and expressed protein stability to achieve a steady state in ENaC surface expression. Any of these regulatory steps can be acted upon acutely or chronically to modulate the rate of Na^+ reabsorption in the nephron. The transcriptional regulation of SGK1 is the principal mechanism by which aldosterone modulates ENaC activity through a regulatory kinase (3, 41). The phosphorylation of Nedd4-2 by SGK1 suppresses its capacity to bind to the COOH-terminal PY motifs of ENaCβ and ENaCγ, suppressing their ubiquitination and promoting the stability of these ENaC subunits in the apical cell membrane (42, 43). Nedd4-2 is also a substrate for phosphorylation at its SGK1 target residue, Ser444, by PKA (42) and the inhibitor of κB kinase (IKK) β isoform (44). ENaC subunit stability is further enhanced by the transcriptional upregulation of ubiquitin-specific proteinase 2-45 (USP2-45) by aldosterone, which deubiquitinates ENaC subunits and rescues the channel from proteolysis (45). Studies using SGK1 knockout mice show differences in the scale of the SGK1 contribution to promoting ENaC activity. Generally, these mice display only a mild salt-wasting phenotype compared with MR and ENaCα knockouts, even under conditions of salt restriction (46, 47), suggesting that the action of SGK1 on channel stability can only partly account for the activation of ENaC by aldosterone.

RAPID REGULATION OF ENaC TRAFFICKING BY ALDOSTERONE

Recent studies have identified novel signaling pathways that modulate ENaC activity by altering the subcellular trafficking of the channel and so can refine the transcriptional effects exerted by aldosterone on ENaCα and SGK1 expression. The Rho subfamily of the Ras small GTPases has been implicated in the regulation of ENaC through the activation of associated kinases that regulate cytoskeletal organization and also influence phosphoinositide signaling. In various tissues, aldosterone activates cascades coupled to the Ras superfamily of small GTPases. These molecular switches have a significant impact on physiological responses to aldosterone. For example, aldosterone induces both the expression and activation of K-Ras in renal cells, which, through stimulation of PI3K, promotes ENaC open probability (48). A constitutively active form of one of the Rho small GTPases, Rac1, amplifies aldosterone-induced gene expression and stabilizes the association of MR with the nucleus following aldosterone treatment (49). In mesangial cells, aldosterone promotes the activation of Rho kinase (ROK), which is coupled to RhoA activation within 10 min of treatment (50). The activation of ROK is required for aldosterone-induced myofibroblastic transdifferentiation of these cells, which is associated with changes in the protein expression profile and with the stimulation of cell hypertrophy. The activation of ROK contributes to both the promotion of cardiac fibrosis (51) and the exacerbation of renal ischemia (52) by aldosterone. These pathological states are marked by the aldosterone-dependent generation of reactive oxygen species, which synergize with ROK activation to inflict tissue damage.

Classically, the activation of different Rho GTPases and their associated kinases is associated with changes in the structural configurations of cytoplasmic actin and consequently impacts subcellular vesicle trafficking. Dynein motor–dependent vesicle trafficking relies on actin polymerization and bundling, and cytoskeletal structures also tether cell-surface ENaC within discrete domains at the apical membrane (53). The activity of phosphatidylinositol-4-phosphate 5-kinase, a type II phosphatidylinositol phosphate (PIP) kinase, is also coupled to RhoA activation and regulates Golgi fission to release ENaC-containing transport vesicles (54). The serine/threonine kinase PKD1 also regulates type II PIP kinases (55). The three members of the PKD family of protein kinases, implicated in a wide range of physiological processes, play a pivotal role in the maintenance of Golgi structure and post-Golgi vesicle trafficking (56, 57). PKD1 activation in response to aldosterone affects the subcellular distribution of preexpressed ENaC subunits in renal collecting duct cells within 5 min of hormone treatment; however, increased apical membrane insertion of ENaC is not observed until after 60 min (58). The activation of PKD1 by aldosterone not only may determine the rate of aldosterone-induced ENaC subunit trafficking to the apical membrane but may also have a more general effect on subcellular trafficking of proteins in the cells of the distal nephron.

Aldosterone transcriptionally upregulates the expression of the 17-kDa glucocorticoid-induced leucine zipper protein (GILZ) in renal cortical collecting duct cells (59), where it suppresses basal ERK1/2 activation to promote the increased surface expression of ENaC and consequently stimulates Na^+ reabsorption (60). GILZ was first identified in the kidney but has been most intensively studied in the context of its role in the anti-inflammatory effects of glucocorticoids on cells of the immune system through its interaction with Ras and nuclear factor κB (NFκB) (reviewed in Reference 61). Multiple isoforms of GILZ are expressed in the kidney and have distinct biological roles, including the modulation of ENaC trafficking and the suppression of cell proliferation (62). In renal cell lines, GILZ1 stimulates the ENaC Na^+ current, whereas GILZ2 and GILZ3 also promote channel trafficking to the apical membrane without a concurrent rise in ENaC

