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REVIEW ARTICLE

Context-dependent mechanisms modulating aldosterone signaling in the kidney

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Abstract The aldosterone–mineralocorticoid receptor (MR) system serves as the major regulator of fluid homeostasis, and is an important drug target for the treatment of hypertension, heart failure, and chronic kidney disease. While the ligand aldosterone plays a central role in facilitating MR activity, recent studies have revealed that MR signaling is modulated through distinct mechanisms at the levels of the receptor and the downstream targets. Notably, phosphorylation of the ligand-binding domain in MR regulates the ability of the receptor to bind to ligand in renal intercalated cells, providing an additional layer of regulation that allows the cell-selective control of MR signaling. These mechanisms are involved in the context-dependent effects of aldosterone in the distal nephron. In this article, the recent progress in the understanding of mechanisms regulating the action of aldosterone is discussed, focusing on the connecting tubules and collecting duct in the kidney.

Keywords Aldosterone · Mineralocorticoid receptor · Intercalated cell · Post-translational modification · Phosphorylation · Renin–angiotensin system

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Introduction

Steroid hormone aldosterone and its receptor the mineralocorticoid receptor (MR) are the central regulators of fluid homeostasis in the body. Mutations in genes encoding the constituents of the aldosterone–MR axis can result in both hypotension and hypertension, clearly illustrating the predominance of this system in regulating blood pressure in humans [1].

Aldosterone is synthesized in the zona glomerulosa cells of the adrenal gland. Once produced, aldosterone enters the systemic circulation and binds to MR in target tissues, inducing downstream signaling. While the recent advances in high-throughput sequencing technology have facilitated the discovery of molecules mediating aldosterone biosynthesis in the adrenal gland [2–6], accumulating studies have also provided insights into how the kidney responds to the elevated plasma aldosterone to produce appropriate homeostatic responses. I here review recent key progress in our understanding of the mechanisms modulating the action of aldosterone in the kidney, especially focusing on electrolyte transport machinery in the connecting tubules (CNT) and collecting duct (CD).

Na–Cl transport mechanisms in CNT and CD

In the kidney, more than 99 % of salt filtered in the glomeruli is reabsorbed by the tubular cells. Although a major part of this process occurs at the level of proximal convoluted tubules, fine tuning of the total amount of salt reabsorption occurs in the aldosterone-sensitive distal nephron (ASDN). Among the cells lining the ASDN, one of the best characterized targets of aldosterone is the principal cells in CNT and CD, which express the

amiloride-sensitive epithelial Na^+ channel (ENaC) and are responsible for electrogenic Na^+ reabsorption. Although MR can bind to both aldosterone and cortisol, selective binding of aldosterone to MR in principal cells are ensured by the expression of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), catalyzing cortisol to inactive cortisone. Upon binding of aldosterone, MR undergoes conformational change, translocates to the nucleus, and regulates transcription of target genes including *SGK1* and *SCNN1A*. SGK1 (Serum and glucocorticoid-induced kinase 1), a Ser/Thr kinase, then phosphorylates ubiquitin ligase NEDD4-2 (neuronal precursor cell expressed developmentally downregulated 4-2), resulting in decreased association between NEDD4-2 and ENaC [7]. This in turn decreases ubiquitination and degradation of ENaC, increasing the number of the channel on the plasma membrane. ENaC mutations in Liddle's syndrome affect the interaction of NEDD4-2 with ENaC, phenocopying the downstream effects of aldosterone in principal cells [7]. Aldosterone regulates ENaC also via proteolytic cleavage [8] and epigenetic modification [9].

In the extracellular fluids, the major cation is Na^+, whereas the major anion is Cl^-. A large body of evidence has indicated the importance of Cl^- in regulating fluid volume homeostasis. The dependence of blood pressure on Cl^- has been demonstrated in well-established models of salt-sensitive hypertension, including DOCA-salt model [10], Dahl salt-sensitive strain [11], and angiotensin II infusion model [12]. Consistently, clinical studies have shown that the anionic component of sodium salt influences its ability to increase blood pressure in hypertensive subjects [13, 14]. In these studies, the pressor effect of high sodium intake is most prominent when Na^+ is administered as sodium chloride, but not as sodium bicarbonate nor sodium phosphate [13, 14].

