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Regulation of epithelial Na channels by aldosterone

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The adrenocortical steroid aldosterone regulates Na transport through the epithelial Na Channel (ENaC) in the kidney and other epithelia. Although this process plays an important role in the control of overall Na and fluid balance in the body, the mechanisms underlying it have not been fully elucidated. In this review we discuss the roles of various regulatory modes including mRNA transcription and translation, protein processing and trafficking, and alteration of channel gating in the response to the hormone. We also discuss the importance of specific aldosterone-induced proteins to the upregulation of channel function in the kidney.

Key words: renal Na transport, amiloride-sensitive channel, hypertension, channel trafficking

Overview

Regulation of renal Na transport is crucial to the maintenance of normal extracellular fluid volume and blood pressure. Although only a small portion of filtered Na is reabsorbed through the epithelial Na channel (ENaC), control of this transport, particularly by the adrenal corticosteroid aldosterone, is an essential component of Na homeostasis. Evidence for this comes from the large number of monogenic forms of hypertension that involve the renin-angiotensin-aldosterone (RAA) axis or ENaC itself.[1] These include glucocorticoid-remediable aldosteronism,[2] 11β-hydroxysteroid dehydrogenase (HSD) deficiency,[3] mineralocorticoid-receptor activating mutations[4] and Liddle's syndrome.[5] Although these monogenic forms are rare, 5%－10% of patients with secondary hypertension have elevated levels of circulating aldosterone,[6] indicating a broader role of this system in human disease. However the involvement of the RAA/ENaC system in essential hypertension remains elusive.

Ion flux and electrophysiological measurements using ex vivo renal tubules from rodents illustrate the extent of regulation of ENaC by aldosterone. In isolated-perfused rat CCDs, net Na transport is negligible in normal animals eating conventional rodent chow, but becomes robust when they are maintained on a low-Na diet or are treated with exogenous aldosterone.[7,8] Similarly, cortical collecting ducts from untreated rats have very little channel activity measured as single-channel currents in cell-attached patches on the apical membrane of principal cells. When animals are fed a low-Na diet to raise endogenous aldosterone levels or infused with the steroid channels are readily observed in most patches.[9] This finding was confirmed using whole-cell recordings. Here the current sensitive to amiloride－the canonical blocker of ENaC－is unmeasurably low (<10 pA/cell) in most cells but can easily reach 500 pA/cell (at a holding potential of -100 mV) in Na-depleted or hormone-treated animals.[10-12] This high degree of regulation extends from the connecting tubule[12] through the outer and inner medullary collecting duct.[10] However, ENaC activity in the late distal convoluted tubule is less dependent on elevated aldosterone levels.[13]

Regulation of ENaC transcription

Aldosterone activates a cytoplasmic receptor in target epithelial cells, initiating translocation to the nucleus and alteration of gene transcription.[14] The most straightforward mechanism of channel regulation would therefore entail changes in the abundance of mRNAs coding for ENaC protein. Of the three essential components of the channel, only the mRNA encoding the αENaC subunit increases with elevated aldosterone; those for βENaC and γENaC are constitutively

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expressed.[15-17] Curiously, the situation is opposite in the colon, another epithelia in which aldosterone regulates ENaC. Here the αENaC subunit is constitutively expressed, while βENaC and γENaC mRNA is stimulated by aldosterone.[15] Why the same hormone regulates the same protein in different tissues through the transcription of different genes remains a mystery.

The αENaC gene contains a glucocorticoid response element that could mediate the effects of steroids on its transcription.[18] Whether mineralocorticoids use this same site to regulate transcription is unclear. An alternative albeit more indirect mechanism involves the induction of the steroid-dependent kinase sgk1. This kinase phosphorylates and activates the transcription factor Af9, de-repressing the αENaC gene.[19]

Regulation of protein expression

Consistent with increased mRNA levels, the αENaC protein content increases when aldosterone levels rise.[20,21] This response is not sufficient to stimulate ENaC activity, as the glucocorticoid dexamethasone increased αENaC to a similar extent but did not activate amiloride-sensitive conductance in the CCD (see below).[22]

In A6 cells, a cell line derived from *Xenopus* kidney, rates of synthesis of αENaC and βENaC also increased acutely in response to aldosterone administration despite constant mRNA levels.[23] This suggests stimulation of channel translation independent of those on transcription. It is not known whether this mode of regulation pertains to the mammalian kidney. βENaC protein levels rose modestly with Na depletion in rats but no change was observed with aldosterone infusion.[20] γENaC undergoes post-translation processing in response to elevated aldosterone levels, as described in the next section, but the overall content of the subunit does not markedly change.[20,21]

