Effect of Mineralocorticoids on Acid-Base Balance

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Key Words
Mineralocorticoids · Acid-base balance · Aldosterone · Acidosis

Abstract
Aldosterone is classically associated with the regulation of salt and potassium homeostasis but has also profound effects on acid-base balance. During acidosis, circulating aldosterone levels are increased and the hormone acts in concert with angiotensin II and other factors to stimulate renal acid excretion. Pharmacological blockade of aldosterone action as well as inherited or acquired syndromes of impaired aldosterone release or action impair the renal response to acid loading and cause hyperkalemic renal tubular acidosis. The mineralocorticoid receptor (MR) mediating the genomic effects of aldosterone is expressed in all cells of the distal nephron including all subtypes of intercalated cells. In acid-secretory type A intercalated cells, aldosterone stimulates proton secretion into urine, whereas in non-type A intercalated cells, aldosterone increases the activity of the luminal anion exchanger pendrin stimulating bicarbonate secretion and chloride reabsorption. Aldosterone has also stimulatory effects on proton secretion that may be mediated by a nongenomic pathway. In addition, aldosterone indirectly stimulates renal acid excretion by enhancing sodium reabsorption through the epithelial sodium channel ENaC. Increased sodium reabsorption enhances the lumen-negative transepithelial voltage that facilitates proton secretion by neighboring intercalated cells. This indirect coupling of sodium reabsorption and proton secretion is thought to underlie the fludrocortisone-furosemide test for maximal urinary acidification in patients with suspected distal renal tubular acidosis. In patients with CKD, acidosis-induced aldosterone may contribute to progression of kidney disease. In summary, aldosterone is a powerful regulator of renal acid excretion required for normal acid-base balance.

Introduction
Systemic acid-base homeostasis is maintained and controlled by the concerted action of various organs including lungs, kidneys, liver, bone, skeletal muscle, and intestine. Among them, lungs and kidneys play arguably the most important role as evident from respiratory disorders and inherited or acquired renal diseases.

Daily metabolism in an adult healthy person with standard diet and average physical activity produces approximately 1 mmol of protons per kilogram body weight which must be buffered or eliminated. Elimination and buffering of protons critically depend on the kidney. Approximately 20 mmol of acid are absorbed from diet and an additional 10 mmol base equivalents are lost due to intestinal secretions. The major acid load comes from
daily metabolism liberating around 15,000 mmol of volatile acid in the form of CO₂ that are eliminated by ventilation and 40 mmol of non-volatile acids that require immediate buffering (mostly by bicarbonate) or direct renal excretion. The kidney contributes to buffering of acids by reabsorbing virtually all filtered bicarbonate and the de novo synthesis of bicarbonate from ammoniagenesis leading to the excretion of approximately 40 mmol of ammonium into urine. Additionally, the kidneys excrete free protons that have to be buffered by urinary buffers, so-called titratable acids (mostly phosphate), thereby buffering and eliminating a total of 70 mmol acid/day [1, 2].

The different nephron segments specifically contribute to the control of acid-base homeostasis by the kidney [1, 3, 4]. Proximal tubule segments are involved in bicarbonate reabsorption, ammoniagenesis, and determination of urinary excretion of titratable acids, the thick ascending limb of the loop of Henle (TAL) mostly reabsorbs bicarbonate, and the collecting ducts excrete protons and ammonium and are the major site of adapting and fine-tuning renal acid-base excretion [4].

Whether the proximal tubule and the thick ascending limb are directly regulated by aldosterone is controversial, whereas the functions of the distal convoluted tubule (DCT), connecting tubule and collecting duct system, collectively referred to as aldosterone sensitive distal nephron (ASDN), are directly and indirectly modulated by aldosterone [5–7].