Among the renal Cl^- flux pathways, accumulating data highlight the role of intercalated cells. In the CNT and CD, Cl^- is reabsorbed either via paracellular route or transcellular route [15]. In the former, Cl^- is transported through tight junctions consisting of several claudins [16]. In the latter, it has been known that the transcellular Cl^- flux occurs via the intercalated cells [17], and Wall et al. revealed that this process is primarily mediated by SLC26A4 (pendrin), a Cl^-/HCO_3^- exchanger selectively present in β-intercalated cells [18, 19]. They found that Cl^- flux in the cortical CD disappears in mice lacking pendrin, resulting in hypotension, especially when challenged with a low Na–Cl diet [19]. Consistent with this finding, Soleimani et al. reported that the double knockout of pendrin and Na–Cl cotransporter (NCC) results in severe volume depletion [20], demonstrating the compensatory roles of these Cl^- flux mediators. Conversely, overexpression of pendrin produces salt-dependent hypertension [21].

These observations from the basic studies are also of clinical relevance. Pendred syndrome is an autosomal recessive disorder featuring thyroid abnormality and hearing impairment that results from the loss of function mutations in *SLC26A4*. Although there seems to be no apparent symptoms attributable to the kidney disorder at baseline, these patients are actually extremely sensitive to diuretics, and show severe chloride depletion in response to thiazide therapy [22].

Importantly, H^+-ATPase is also involved in fluid volume homeostasis. In intercalated cells, H^+-ATPase controls the membrane potential difference and critically regulates the cell function [23]. B1 subunit of H^+-ATPase is predominantly present in the apical membrane of intercalated cells, mediating the acid secretion; mutations in *ATP6V1B1* (encoding B1 H^+-ATPase) cause type I (distal) renal tubular acidosis [24, 25]. The mouse model (*Atp6v1b1*$^{-/-}$) is also created [26], which consistently shows impaired acid secretion in the kidney. Using this model, Gueutin et al. recently reported that the *Atp6v1b1* deletion also results in renal loss of Na–Cl, K^+, and water [27]. Notably, they reported that the levels of ENaC-α, ENaC-γ, aquaporin 2, and pendrin are reduced in the cortex (but not in the medulla in the case of ENaC) in *Atp6v1b1*$^{-/-}$, which is abolished by the inhibition of prostaglandin E2 synthesis. The authors further showed in the isolated microperfused cortical CD that the inhibition of H^+-ATPase by bafilomycin A1 increases prostaglandin E2 levels [27]. Thus, evidence indicates that H^+-ATPase in intercalated cells, as well as Cl^-/HCO_3^- exchanger, mediates Na–Cl and water reabsorption, and intercalated cells and principal cells can communicate via a paracrine mechanism involving prostaglandins. These studies clearly establish that intercalated cells are key components of the kidney-fluid mechanism.

Thiazide diuretics are widely used to treat hypertension. The primary target of thiazide is considered to NCC, which is selectively present in distal convoluted tubules (DCTs). However, Terada et al. showed that Na–Cl transport sensitive to thiazide also occurs in the cortical CD [28]. The recent study confirmed this observation, and demonstrated that Na^+-dependent Cl^-/HCO_3^- exchanger (NDCBE; encoded by *SLC4A8*) is involved in this process [29]. In the proposed model, NDCBE and pendrin are considered to act in tandem to regulate Na–Cl reabsorption in β-intercalated cells.

Context-dependent action of aldosterone in CNT and CD: possible role of intercalated cells

It has long been known that aldosterone is produced both in hypovolemia and hyperkalemia [30]. In hypovolemia, the activation of the renin–angiotensin system stimulates aldosterone secretion from adrenal glomerulosa cells via

AT1 receptor and Ca^{2+} signaling [30]. Hyperkalemia also increases aldosterone production, in this case by directly depolarizing glomerulosa cells [31]. In the setting of volume depletion, aldosterone increases Na–Cl reabsorption without increasing K^+ secretion, whereas in the setting of hyperkalemia, aldosterone maximizes K^+ secretion without increasing Na–Cl reabsorption. Thus, the kidney produces distinct responses in hyperkalemia and in hypovolemia, even though plasma aldosterone levels are similarly elevated.