activity. GILZ1 and GILZ3 effectively inhibit ERK1/2 phosphorylation, whereas GILZ4 suppresses cell proliferation but promotes ERK1/2 phosphorylation. GILZ binds to activated Ras to suppress ERK1/2 and Akt activation (63) and binds to Raf to block only the ERK1/2 pathway (63). Different amino acid residues are involved in each of these interactions, and consequently GILZ can also form heterotrimeric complexes with Ras and Raf. GILZ has potential phosphorylation sites that may determine its molecular interactions; however, the kinases that determine GILZ isoform phosphorylation states are yet to be determined. The characterization of these signaling intermediates will be important because it will help to discern why aldosterone appears to have differing effects on Ras-coupled signaling in the context of the upregulation of K-Ras expression and the simultaneous suppression of interactions of Ras with downstream effectors like ERK1/2 and Akt.

The explanation for aldosterone activation of antagonistic signaling events probably lies in the subcellular localization of signaling intermediates that regulate particular transporters to discrete complexes, although the same signaling intermediates may also be localized to other complexes that are differentially regulated (64). Nedd4-2 resides in an inhibitory complex with SGK1, Raf-1, and GILZ1 that is associated with ENaCβ (65). Increased expression of SGK1 and GILZ suppresses Raf-1 and Nedd4-2 activity in this complex and promotes ENaC trafficking to the membrane by suppressing channel ubiquitination. The effects of heterologous expression of SGK1 and GILZ on this protein complex can be replicated in polarized cortical collecting duct (CCD) cells by aldosterone treatment for 4 h. PKD is activated in response to aldosterone treatment of CCD cells, and the interaction of PKD with ERK1/2, PKCδ, and PKCε is altered following activation, illustrating a change in the complement of a further multicomponent signaling complex linked to the Raf–MAP/ERK kinase (MEK)–ERK cascade (58, 66).

RAPID MODULATION OF Na^+/K^+-ATPASE BY ALDOSTERONE

CCD: cortical collecting duct

TAL: thick ascending limb of the loop of Henle

The circulatory K^+ concentration is precisely regulated to maintain efficient functioning of excitable cells. The kidney contributes to K^+ homeostasis through K^+ reabsorption from the renal ultrafiltrate passing through the proximal tubule and the thick ascending limb of the loop of Henle (TAL). Hormone-regulated K^+ excretion is maximal in the principal cells of the ASDN. The activity of the Na^+/K^+-ATPase provides the electrochemical driving force for aldosterone-induced apical Na^+ reabsorption through ENaC (67). This provides a favorable electrochemical gradient for K^+ transported into the cytoplasm to be secreted across the apical membrane into the ultrafiltrate through K^+ channels, principally ROMK (Kir1.1), or alternatively recycled back into the blood through basolateral K^+ channels. The route taken by K^+ is determined by other aldosterone-regulated K^+ channels and the concurrent activation of transporters regulated by angiotensin under conditions of hypotension. There are contradictory reports in the literature going back to the early 1980s as to whether aldosterone can acutely affect Na^+/K^+-ATPase activity in adrenalectomized animals (68–70). The discrepancies may be due to differences in the time of adrenalectomy and so to the period of aldosterone depletion prior to experimentation (71). Under conditions in which ENaC activity was blocked or Na^+ was absent from bath solutions, aldosterone still stimulated Na^+/K^+-ATPase activity within 30 min of treatment (71). Na^+/K^+-ATPase activity is also sensitive to intracellular pH, which affects the cation-binding specificity of the pump (72, 73). Transient activation of NHE1 is one of the earliest physiological responses observed in aldosterone-treated CCD cells (74–76) and may contribute to the earliest phase of Na^+/K^+-ATPase modulation by aldosterone.

Aldosterone induction of Na^+/K^+-ATPase activity is at least biphasic; the initial phase