Reabsorption of bicarbonate in the proximal tubule is mediated by the secretion of protons into primary urine by Na⁺/H⁺ exchangers (NHEs) and proton pumps situated at the apical brush border membrane [8]. NHE3 (SLC9A3) is the major NHE isoform in adults [9]. NHE3 contributes also as a major mechanism to sodium reabsorption in the proximal tubule. Protons for NHEs and proton pumps are generated by intracellular carbonic anhydrases (mostly carbonic anhydrase II, CAII) [8]. The secreted protons combine in the luminal fluid with filtered bicarbonate to form carbon dioxide (CO₂) and water (H₂O). This reaction is catalyzed by membrane-anchored extracellular carbonic anhydrases (mostly carbonic anhydrase IV, CAIV) [8]. CO₂ diffuses into proximal tubule cells. Bicarbonate formed by rehydration of CO₂ by intracellular carbonic anhydrases leaves proximal tubular cells across the basolateral cell membrane into blood via the electrogenic Na⁺-HCO₃ cotransporter NBCe1 (SLC4A4) [10]. Another part of bicarbonate reabsorption occurs through the paracellular pathway due to solvent drag driven by the lumen-negative potential and the osmotic gradient built by active solute transport [8].

Bicarbonate not reabsorbed by the proximal tubule is reabsorbed in the TAL and the DCT. Thus, urine entering the collecting duct system contains only minute amounts of bicarbonate under conditions of acid-base balance. The mechanisms mediating bicarbonate reabsorption in the TAL are similar to the proximal tubule involving also NHE3 and proton pumps on the luminal membrane [11]. The exit pathways for bicarbonate across the basolateral membrane are not well defined. The electroneutral Na⁺-HCO₃ cotransporter NBCn1 is localized at the basolateral membrane but is thought to mediate rather bicarbonate uptake from blood required for ammonium reabsorption by the TAL [12].

The late section of the DCT is characterized by the presence of the first intercalated cells, which express luminal proton pumps (V-type H⁺-ATPases) and the basolateral chloride-bicarbonate exchanger (AE1).

Ammoniagenesis takes place only in the proximal tubule and serves the elimination of protons and the de novo generation of bicarbonate from the metabolism of glutamine. Glutamine is extracted from peritubular capillaries, in part mediated by the glutamine transporter SNAT3 (SLC38A3) at the basolateral membrane of proximal tubule cells [13, 14]. Glutamine is further imported into mitochondria and metabolized by mitochondrial phosphate-dependent glutaminases and glutamate dehydrogenases to yield α-ketoglutarate. These steps liberate two NH₃ and one bicarbonate ion per glutamine. α-Ketoglutarate can then be further metabolized during gluconeogenesis or be converted to yield additional two HCO₃⁻ ions [2]. The bicarbonate synthesized during ammoniagenesis is exported via the basolateral NBCe1 bicarbonate transporter whereas NH₃ either diffuses into urine and is trapped as NH₄⁺ after protonation or binds intracellularly protons and may be excreted into urine by the NBCe1 exchanger instead of a proton.

Most of the ammonium excreted into urine is reabsorbed in the TAL by the NKCC2 cotransporter. NH₄⁺ is accumulated in the interstitium, a process that may require sulfatides binding ammonium [15, 16].

In the DCT and collecting duct system, at least two types of intercalated cells mediate acid or alkali excretion, respectively. Type A intercalated cells secrete protons and ammonium whereas type B intercalated cells excrete bicarbonate [3]. Type A intercalated cells produce bicarbonate from the hydration of CO₂ and the excretion of protons by V-type proton pumps (V-ATPases or H⁺-ATPases) into urine [4, 17]. Bicarbonate generation is catalyzed by the intracellular carbonic anhydrase II (CAII). The newly formed bicarbonate is secreted into the inter-

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stition/blood across the basolateral membrane by the anion exchanger 1 (SLC4A1, AE1) [4]. Proton secretion drives ammonium excretion along the collecting duct. Ammonium excretion is mediated by specific transport proteins of the rhesus protein family, RhBG and RhCG [4, 15, 18]. RhCG is critical for renal ammonium excretion [19–21] and upregulated during acidosis [18].

Titratable acids are alkali buffers neutralizing protons in urine. The major urinary titratable acids are inorganic phosphate (HPO$_4^{2-}$ and H$_2$PO$_4^-$) and citrate [22]. The amount of available titratable acids depends mostly on their reabsorption in the proximal tubule since both phosphate and citrate are freely filtered and actively re-absorbed to some extent by the proximal tubule. The rate of reabsorption of phosphate by its major transporter NaPiIIa and citrate by NaDC1 is highly pH-sensitive and in the case of phosphate reabsorption regulated by various hormones and factors [23–25]. Titratable acids are required to buffer protons as proton secretion by proton pumps is limited by the inability to pump protons against a proton gradient >3.5 pH units (intracellular pH approx. 7.2 vs. maximal urinary pH 4.5).