A rational explanation has been that the amount of Na–Cl delivered to the CNT and CD controls the actions of aldosterone in these segments. In volume depletion, Na–Cl reabsorption in more proximal portion of the renal tubules (most importantly DCTs) reduces the amount of Na^+ delivered to the CNT and CD, diminishing the electrogenic Na^+ reabsorption via ENaC. Reduced ENaC activity results in decreased K^+ secretion [32, 33], because potassium secretion through ROMK (renal outer medullary K^+ channel) in principal cells is primarily driven by the electrochemical gradient created by Na^+ reabsorption [32, 33]. While this model well explains a mechanism of how the kidney limits K^+ secretion in the setting of volume depletion, more "active" mechanisms seem to be necessary to optimize electrolyte transport in CNT and CD, given that increased ENaC activity in high aldosterone status would facilitate K^+ secretion as well as Cl^- reabsorption [15].

As stated above, intercalated cells regulate Cl^- flux in CNT and CD. Despite the importance in blood pressure homeostasis, however, little has been known about the regulation of this process. This is in sharp contrast to the well-characterized Na^+ reabsorption machinery in principal cells. Although previous studies demonstrated that MR is present in intercalated cells [34, 35], they express much lower levels of 11βHSD2 than principal cells [34, 36]. Nonetheless, aldosterone seems capable of regulating electrolyte flux mediators in these cells [37]; functional studies in the 1980s have revealed that aldosterone stimulates acid secretion in CNT and CD [38]. In β-intercalated cells, on the other hand, mineralocorticoid DOCP increases Cl^-/HCO_3^- exchanger pendrin at the apical membrane as evaluated by electron microscopy [39]. Physiological significance of these observations has been obscure, especially in terms of acid–base regulation. Interpretation of these data is further complicated by the finding that pendrin expression is not altered by DOCA in wild-type mice [40], suggesting the complex regulation of MR signaling in intercalated cells.

Mechanisms modulating MR function

In addition to circulating ligands, a growing body of evidence suggests that the signaling of nuclear receptors (including MR) is modulated by multiple factors, including receptor expression [41], recruitment of co-regulator molecules [42, 43], and the interaction with other signaling pathways [44]. Regarding mechanisms modifying MR function, we have previously shown that the constitutive activation of small GTPase Rac1 facilitates MR nuclear accumulation and signaling, resulting in salt-sensitive hypertension and chronic kidney disease [45, 46].

Post-translational modification can significantly modify nuclear receptor activity [47]. Indeed, several studies have indicated that MR undergoes phosphorylation [48, 49], and that CDK5 and MAPK may be responsible for the phosphorylation [50, 51]. However, the precise roles of phosphorylation in regulating MR function have remained largely unknown. Using phospho-proteomics [52], we have comprehensively analyzed phosphorylation sites in full-length human MR and identified 16 phosphorylation sites (of which 14 sites are not previously described) [53]. After the functional screening, we noted with interest the phosphorylation at S843, the only site present in the ligand-binding domain. MR and GR evolved from a common MR-like ancestor, and serine at this position is also conserved in the ancestral corticoid receptor [54]. Previous studies have indicated that the difference in ligand selectivity between GR and MR is driven by two amino acid substitutions in the ligand-binding domain, and interestingly, one of the two substitutions is serine changing to proline at position corresponding to S843 in human MR.

Binding assay using phosphomimetic MR^{S843E} revealed that phosphorylation severely impairs aldosterone binding, increasing the dissociation constant by more than 100 fold. This indicates that the phosphorylated form of MR is virtually incapable of binding ligands at a physiological concentration. Consistently, MR^{S843E} is not activated by either aldosterone or cortisol as assessed by luciferase assay, and is exclusively cytoplasmic in the presence of a sufficient amount of ligand. Together, these data demonstrate an additional mechanism regulating nuclear receptor signaling, whereby phosphorylation reversibly regulates the ability of the receptor to bind to ligand.

Cell-selective regulation of MR by phosphorylation in intercalated cells

To explore the in vivo significance of MR phosphorylated at S843 (MR^{S843-P}), we surveyed tissues using phospho-specific antibodies. By Western blotting, MR^{S843-P} is identified in the kidney lysates but not in other tissues known to express MR, including brain, heart, colon, and vasculature. Surprisingly, immunofluorescent studies revealed that MR^{S843-P} is present in renal intercalated cells, but not in principal cells nor DCT cells. Importantly, MR^{S843-P} is seen only in the cytoplasm, consistent with the

in vitro analysis in COS-7 cells expressing phosphomimic MR mutant.