occurs in advance of detectable increases in α-subunit expression 6 h after aldosterone treatment (77). The later phase results in the accumulation of a Na^+/K^+-ATPase pool that can be recruited to the basolateral membrane in response to agonists such as vasopressin (78, 79). Aldosterone can also induce a very rapid translocation (<1 h) of α-Na^+/K^+-ATPase into the cell membrane with a concurrent rise in pump current that is maximal after 2 h in a colon cell line (80). Here a significant rise in SGK1 activity was measurable after aldosterone treatment for 1 h; however, there was a twofold rise in PI3K activity within 30 min of aldosterone treatment. PI3K is an important regulator of Na^+/K^+-ATPase trafficking and regulates incorporation of the pump into endosomes. The p85 subunit of PI3K directly binds the proline-rich motif of α-Na^+/K^+-ATPase when the pump subunit has undergone PKC-dependent phosphorylation at residues Ser11 and Ser18 (81). Aldosterone treatment of CCD cells results in the rapid activation of multiple PKC isoforms: PKCα (82), PKCδ, and PKCε (58). PKCε activation suppresses pump current in cardiac myocytes (83). There are four isoforms of α-Na^+/K^+-ATPase. Isoform α1 is ubiquitous in its expression and is the isoform regulated by aldosterone in the nephron; α2 is expressed in the brain, skeletal muscle, and cardiac myocytes, and α3 and α4 are expressed in the brain and testis, respectively (84). Structural differences between the α-Na^+/K^+-ATPase isoforms contribute to differences in their mechanism of regulation. For example, α1 and α3 differ at sequences proximal to the ATP-binding site so that α1 is activated by PKC whereas α3 is not (85). Ca^{2+}-dependent isoforms PKCα and PKCβ mediate the phosphorylation of α-Na^+/K^+-ATPase at its proline-rich motif (86), whereas PKA-dependent phosphorylation occurs at Ser943. The PKA phosphorylation event has variously been linked to the promotion (87) and inhibition (88) of pump activity in response to different agonists. The most likely explanation is that the acute regulation of pump activity through direct phosphorylation and trafficking represents the summation of multiple signaling cascades. The finding that intracellular Na^+ concentration ($[Na^+]_i$) can affect pump surface recruitment through the cAMP-independent stimulation of PKA (89) raises the question of whether aldosterone-induced NHE activity can activate the pump. This finding also highlights the importance of Na^+ self-inhibition of ENaC to stop an uncontrolled rise in $[Na^+]_i$ when ultrafiltrate Na^+ concentration is high.

RAPID EFFECTS OF ALDOSTERONE ON INTERCALATED CELL H^+-ATPASE

The major mechanism of tubular acidification in the CCD involves active, electrogenic H^+ secretion through vacuolar-type H^+-ATPase pumps located in intercalated α-type cells. It has been known since the early 1990s that corticosteroids can enhance late distal tubule acid secretion (90). Aldosterone acts on H^+ transport in CCD cells, resulting in rapid nongenomic activation of H^+-ATPase pumps (91), H^+ conductance (92), and Na^+/H^+ exchange (74). The molecular mechanisms of rapid nongenomic actions of aldosterone on the various acid extrusion transporters and the consequences for whole-body acid-base balance and the regulation of pH-sensitive ion transporters and signaling enzymes are still largely unknown and provide a ripe area for future research. The earliest reports of aldosterone stimulation of the vacuolar-type H^+-ATPase pump came from studies in amphibian skin and urinary bladder, which revealed a rapid action of the hormone on proton flux through H^+-ATPase pumps in mitochondria-rich intercalated cells (93). These early studies showed that aldosterone stimulated H^+ secretion independently of Na^+ transport as a result of trafficking of proton pumps stored in cytosolic vesicles into the apical membrane of intercalated cells via a PKC-dependent signaling pathway (94). Morphological changes in intercalated cell number and apical membrane area were also found to be a

feature of long-term exposure to aldosterone or deoxycorticosterone (95).

In Madin-Darby canine kidney (MDCK) cells, both aldosterone and vasopressin activate a bafilomycin-sensitive proton flux (vacuolar H^+-ATPase) acting separately through MR and V1 receptors, respectively (96). The sensitivity of the aldosterone effect to spironolactone in the MDCK cell line would indicate a genomic pathway. However, the rapid nongenomic action of aldosterone on H^+-ATPase pump activity described in mouse outer medullary collecting duct (OMCD) intercalated cells was insensitive to inhibitors of aldosterone interaction with MR and was unaffected by inhibition of transcription and translation (91). Both in amphibian skin and in renal intercalated cells, the rapid actions of aldosterone were prevented by incubation with microtubule-disrupting agents and inhibitors of PKC. The half-time for activation of H^+ flux and insertion of proton pumps into the apical membranes of intercalated cells was 12 min in the amphibian skin epithelium (94). A threefold stimulation of H^+ extrusion through the vacuolar-type H^+-ATPase pump was detected within 15 min in mouse OMCD cells (91). The rapidity of these responses precludes a genomic pathway but does not rule out signal transduction via MR. Rapid trafficking of vacuolar H^+-ATPase pump units into the luminal membrane is a major regulatory mechanism to enhance urinary acidification in response to elevated plasma pCO_2 or luminal HCO_3^- (97). A similar mechanism may explain the rapid nongenomic stimulation of H^+ extrusion by aldosterone, but the details of the protein kinase signaling network are still not worked out and provide an exciting challenge for future research. Apart from the lack of knowledge about the physiological role of rapid actions of aldosterone in regulating urinary acidification, little is known about the consequences of the nongenomic responses on the latent genomic effects of aldosterone. Moreover, little is known of the contribution, if any, of dysregulation of the early trafficking effects induced by aldosterone on proton pumps to disease states that affect distal tubular acidosis and that result from inborn errors of metabolism involving vacuolar H^+-ATPase subunits in the CCDs.