Bicarbonate excretion is mediated by type B and non-A/non-B intercalated cells that are present in the late DCT, CNT, and CCD. These cells express the chloride-bicarbonate exchanger pendrin (SLC26A4) on the luminal membrane secreting bicarbonate into urine in exchange for urinary chloride [26, 27]. Pendrin serves also chloride reabsorption and blood pressure regulation [28, 29].

A variety of factors, such as electrolyte status and hormones, influence renal acid-base metabolism and handling. Among them, angiotensin II and aldosterone play a very prominent role as discussed below. In addition, endothelin is a potent stimulus for renal acid excretion and may also enhance aldosterone secretion during acidosis [30–35]. Also insulin, proangiotensins, noradrenaline, glucocorticoids, and many more hormones modulate renal acid-base excretion [1]. This review will focus on the role of aldosterone in renal acid-base handling.

**Regulation of Aldosterone by Acid-Base Status**

Aldosterone secretion and levels are elevated during acidosis in humans and small and large animal models [36–44]. This increase in aldosterone levels is at least in part independent from changes in plasma levels of potassium and occurs even in the presence of a blockade of angiotensin II synthesis or action and with suppressed ACTH levels, factors known to stimulate aldosterone secretion [38, 42, 45].

TASK1 and TASK3 potassium channels that are very sensitive to external pH changes and whose activity is suppressed by physiological proton concentrations are highly expressed in zona glomerulosa cells and involved in the regulation of aldosterone secretion. However, their presence in the adrenal gland is not required for the aldosterone secretion stimulated by acidosis [45]. Thus, the acid-sensing mechanism responsible for aldosterone secretion by zona glomerulosa cells remains unknown but may reside in these cells as zona glomerulosa cells cultured in vitro are responsive to changes in pCO$_2$ and pH of culture media [46].

**Aldosterone-Sensitive Regulation of Acid-Base Homeostasis**

Acidosis increases the circulating levels not only of aldosterone but also of angiotensin II [41, 42]. Angiotensin II acts on renal acid excretion by stimulating NHE3, NBCe1 and H$^+$-ATPase-dependent bicarbonate reabsorption as well as ammonium excretion in the proximal tubule, and by stimulating H$^+$-ATPase-mediated urinary acidification in the collecting duct [47–51]. Angiotensin II may also induce phosphate reabsorption thereby decreasing its delivery to the collecting duct and availability as buffer. Consequently, blockade of the angiotensin converting enzyme (ACE) or angiotensin II type 1 receptors delays the renal adaption to acidosis in healthy humans as well as in various animal models [42, 52–57].

Similarly, impaired aldosterone release or signaling affects the ability of the kidney to excrete acid and to respond to an acid load causing hyperkalemic distal renal tubular acidosis, also called type IV RTA [58]. Inhibition of aldosterone actions by mineralocorticoid receptor (MR) antagonists (e.g. spironolactone, eplerenone) reduces renal acid excretion in healthy humans [42, 59]. Also patients with aldosterone deficiency due to adrenal-ectomy show type IV dRTA which is reversed with aldosterone substitution [60]. Mutations in the gene CYP11B2 encoding for the enzyme aldosterone synthase (corticosterone methyl oxidase) catalyzing the last steps in the synthesis of aldosterone cause hyperreninemic hypoaldosteronism with hyperkalemia, low blood pressure, and metabolic acidosis [61]. Also inactivating mutations in the MR impairing aldosterone signaling cause similar symptoms [62].
In contrast, syndromes of aldosterone excess cause metabolic alkalosis due to inappropriately high acid excretion [63] such as in Conn syndrome or syndromes of pseudohyperaldosteronism (with the exception of pseudohypoaldosteronism type II (Gordon syndrome) which features metabolic acidosis [64, 65]). Mutations in CYP11B2 leading to aberrant stimulation by ACTH or CYP11B2 gene duplication can cause uncoupled and excessive aldosterone synthesis in [66, 67]. More recently, mutations in the KCNJ5 potassium channel or the ATP1A1 (Na+K+-ATPase α-subunit) and ATP2B3 (a Ca2+-ATPase) have been found that cause adrenal gland adenomas overproducing aldosterone [68, 69]. In states of volume depletion and contraction of the extracellular fluid volume, the physiologic increase in aldosterone secretion contributes to the development of metabolic alkalosis as salt retention by the kidneys is linked to increased bicarbonate reabsorption and acid excretion (see also below). The mechanism has been attributed mostly to the depletion of chloride [70].