Regulation of ENaC processing

Both Na depletion and aldosterone administration, promote the appearance of a reduced molecular mass (-65 kDa) form of γENaC, with a concomitant decrease in the full-length (-80 kDa) peptide.[20,21] This is consistent with the removal of the first -200 amino acids of the subunit, including the cytoplasmic N-terminus and first transmembrane domain. A similar proteolytic cleavage occurred in the αENaC subunit[20] although in this case,

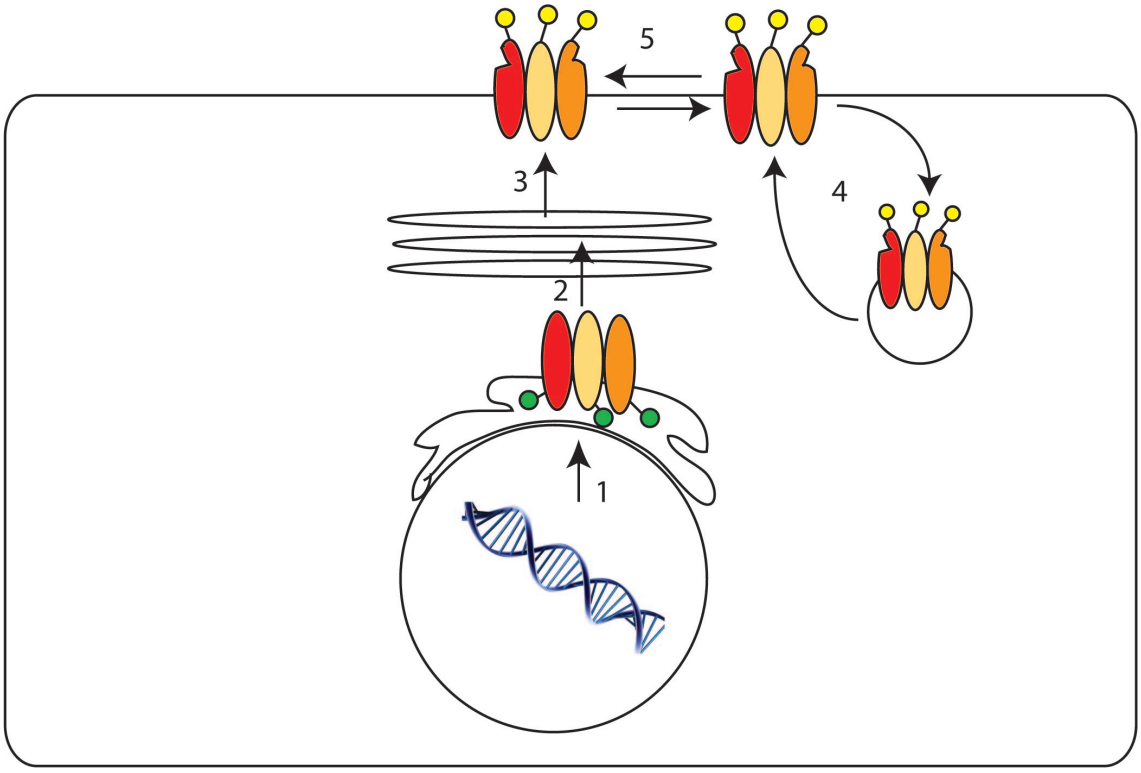

Figure 1. Possible aldosterone-dependent steps in ENaC processing in the kidney. 1. Increased gene transcription of the channel subunits. 2. Increased rates of protein synthesis. 3. Forward trafficking through the Golgi to the plasma membrane, together with increased proteolytic processing. 4. Decreased rates of internalization or increased rates of recycling to the apical membrane. 5. Activation of channels at the surface.

as discussed above, the abundance of the full-length form of the peptide also increased secondary to elevated mRNA levels.

The cleaved species of ENaC subunits almost certainly represent activated forms of the channel. In heterologous expression systems, ENaC activity increases with coexpression of channel-activating serine protease (CAP1), also known as prostasin,[24] or with application of an extracellular protease.[25] Under these conditions cleavage of αENaC and γENaC occurs at sites in the extracellular domain of the subunits close to the first transmembrane segment, consistent with the cleaved forms observed in the kidney.[26]

Activation by proteolytic processing may require two cleavage events, excising an inhibitory domain of the subunits.[27] In the case of γENaC this appears to involve both an intracellular cleavage by furin, an protease expressed primarily in the Golgi apparatus, and by an extracellular protease acting on channels at the cell surface. Evidence that these two steps can be separable *in vivo* comes from experiments in which animals are treated with the serine protease inhibitor camostat, resulting in the appearance of a partially cleaved γENaC subunit.[28] For αENaC both sites are cleavable by furin.[27] We know of no evidence for increased furin activity in the response to aldosterone. The hormone apparently also does not increase protasin expression, at least in M1 cells, a cell line from mouse kidney.[29] Tissue kallikrein is secreted into the urine at rates that are enhanced by elevation of circulating aldosterone.[30,31] Kallikrein can cleave and activate ENaC in the Xenopus oocyte expression system.[32] Furthermore the cleaved form of γENaC is diminished in kidneys of mice lacking the tissue kallikrein-1 gene.[33] It remains to be seen whether kallikrein is essential for activation of the channel *in vivo*. It is also not known if the increased levels of the protease observed in animals with elevated mineralocorticoid status are essential for the response to the hormone.