Another area of unexplored aldosterone physiology is the functional interaction between acid-secreting intercalated cells and Na^+-absorbing principal cells. In amphibian skin epithelium in vivo, when the luminal Na^+ concentration is low (<10 mM), as is the case in the distal tubule, proton secretion through intercalated cells provides the transepithelial charge compensation for Na^+ absorption across the principal cells (98). Under low external Na^+ conditions on the apical side of the epithelium, Na^+ uptake through principal cells is indirectly electrically coupled to proton secretion through intercalated cells. Therefore, aldosterone may induce a simultaneous rapid nongenomic effect on both H^+ secretion and Na^+ absorption in the CCD under physiological conditions of low external NaCl. Testing the hypothesis that aldosterone activation of electrogenic proton flux via H^+-ATPase pumps in α-type intercalated cells energizes Na^+ absorption through ENaC in neighboring principal cells may reveal a novel physiological role for rapid nongenomic effects of aldosterone in the CCD under conditions of physiologically low luminal Na^+ concentrations.

EGFR ACTIVATION VERSUS EGFR TRANSACTIVATION

Epidermal growth factor (EGF) suppresses Na^+ transport in the CCD (99). EGF treatment of isolated CHO cells heterologously expressing ENaC and EGFR resulted in a decrease in ENaC current, as measured in patch clamp experiments, through the suppression of channel open probability (100). This effect was detectable within 1 to 2 min of treatment and was tyrosine kinase dependent but did not involve direct tyrosine phosphorylation of ENaC subunits and was not blocked by c-Src antagonism. Chronic treatment of renal A6 cells with EGF or the more potent EGFR ligand transforming growth factor α (TGFα) also suppressed ENaC activity but through the

downregulation of channel subunit mRNA transcription, rather than through changes in channel open probability (101). Acute treatment of a confluent A6 cell epithelium with TGFα stimulated a rise in ENaC current, which peaked within 15 min of treatment and was attributed to a PI3K-dependent increase in open probability (101). EGFR ligands can thus stimulate or suppress ENaC activity, depending on the experimental model employed and the duration of exposure. EGFR is acutely transactivated by aldosterone, but this transactivation does involve receptor phosphorylation by c-Src, which contributes to stimulating at least part of the downstream repertoire of signaling events.

A couple of important questions are, How does the activation of EGFR by EGF differ from its transactivation by aldosterone, and what are the signaling and physiological consequences of this differential receptor activation? EGFR has multiple amino acid residues that can serve as targets for phosphorylation and as sites of protein interaction for the recruitment of specific signaling intermediates including phospholipase Cγ (PLCγ), growth factor receptor bound protein 2 (GRB2), cellular Casitas B-lineage lymphoma protein (c-Cbl), and Src homology 2 domain–containing transforming protein 1 (Shc1) (102). The EGFR phosphorylation state at these different sites may vary according to the manner of activation, and accessory signaling cascades are activated in parallel with EGFR activation. Human epidermal growth factor receptor 2 (HER2), rather than EGFR, is the erythroblastic leukemia viral oncogene B (ErbB) family member most highly expressed in the CCD; this may differ from the expression profile in immortalized cell lines and may contribute to the dichotomy in EGF effects found in different experimental models.

Na^+/H^+ EXCHANGER REGULATION BY RAPID ALDOSTERONE SIGNALING

The individual NHE isoforms have different functions in the nephron. These functions are reflected in their differential expression along the segments of the nephron, their localization in renal epithelial cells at their basolateral or apical surfaces, and their distinct activation in response to agonists. Acute and chronic treatment with aldosterone has a pronounced effect on NHE activity in established renal cell lines and in isolated nephron segments. However, published data show some contradiction in terms of whether the effects are stimulatory or inhibitory and which signal transduction cascades are coupled to NHE modulation in the different experimental models. The investigations do consistently show that the duration of exposure to elevated aldosterone concentrations is important in determining whether an inhibitory effect or a stimulatory effect on NHE activity is observed. Opposing effects of aldosterone on NHE activity may reflect the differential expression of MR and GR along the nephron with their differing affinities for aldosterone as well as the coupling of NHE isoforms to different signaling cascades and regulators in different cell types. NHE can be directly phosphorylated to modify its activity, as can its accessory proteins, the NHE-regulatory factors (NHERFs). NHE can be directly phosphorylated by ERK1/2, and the activation of NHE1 in CCD cells is ERK1/2 dependent (103). Similarly, NHE activity can be linked to PKC activation through increases in $[Ca^{2+}]_i$ (104, 105).

Nonelectrogenic Na^+ transport in the proximal renal tubule is subject to regulation by aldosterone. NHE3 is the main transporter facilitating Na^+ reabsorption in the proximal tubule, where >60% of the NaCl conservation by the nephron occurs. NHE3 activity in human primary renal proximal tubule epithelial cells (RPTECs) was sensitive to aldosterone treatment over 72 h (106). However, MR is expressed only at very low levels in the proximal tubule, whereas GR is at much higher abundance (19). In spite of this receptor expression profile, NaCl reabsorption by the proximal tubule is spironolactone sensitive, confirming that aldosterone acts through MR to promote NHE3 activity (107). Furthermore, aldosterone treatment of adrenalectomized rats over five days did not promote an increase