Because aldosterone secretion is stimulated by the activation of the renin-angiotensin system and separately by hyperkalemia, we evaluated whether volume depletion (by Na–Cl restriction and separately by genetic ablation of NCC) and potassium loading change MR^{S843-P}. We found that hypovolemia via angiotensin II signaling reduces MR^{S843-P} levels, whereas potassium loading increases MR^{S843-P}. The decrease in MR^{S843-P} in hypovolemic condition is associated with the increase in nuclear accumulation of MR in intercalated cells. Notably, consistent with previous studies showing that aldosterone can regulate H^+-ATPase and pendrin, the upregulation of apical B1 H^+-ATPase and pendrin associated with MR^{S843-P} dephosphorylation in volume depletion is blocked by MR antagonist spironolactone. Furthermore, constitutive dephosphorylation of MR^{S843-P} increases the sensitivity of MR to aldosterone in intercalated cells [53]. Thus, volume depletion induces MR dephosphorylation, which, in turn, allows aldosterone signaling in intercalated cells, resulting in the activation of Na–Cl transport mechanisms involving these cells.

In the CNT and CD, Na^+ is reabsorbed via principal cells, whereas Cl^- is transported through paracellular or transcellular pathways. Evidence indicates that H^+-ATPase is also involved in these processes [27]. Using a mathematical model, Weinstein AM demonstrated physiological conditions that maximize Na–Cl reabsorption in the cortical CD, while minimally affecting the handling of other ions (K^+, H^+, and HCO_3^-) [15, 55]. According to the model, activation of ion flux pathways in principal cells increases both Cl^- reabsorption and K^+ secretion, along with the electrogenic Na^+ reabsorption. However, when transporters in principal cells and those in intercalated cells (both α- and β-intercalated cells) are activated simultaneously, maximal Na–Cl reabsorption occurs without significantly affecting K^+ or H^+ flux in the CD [15, 55]. Our data provide insight into the mechanism of how the activities of diverse electrolyte flux pathways in the distinct cells are orchestrated to achieve appropriate homeostatic responses.

Other mechanisms regulating balance between Na–Cl reabsorption and K^+ secretion in the distal nephron

Accumulating data demonstrate that the alternation in fluid volume or in electrolyte composition can modify the function of renal tubular cells independently of aldosterone. Changes in potassium balance can directly modify the function of thiazide-sensitive Na^+–Cl^- cotransporter (NCC) in the DCT, which plays a key role in determining the balance between NaCl reabsorption and K^+ secretion [56, 57]. Recent studies demonstrated that these effects on NCC are mediated by the change in serum K^+ levels, which in turn modulates DCT cell membrane voltage [58]. Hyperpolarization of the cells enhances Cl^- exit and finally alters the function of WNK kinase, a Cl^--sensing kinase [58–60].

Angiotensin II signaling also regulates electrolyte flux mediators in the distal nephron via mechanisms that do not require aldosterone [61]. For example, angiotensin II increases NCC phosphorylation in adrenalectomized rats [62]. In mice lacking aldosterone synthase, angiotensin II receptor blocker reduces ENaC at the plasma membrane, indicating the compensatory and aldosterone-independent action of angiotensin II signaling [63]. Recently, a novel effector mechanism mediating AT1 receptor signaling in the distal nephron has been discovered [64]. Kelch-like 3 (KLHL3) and cullin 3 (CUL3) are two partners in a cullin-RING (really interesting new gene) E3 ubiquitin ligase complex (CRL). In 2012, mutations in *KLHL3* and *CUL3* are demonstrated to cause pseudohypoaldosteronism type II (PHAII, aka familial hypertensive hyperkalemia or Gordon syndrome), accounting for ~79 % of kindreds [65, 66]. Subsequently, it was demonstrated that KLHL3-CUL3 CRL bind and degradate WNK kinase [67–70] and claudin-8, a regulator of paracellular Cl^- flux [71]. Because mutations in *KLHL3* and *CUL3* alter the balance between Na–Cl reabsorption and K^+ secretion in the kidney, resulting in hypertension and hyperkalemia, a key remaining question was how this CRL is regulated in a physiological context.