**Expression of Key Molecules of the Mineralocorticoid Response in Kidney Segments Involved in Acid-Base Handling**

The response of the kidney to mineralocorticoids depends on the expression of several key molecules required for aldosterone sensitivity and selectivity towards mineralocorticoids over glucocorticoids. Mineralocorticoids bind to the MR and induce its nuclear translocation where the ligand-receptor complex acts as transcription factor. Mineralocorticoid over glucocorticoid selectivity is ensured by the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) inactivating 11β-hydroxylglucocorticoids and thereby protecting the MR from activation by glucocorticoids. At least in rat and mouse kidney, the MR is found in the cells lining the TAL, DCT, connecting tubule (CNT), and collecting duct system [5, 71]. In the DCT, CNT and CD all cell types, e.g. principal (segment-specific) cells as well as all subtypes of intercalated cells, express MR. No MR was detected by PCR and immunohistochemistry in proximal tubules and thin loops of the limb of Henle. Similarly, 11β-HSD2 is abundant in the cells of the TAL, DCT, CNT and CD but appears not to be expressed in intercalated cells. Its expression in the rat collecting duct, however, is downregulated during metabolic acidosis [72]. The glucocorticoid receptor (GR) is found in most cells along the entire nephron including also all subtypes of intercalated cells. Infusion of aldosterone into adrenalectomized rats induces translocation of both GR and MR into the nucleus, suggesting that the GR may mediate some effects of aldosterone in MR-negative cells. In contrast, corticosterone induces GR and MR translocation into the nucleus in all cells, positive or negative for 11β-HSD2, only at high concentrations whereas low doses have no effect on MR translocation in 11β-HSD2-positive cells [5]. Thus, glucocorticoids may stimulate MR in 11β-HSD2-negative intercalated cells and be responsible for some of the effects attributed to mineralocorticoids [71].

The MR is phosphorylated at various sites including at serine 483, a site that modifies binding of aldosterone and glucocorticoids to the receptor. Phosphorylation of S483 reduces affinity and activation of the receptor. In kidney in vivo, most MR is in the non-phosphorylated form except in intercalated cells that show a high degree of S483 phosphorylation [71]. MR phosphorylation is decreased in states of elevated aldosterone such as in volume depletion [71]. In contrast, in hyperkalemia, MR phosphorylation is increased and the receptor found mostly in the cytoplasm of intercalated cells whereas it is nuclear in principal cells. Angiotensin II may play a key role in regulation of the phosphorylation status of MR in intercalated cells and high angiotensin II reduces MR phosphorylation thereby providing a switch in the responsiveness of intercalated cells to aldosterone. The effects of angiotensin II may in part be mediated by the WNK4 kinase and the PP1 phosphatase [71].

The molecules mediating non-genomic rapid effects of aldosterone have not been identified to date. Possible candidates have been proposed and include GPR30 (GPER), a membrane-associated estrogen receptor. However, its role in physiological processes regulated by aldosterone in the kidney remained elusive [73].

**Regulation of Renal Acid-Base Transporters by Aldosterone**

Regulation of renal acid-base handling by aldosterone may occur by direct effects of aldosterone on target cells and molecules as well as by indirect effects requiring the crosstalk between different cells within a given nephron segment or between different nephron segments.

**Direct Regulation**

Genomic Effects of Aldosterone

Aldosterone stimulates urinary acidification and type A intercalated cell function. It may act directly on H+-.
ATPases and AE1 activity [74–79]. In aldosterone-deficient animals (e.g. due to adrenalectomy and repletion of glucocorticoids) urinary ammonium excretion is reduced but this may be mostly the result of reduced proton secretion and hyperkalemia impairing proximal ammoniagenesis [78].