in total NHE3 abundance. Rather, aldosterone treatment promoted the association of NHE3 with the apical brush border, suggesting either the activation of subcellular vesicle trafficking to the cell membrane or the suppression of NHE3 recycling from the membrane (108). Aldosterone treatment of RPTEC cells for 72 h maximally stimulated NHE3 activity at a 1-nM concentration, whereas maximum expression required the application of tenfold-higher concentration of aldosterone. The action of aldosterone on NHE3 activity at 100 nM was entirely blocked by spironolactone, but NHE3 surface expression was only partially suppressed, raising the possibility that at this high steroid concentration there may be some GR involvement in NHE3 trafficking. The stimulation of NHE3 surface expression by aldosterone was completely abolished by antagonism of EGFR, and EGFR expression was elevated in response to aldosterone treatment. EGFR transactivation in some way contributed to both the activation and the trafficking of NHE3, which was stimulated by aldosterone in this experimental model. A similar biphasic effect of aldosterone was observed in the colon, where initial NHE3 membrane insertion was the result of increased $[Na^+]_i$ that resulted from a PI3K-dependent rise in Na^+/K^+ pump activity. The second phase of NHE activation was attributed to a SGK1-dependent rise in the expression of NHE3 and Na^+/K^+-ATPase (80).

Over a shorter time course of 15 to 20 min, the nongenomic actions of aldosterone augmented 1,25 dihydroxyvitamin D3 suppression of NHE3 activity in the TAL through a mechanism that is ERK1/2 dependent but PI3K independent (109). The inhibition of exchanger activity reduces the rate of apical HCO_3^- absorption, which is driven by H^+ secretion. ERK activation in response to α-adrenergic receptor agonists promotes NHE3 activity, suggesting that other signals distinguish the effects of these ligands from the effects of aldosterone (110). Nerve growth factor (NGF) also antagonizes NHE3 activity in the TAL (111) but does so indirectly. Here ERK1/2 is also activated but in turn suppresses basolateral NHE1 activity, which indirectly inhibits apical NHE3. Aldosterone infusion promotes a transient rise in Na^+ excretion from the kidneys of experimental animals within 15 min of commencement; this transient rise subsides when infusion is stopped (112). This early phase of increased Na^+ excretion was not mirrored by a change in HCO_3^-, K^+, or Cl^- excretion or a change in urinary pH. The rapid activation of NHE3 in the proximal nephron that precedes the later stimulatory effects on ENaC in the ASDN and on NHE3 may account for this effect of aldosterone on Na^+ secretion. However, the physiological usefulness of such activation in response to a stimulus aimed at promoting Na^+ conservation is unclear. The $[Na^+]$ in the renal ultrafiltrate reaching the CCD rises, resulting in increased self-inhibition of ENaC and a shift in the burden of Na^+ reabsorption from the proximal nephron to the distal nephron, where Na^+ reabsorption is more tightly controlled by corticosteroids. There is thus a brief transient rise in Na^+ excretion as this physiological shift is achieved.

Biphasic responses to aldosterone have also been reported in the microdissected S3 (pars recta) segment of the proximal tubule, which displays concentration dependency and the involvement of MR and GR as the signal-initiating receptors (104). Aldosterone (1 pM) stimulated an initial rise in $[Ca^{2+}]_i$ within 1 min, followed 6 min later by a second increase that was sustained for at least 1 h. This rise in $[Ca^{2+}]_i$ was mirrored by a stimulation of NHE1-mediated pH_i recovery rate under conditions of acid loading, which was sensitive to GR antagonism but not to MR antagonism. This differs from the situation in mesenteric blood vessels, where the aldosterone-induced rise in $[Ca^{2+}]_i$ and NHE1 activity was sensitive to MR antagonism with eplerenone (105). The responses in the S3 nephron segment after 1 h of aldosterone treatment were spironolactone sensitive, indicating MR dependency, and sensitive to inhibitors of transcription and translation. The conclusion based on these data is that GR is responsible for transducing an early nongenomic $[Ca^{2+}]_i$ signal, whereas the

later phase of NHE1 stimulation depends on changes in gene expression that are mediated by MR. Treatment of the isolated S3 segments with aldosterone at a supraphysiological concentration (1 μM) resulted in an amplified Ca^{2+} response but a suppression of NHE activity to less than basal levels rather than the stimulation seen at lower concentrations.

Modulation of NHE activity by aldosterone may also contribute to the activation of pH-sensitive K^+_{ATP} channels in the basolateral membrane to promote K^+ recycling (113). Other mechanisms for the regulation of apical K^+ channel activity rely upon elevated SGK1 activity either to suppress Nedd4-2, which ubiquitinates KCNQ2- and KCNQ3-type K^+ channels (114, 115), or to modulate ROMK activity through direct phosphorylation of WNK4 by SGK1 (14). ROMK is the principal K^+-secreting channel in the kidney and is expressed apically along the ASDN (116). Phosphorylation of ROMK by protein kinase A (PKA) affects the pH sensitivity of the channel (117) and also uncouples it from CFTR, rendering ROMK activity insensitive to ATP. The dynamic interaction of ROMK with CFTR is mediated by NHERF-2, which also binds SGK1 and PKA (118). It remains to be established whether the rapid aldosterone-induced kinases play a direct role in regulating ROMK activity.