S433 in the Kelch domain of KLHL3 is recurrently mutated in autosomal dominant form of PHAII [65, 66]. Interestingly, we found that this site is actually phosphorylated in cells and in vivo, and angiotensin II via protein kinase C increases the phosphorylation [64], providing the signal that prevents KLHL3/CUL3-mediated degradation of WNK kinase. These mechanisms are also involved to achieve the appropriate balance between Na–Cl reabsorption and K^+ secretion in the kidney. It is currently not known whether aldosterone directly regulates the activity of KLHL3/CUL3-CRL.

Aldosterone signaling in DCT cells: direct or indirect effects?

In addition to CNT and CD, MR is highly present in DCT cells in the distal nephron. While 11βHSD2 is present only in the terminal portion of the DCT (DCT2) [72], aldosterone increases expression and activity of NCC [73, 74]. Ser/Thr kinases oxidative stress response kinase-1 (OSR1)

and STE20/SPS1-related proline alanine-rich kinase (SPAK), the upstream regulators of NCC [75], seem to be involved in this process, because NCC induction in low salt condition is accompanied by the phosphorylation of OSR1/SPAK, which is blocked MR antagonist spironolactone [76]. Given these data, it is generally accepted that aldosterone has a direct effect on NCC.

As noted previously, however, emerging evidence points to the importance of K^+ as a powerful modulator of DCT cell function. Potassium loading decreases NCC phosphorylation in DCT even when plasma aldosterone is elevated [56, 57]. Consistently, in a model of pseudohypoaldosteronism type I (PHAI) that lacks ENaCα in the kidney, hyperkalemia determines the activity of NCC regardless of salt wasting and high plasma aldosterone [77]. Conversely, potassium restriction increases NCC activity under aldosterone suppression [78]. Thus, an important unanswered question is how much the effect of aldosterone on NCC is mediated by the change in serum K^+ levels. A very recent research from Dr. Ellison's group addressed this issue in detail [79]; using kidney-specific MR knockout mice, the authors delineated the direct and indirect effects of aldosterone in principal cells and in DCT cells, respectively. They first found that both ENaC and NCC were decreased in their salt-wasting model, which is in line with the current understanding. Notably, however, downregulation of the latter was reversed by restricting dietary potassium. Conversely, potassium supplementation completely prevented the upregulation of NCC (but not ENaC) in aldosterone-infused animals. These data led authors to conclude that in a state of aldosterone excess, the mineralocorticoid stimulates ENaC directly, whereas low K^+ levels increase NCC secondarily, causing salt retention and hypertension [79]. These data indicate that a major part of aldosterone effects on NCC is mediated by the changes in serum K^+ levels. Given that the serum K^+ alters the function of WNK kinase [58], it is worth testing whether WNK-OSR1/SPAK-NCC cascade is regulated directly by aldosterone or indirectly through changes in potassium status. It is also interesting to delineate how intercalated cell function and paracellular Cl^- flux mediators are regulated in this context.

Summary and future directions

In this review, I have summarized recent advances in the understanding of the mechanisms modifying the action of aldosterone in the distal nephron. In the CNT and CD, elevated circulating aldosterone increases MR signaling in principal cells, whereas MR in intercalated cells is regulated at an additional level, through the phosphorylation of the ligand-binding domain in MR. The phosphorylation levels are counter-regulated by angiotensin II and high potassium, controlling Cl^- flux mediators in intercalated cells. In the DCT, serum potassium regulates NCC activity independently of aldosterone and MR. Angiotensin II also directly regulates Na–Cl transport mechanisms partly via phosphorylating KLHL3. These mechanisms act in concert to regulate the balance between Na–Cl reabsorption and K^+ secretion in the distal nephron. In the DCT, changes in serum potassium levels modulate NCC activity by altering the resting membrane potential. Whether the same or similar mechanisms mediate the effect of potassium on MR^{S843-P} levels remain to be determined. Future studies are also required to determine the pathways and kinases responsible for MR phosphorylation.

Aldosterone and MR are the important therapeutic targets in hypertension [80–82] and chronic heart failure [83]. Accumulating data also indicate that MR antagonists can be protective against the chronic kidney disease [84–86]. Detailed characterization of the molecular mechanisms regulating MR function in the kidney and in other tissues may reveal new targets that might be exploited for therapeutic purposes.

Compliance with ethical standards

Conflict of interest The author has declared that no conflict of interest exists.

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