Treatment of mice with the aldosterone analogue deoxycorticosterone acetate (DOCA) or NaHCO3 alone had no effect on the expression of the AE1 anion exchanger mRNA and protein abundance [80]. However, the combination of DOCA with NaHCO3 increased AE1 mRNA and protein, suggesting a combined action. Similarly, DOCA has no or only very little effects on mRNA and protein abundance of H+ -ATPases in the collecting duct, suggesting that the stimulatory effects of aldosterone on proton secretion must be mediated by other mechanisms [Daryadel, Mohebbi, Wagner, unpubl. data]. Whether a combined treatment of angiotensin II and aldosterone would be able to increase AE1 and H+ -ATPase abundance has not been directly tested to our knowledge. However, infusion of angiotensin II over several days increases protein abundance of the intercalated cell-specific B1 H+ -ATPase subunit [71]. Moreover, in mice lacking the NaCl cotransporter NCC, B1 expression is increased and this effect is abolished by either angiotensin receptor antagonists or MR blockers, suggesting that the combined action of angiotensin II and aldosterone may be required [71].

Similarly, regulation of pendrin in non-type A intercalated cells by aldosterone has been described [81, 82]. Whether this effect involves only redistribution of pendrin or increased abundance remained open. However, a subsequent detailed analysis suggested that DOCA alone does not alter pendrin abundance but that a combined effect of NaHCO3 and aldosterone could be observed [80]. Interestingly, expression of pendrin in other tissues such as kidney, heart, lung and thyroid gland is stimulated by aldosterone alone [83]. These in vivo data are in contrast to results obtained from in vitro promoter studies demonstrating reduced pendrin promoter activity with aldosterone [84]. As for the expression of the B1 H+ -ATPase, pendrin expression is regulated by angiotensin II [85] and its expression is sensitive to angiotensin II and MR blockers [71].

Thus, there is mounting evidence for the genomic regulation of intercalated cell function by aldosterone and that complex interactions of aldosterone with angiotensin II and possibly further factors are involved. However, the exact pathways and the role of the MR in overall intercalated cell function remains to be further explored.

Non-Genomic Effects of Aldosterone on H+ -ATPases

Aldosterone rapidly stimulates within 5 min H+ -ATPase activity in acid-secreatory type A intercalated cells of the mouse and human outer medullary collecting duct [75, 76]. Stimulation occurred also in the presence of the MR antagonist spironolactone and was thus considered to be non-genomic. Moreover, corticosterones had no effect on H+ -ATPase activity, suggesting specificity for aldosterone [75, 76].

The receptor mediating the rapid effects of aldosterone in intercalated cells is unknown. Interestingly, inhibition of Gαq proteins or phospholipase C activity blocked the effects of aldosterone, suggesting that either G protein-coupled receptors (GPCRs) mediate the effects or that additional signals through a GPCR play a permissive role in aldosterone signaling.

The MR may interact with epidermal growth factor receptor (EGFR) signaling by transactivation of EGFR-dependent signaling cascades [86]. Whether such a crosstalk between MR and EGFR signaling is responsible for the rapid effects of aldosterone on H+ -ATPase activity remains to be examined.

Injection of aldosterone in mice and rats caused a translocation of H+ -ATPases to the luminal membrane with massive protrusion of the apical membrane indicating cellular remodeling and increased H+ -ATPase density at the luminal membrane [75, 76]. A similar stimulation of H+ -ATPase activity, translocation of H+ -ATPases, and remodeling of intercalated cells is observed after direct stimulation with PKA agonists [87]. Indeed, blockade of PKA prevented the stimulatory effect of aldosterone. Moreover, the signaling cascade involves rises in intracellular calcium, PKC and ERK1/2 [75, 76].

Indirect Regulation

Aldosterone stimulates sodium reabsorption in the late DCT and collecting duct through the epithelial sodium channel ENaC expressed in principal cells [88, 89]. Increased electrogenic sodium reabsorption renders the transepithelial potential in the collecting duct more negative in the lumen. This potential difference facilitates proton secretion by neighboring intercalated cells [90]. Consequently, blockade of ENaC channels with inhibitors such as amiloride or triamterene reduces urinary acidification [90].