ALDOSTERONE AND RENAL CELL HYPERTROPHY AND PROLIFERATION

Aldosterone stimulates a rapid rise in Na^+/H^+ exchange in renal CCD and MDCK cells. This rise is due to an ERK1/2-dependent rise in NHE1 activity (103, 119). The resulting transient rise in cytosolic pH may facilitate the activation of pH-sensitive signaling intermediates that are involved in cell cycle progression, such as the ribosomal S6 kinase (RSK) family protein kinases (120). This coupling of ERK1/2 to cell cycle advancement through NHE1 activation is also a facet of growth factor–stimulated cell proliferation (121, 122). Recent evidence points to aldosterone as a regulator of cell growth and differentiation in various tissues, including the nephron. Circumstantial evidence—e.g., the signaling cascades that are sensitive to aldosterone treatment, in particular the ERK1/2 MAP kinases, PKCs, and the transactivation of growth factor receptors—additionally indicates that aldosterone may affect the growth of responsive cells. The ERK1/2 signaling cascade is a key regulator of many cellular processes in diverse tissues, especially those linked to cell growth and proliferation. The ERK1/2 cascade is rapidly activated in response to aldosterone treatment of cell line models for different segments of the nephron, and this activation is generally coupled to EGFR transactivation (123). Aldosterone also stimulates the expression of EGFR in renal tubules of adrenalectomized rats, which increases the EGF and TNFα sensitivity of the renal cells and demonstrates the coupling of corticosteroid and growth factor signaling. SGK1 suppresses apoptosis-associated gene expression by phosphorylating and inhibiting the forkhead box OA3 (FOXO3) transcription factor (124).

Recently published data show that aldosterone affects the proliferation and differentiation of isolated renal stem cells (125, 126) as well as the proliferation of transformed CCD cell lines (127). Evidence also points to aldosterone involvement in the upregulation of renin-secreting mesangial cell proliferation; again a rapid increase in ERK1/2 activity is implicated (128). There are, however, no experimental data from animal models indicating a role for aldosterone in initiating cell proliferation in the adult, healthy distal nephron. The situation in diseased kidneys may be different. Dysregulation of epithelial cell proliferation is an important factor in chronic renal conditions such as polycystic kidney disease (129), which is a genetic disorder associated with mutations in genes encoding polycystin I and polycystin II (130, 131). However, hypertension is a significant factor contributing to cyst enlargement and the resulting decline in renal function. EGFR expression is elevated in cells forming these cysts (132). Transactivation of EGFR by

aldosterone resulting in stimulation of ERK1/2 may be another factor influencing the rate of disease progression and supports the rationale for using MR antagonism at the earliest stages of treatment (133).

CROSS-TALK BETWEEN ALDOSTERONE-INDUCED SIGNALING CASCADES AND TRANSCRIPTION REGULATION

A key question is how rapidly induced signal transduction cascades involving diverse intermediates such as ERK1/2, PKC isoforms, and Ca^{2+} mobilization, which are detectable within a few minutes, can impact major transcriptional events such as SGK1 and ENaCα expression, which occur much later (**Figure 2**). The answer may be that the initiation of aldosterone-induced gene expression is a synthesis of multiple events that include MR nuclear stabilization and cofactor recruitment. The binding of MR (and GR) by aldosterone directly affects gene expression through the DNA-binding properties of these receptors and through the capacity of the receptors to recruit coactivators and corepressors to promoter sites in order to modulate transcription (134). MR resides in a multiprotein complex in the cytoplasm with partners including Hsp90 (135). The displacement of Hsp90 from this complex following aldosterone binding exposes a nuclear localization signal on MR that promotes its translocation. The induction of MR-dependent transcription is sensitive to PKA antagonism with 8-bromo-cAMP; a PKA-mediated phosphorylation event may facilitate the release of MR from its cytoplasmic protein complex (136).

Nuclear receptors can also be phosphorylated. For example, estrogen receptor α is phosphorylated on Ser118 by ERK1/2 in breast carcinoma cells (137). This modification can promote ligand-independent nuclear translocation of the receptor when malignant cells switch from an endocrine-dependent to a growth factor–dependent proliferative phenotype. Similarly, MR also undergoes phosphorylation in CCD cells following

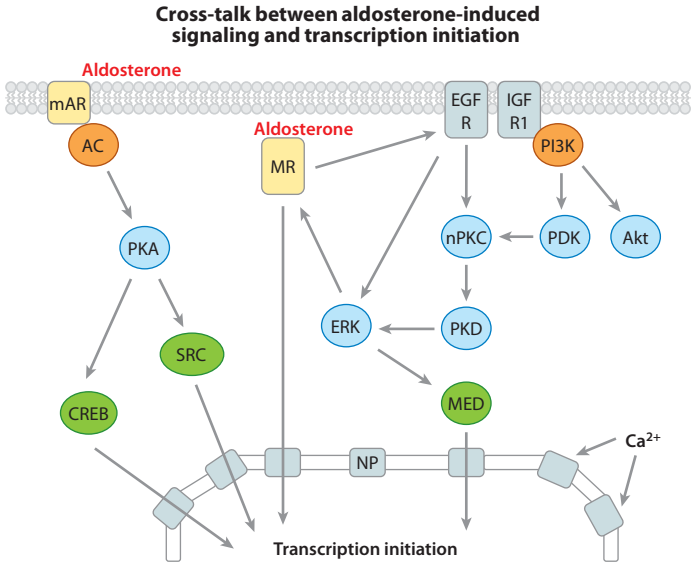

Figure 2

Aldosterone-induced signaling cascades modulate mineralocorticoid receptor (MR)-dependent transcription. Aldosterone initiates the activation of signaling cascades through interaction with MR or a membrane aldosterone receptor (mAR). Activation of the adenylate cyclase (AC) protein kinase A (PKA) affects nuclear translocation of cAMP-responsive element–binding protein (CREB) and steroid receptor coactivators (SRC). Transactivation of epidermal growth factor receptor (EGFR) or insulin-like growth factor receptor (IGFR) results in the activation of the phosphoinositide 3-kinase (PI3K)-coupled protein kinases phosphoinositide-dependent kinase (PDK) and Akt and activation of the novel protein kinase C (nPKC)/protein kinase D (PKD) cascade, which in turn stabilize extracellular stimulus–regulated kinase (ERK). ERK can phosphorylate MR to promote its nuclear translocation and recruitment of the transcription initiation mediator (MED) complex. Aldosterone-induced Ca^{2+} store depletion influences the trafficking of macromolecular complexes through nuclear pores (NP) to modulate gene expression.

aldosterone treatment, pointing to a coupling of the early protein kinase signal transduction cascades to the direct transcriptional effects of aldosterone (82). The activation of p21-activated kinase (PAK) through Rac1 small GTPase stimulation augments the nuclear translocation of MR and the induction of transcription in response to aldosterone treatment (49). It is not clear whether PAK directly phosphorylates MR or whether its effects on other signaling intermediates contribute to the subcellular redistribution of MR. The protein kinase cascades activated by aldosterone can also impact transcription through the phosphorylation of (*a*) the cofactors that are recruited

SRC: p160 steroid receptor coactivator

by direct interaction with MR or (*b*) transcription factors that modulate gene expression independently of MR binding to DNA.

All steroid nuclear receptors require coactivator transcription factors to promote gene expression. Included among these transcription factors are the p160 steroid receptor coactivator (SRC) family of cofactors, which form the core of the transcription preinitiation protein complex, and the mediator (MED) protein complex, which serves as the molecular interface with RNA polymerase II. The phosphorylation profile of these coactivators is determined by the intracellular signaling environment, which in turn is influenced by different factors (including circulating steroids). Consequently, the phosphorylation profile carries information about the environmental stimuli to which cells are exposed. Coactivator phosphorylation influences the specificity of protein-protein interactions and determines cofactor recruitment into the preinitiation complex. Phosphorylation also determines subcellular distribution and SRC sensitivity to ubiquitination and degradation by the S20 proteasome. The phosphorylation profile of SRCs in response to estrogen exposure in the context of breast cancer is the best-studied model of SRC posttranslational modification. These cofactors are required for aldosterone-dependent transcription, and aldosterone-induced signaling events also have the potential to influence the phosphorylation of the SRCs and other cofactors. Aldosterone-induced expression of SGK1 and ENaCα requires the recruitment of SRC1 to the promoter elements of these genes, and SRC1 colocalizes with MR in the nuclei of cells in the murine CCD (134). Each of the coactivators has multiple phosphorylation sites that are targeted by different kinases, and some of these kinases are rapidly activated in response to aldosterone treatment in the nephron. PKA and p38 MAPK phosphorylate SRC2 and SRC3, respectively, to affect ubiquitination and stability (138, 139), whereas the recruitment of MED1 into the mediator complex requires phosphorylation by ERK1/2 (140).

The transient activation of cAMP signaling by aldosterone was reported in CCD cells, and PKA-dependent phosphorylation and activation of the CREB transcription factor following aldosterone treatment were detected (141). This has been proposed as the mechanism underlying the MR-independent upregulation, following aldosterone treatment, of *gadd*153 gene expression through activation of its CRE/ATF promoter element (142). The induction of gene expression by aldosterone was antagonized with spironolactone or RU486 for all but a small subset of aldosterone-responsive genes that included *gadd*153. A number of researchers have reported aldosterone-induced PKA activation; however, investigators have also described antagonism between the physiological responses stimulated by forskolin and aldosterone. More recent evidence points to a suppression of CREB-dependent transcription through the upregulation of protein phosphatase 2β (PP2B) activation by aldosterone (143). This phenomenon may be due to the specific activation of different subpopulations and isoforms of adenylate cyclase and PKA by forskolin and aldosterone; such activation may lead to compartmentalized signaling within cells. Alternatively, such suppression may reflect a built-in negative feedback response that is intrinsic to aldosterone signaling and that makes cells refractive to further PKA stimulation following the initial aldosterone-induced response. The rapid activation of PP2B by aldosterone also emphasizes the need to understand how signaling cascades are rapidly suppressed by aldosterone, as well as the need to determine the targets of aldosterone-induced kinases.