The aldosterone stimulated urinary acidification depending on ENaC activity forms the basis for a clinical test examining the ability of the connecting tubule and collecting duct to maximally acidify urine. This test is used in patients suspected to suffer from forms of incom-
plete or complete distal renal tubular acidosis as an alternative for the classic ammonium chloride loading test that is often not well tolerated. Patients are given a combination of mineralocorticoid analogues such as fludrocortisone to stimulate ENaC activity and a loop diuretic such as furosemide. Furosemide blocks the Na/K/2Cl– cotransporter NKCC2 in the TAL and delivers more sodium to downstream segments thereby increasing sodium reabsorption by ENaC. In healthy subjects, urinary pH acidifies under such treatment below pH 5.4 whereas in patients with impaired distal urinary acidification urinary pH remains more alkaline [91, 92].

This crosstalk between principal cells and intercalated cells may be even more complex as indicated above. In type I distal renal tubular acidosis, due to mutations of AE1 or the a4 and B1 H+-ATPase subunits, urinary salt and potassium wasting has been described which is not fully reversed by correction of acidosis [93, 94]. Experiments in mice lacking the B1 H+-ATPase subunit demonstrated an impaired ability to conserve NaCl and concentrate urine together with increased kaliuresis in view of elevated angiotensin II and aldosterone levels [28]. Defective intercalated cells may secrete ATP, increase prostaglandin production and thereby signal to neighboring principal cells where ENaC expression was reduced. Thus, potassium wasting in distal renal tubular acidosis may be at least in some patients caused by elevated aldosterone levels in combination with direct interactions between intercalated and principal cells [28, 94, 95].

Role of Aldosterone in Acid-Base Balance in CKD

In animal models and patients with reduced kidney function, renal acidosis develops and may contribute to the further decline in renal function [96–99]. In an earlier stage of reduced nephron numbers, acid-base balance is maintained and may depend on elevated angiotensin II levels [100]. In later stages of CKD (e.g. stage 4) enhanced aldosterone secretion may be promoted by stimulated endothelin production due to accumulation of acid in kidney and other tissues. Treatment of acidosis with alkali substitution reduces aldosterone in these patients [98]. Blockade of MR appears to slow down the rate of loss of GFR in a rat model of CKD [97]. How aldosterone alone or in conjunction with acidosis and other factors contributes to decline of renal function is an open question.

Summary and Outlook

Aldosterone is a very powerful regulator of acid-base balance and links this important function to the regulation of salt and potassium homeostasis. However, whereas acidosis and volume depletion are associated with elevated angiotensin II levels, hyperkalemia is not. Angiotensin II has a very strong direct stimulatory effect on renal excretion and appears to have also a permissive effect on genomic effects of aldosterone in intercalated cells by dephosphorylating the MR. Aldosterone stimulates both type A intercalated cells increasing urinary acid excretion as well as non-type A intercalated cells leading to enhanced activity of pendrin. Depending on the coupling of type A and non-type A intercalated cells this may result either in increased acid secretion, alkali secretion or acid-base neutral chloride reabsorption. However, the exact role of aldosterone and its interaction with other hormones and factors are only partly understood. Along the same line, the mechanisms that increase aldosterone secretion during acidosis are mostly unknown.

The molecular mechanisms of the non-genomic rapid effects of aldosterone as well as their physiological relevance are not fully explored. The effects occur in vitro and in vivo but whether it requires the MR or which other receptor(s) is/are involved has not answered. In view of the increasing number of non-genomic effects of steroid hormones, it is likely that aldosterone and other steroid hormones are able to activate alternative signaling routes [101, 102].

In patients with CKD, the increased secretion of aldosterone induced by the renal retention of acid may turn from a protective mechanism into a factor contributing to the progression of disease. Whether blockade of aldosterone secretion or action, possibly in combination with neutralizing the acid overload, could be beneficial for patients with CKD remains to be shown.

Acknowledgements

Work in the laboratory of the author has been supported by grants from the Swiss National Science Foundation (SNSF) and the European Union FP7 COST action BM1301 (ADMIRE: Aldosterone and mineralocorticoid receptor: Pathophysiology, clinical implication and therapeutic innovations).
References


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DOI: 10.1159/000368266