The nuclear envelope forms an effective barrier between cytosolic signaling events and the genome. The nuclear pore complex (NPC) gates the movement of macromolecules such as transcription factors, kinases, and phosphatases into the nucleus and transcribed mRNA into the cytoplasm. The small size of the nuclear pore means that the transport of macromolecular complexes, millions of Daltons in size,

requires dramatic changes in pore structure. Aldosterone treatment can modulate the channel dimensions of NPC to facilitate cytosol-to-nucleus trafficking and to increase nuclear volume (144). The enhanced macromolecular transport detected in *Xenopus laevis* oocytes following aldosterone treatment was transient, lasting up to 15 min after hormone treatment. This may represent a discrete time window when certain cytosol-to-nucleus trafficking events can occur (145). The structural changes in the NPC are influenced by nuclear Ca^{2+} store depletion (146) and ATP availability (147), factors that the rapid actions of aldosterone may impact and that can consequently affect the expression of genes independently of direct MR-mediated transcription.

CONCLUSION

Many questions remain to be addressed concerning the physiological importance of rapid signaling events stimulated by aldosterone in the renal tubule and at other aldosterone target sites within the body. Points for consideration include simultaneous stimulation of apparently antagonistic signaling responses and discrepancies between observations made in vivo when compared with established renal cell line models. An important consideration is that the rapid responses to aldosterone are not necessarily transduced to the same effectors as the latent genomic effects. This raises the prospect that at least some of these rapid effects potentiate later events or impose an additional level of regulation on aldosterone actions that are dependent upon changes in gene transcription. Rapidly activated signaling cascades couple the activity of transport proteins at opposite poles of the cell and may result in fine adjustments to physiological processes that can respond more quickly to changes in circulatory aldosterone than to changes in protein expression (**Figure 1**). Aldosterone-induced signaling should not be thought of as a rapid response from zero to a high physiological concentration of hormone, which is often the approach used in experimental models, but rather a continually changing response to subtle changes in aldosterone secretion that is a reflection of the status of electrolyte homeostasis within the body over time. The secretion of corticosteroids was regarded as a smooth curve that reflected the stage in the body's diurnal cycle. It is now known that in rodents, corticosterone is released according to an ultradian rhythm: Brief peaks of varying amplitude occur with a 50-min periodicity throughout the diurnal cycle (148). This may also be the case for aldosterone, for which the amplitude of such peaks is determined by physiological sensors affecting the RAAS. The peaks in the ultradian rhythm of corticosterone release result in a pulsatory pattern of GR-dependent transcription, with mRNA abundance related to hormone concentration (149). The interaction of steroid receptors with their target DNA sequences is very transient and, in synchronized cells, displays a very precise periodicity of receptor recycling between the nucleus and cytoplasm (150). In this context the cell is continually monitoring its external environment for fluctuations in steroid concentration. The differential rapid activation of signaling cascades that determine the phosphorylation state of transcription factors can confer a crucial level of sensitivity to the regulation of aldosterone-dependent gene transcription (**Figure 2**).

SUMMARY POINTS

1. Aldosterone induces the rapid nongenomic activation of signaling cascades in tissues that express the mineralocorticoid receptor (MR), including the renal tubule.
2. Aldosterone-responsive signaling intermediates modulate the subcellular trafficking, proteolytic stability, and activation of membrane protein targets to regulate electrolyte transport in synergy with the chronic genomic effects of aldosterone.

3. The activation of protein kinases by aldosterone is coupled to transactivation of growth factor receptors at the cell membrane, with implications for modulating cell growth.
4. Steroid hormones, including aldosterone, may enhance their own transcriptional responses through the phosphorylation of the nuclear steroid receptor and key transcription factors, such as the steroid receptor coactivators.
5. Aldosterone-induced signaling cascades provide a mechanism for fine-tuning the transcriptional responses of the renal tubule cells by continually sensing the extracellular endocrine environment.

FUTURE ISSUES

1. The nature of the receptor initiating rapid nongenomic aldosterone–induced signaling remains to be clarified. Data support the initiation from MR in most instances, but other data support the existence of a plasma membrane–binding site for aldosterone.
2. The activation of apparently antagonistic signaling cascades by aldosterone may reflect intrinsic negative feedback mechanisms or may be the result of localized sites of signal activation leading to the activation of a very precise subset of molecular targets rather than of all potential aldosterone targets within the cell.
3. The modulatory effect of the rapid signaling responses on the transcriptional effects of MR is an emerging theme. The relevance of these events to the effects of aldosterone on whole-body electrolyte and acid-base homeostasis remains to be understood.

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