Regulation of trafficking

Immunocytochemical images show that Na restriction or aldosterone administration lead to a migration of ENaC protein from a diffuse distribution within the cytoplasm toward the apical pole of the cell.[21,34,35] The specific organelles in which the channels are sequestered under control conditions have not been identified. Also, to what extent the channels were actually at the apical surface as opposed to in subapical vesicles could not be determined.

The latter problem was addressed using an in situ biotinylation approach. Kidneys are perfused through the renal artery with a biotinylating reagent that is cell-impermeant but freely filtered by glomeruli. Proteins at the surface could then be isolated using neutravidin-coated beads. Na depletion or aldosterone administration resulted in -3-fold increases in the surface expression of γENaC that were specific for the cleaved form of the subunit.[36] Smaller increases in βENaC expression were observed, while αENaC could not be assayed for technical reasons. Similar results were obtained in animals fed a diet rich in K.[37] Under these conditions aldosterone levels also rise, presumably due to activation of hormone production in the adrenals by elevated plasma K^+.

Most of the γENaC at the surface is in the cleaved form.[36] The amount of the uncleaved full-length subunit actually decreases both in total kidney extracts[20,21] and in surface fractions[36] when circulating aldosterone concentrations are high. This could be explained by more rapid processing of ENaC through the Golgi under these conditions, leaving less protein in the unprocessed form.

ENaC activation

Functional measurements of ENaC in the rat CCD and CNT indicate that under basal conditions, with untreated animals eating normal chow, the activity of the channels is undetectably low. This situation pertains to isolated-perfused tubules with measurements of net Na fluxes[7,8] as well as to split-open tubules with electrophysiological assays.[9,10,12] ENaC currents probably increase by 50-fold or more with Na depletion or steroid treatment. In contrast, although surface expression of ENaC subunits increases 3- to 4-fold with elevated mineralocorticoid status, they can be detected, albeit at low levels, at the surface under these same basal conditions.[36]

One explanation for this discrepancy is that biochemical and functional assays measure ENaC in different parts of the nephron. Assessment of activity in late DCT indicates that in this segment ENaC function is to a large extent constitutive and independent of aldosterone.[13] This could account for the surface expression of ENaC measured under basal conditions in whole-kidney extracts.

It is also likely, however, that aldosterone controls not only the amount of protein at the surface but also the function of those channels in the apical membrane. Mamenko et al.[38] assessed single-channel activity in the mouse CCD. They found that ENaC open probability (Po) increased after treatment with a mineralocorticoid.

Furthermore, P_o could be increased further by acute application of angiotensin II, a hormone whose concentration increases in parallel with that of aldosterone when dietary Na intake is low.

How this activation comes about is not known. One possibility, however, is that changes in the composition of the apical membrane could underlie this effect. Methylating enzymes play a role in the aldosterone response in at least some epithelia,[39-41] and these could alter membrane lipids as well as proteins. The signaling molecule IP3 enhanced basal but not aldosterone-stimulated ENaC activity under whole-cell recording conditions, consistent with channel activation involving IP3 generation.[42] This could be due in part to activation of channels in the membrane. Finally, interactions with small G-proteins such as RAS could open channels in the membrane.[43]

Aldosterone-induced proteins

Aldosterone exerts its effects on ENaC primary through mineralocorticoid receptors that bind the steroid in the cytoplasm, translocate to the nucleus and act as transcription factors.[14] This drives enhanced translation of specific gene products known as aldosterone-induced proteins. A number of these proteins have been identified but thus far none of them completely explains the physiological response of the renal cells to aldosterone. Some of these are discussed below.

αENaC.

As discussed above, mRNA levels for αENaC (but not β or γENaC) increase in the kidney in response to aldosterone, and the protein content increases several fold.[16,17,20,21] This effect is not sufficient to drive the response to the hormone as the glucocorticoid dexamethasone elicits comparable increases in αENaC protein without changing processing or surface expression of ENaC or activating ENaC-mediated currents.[22] Whether induction of αENaC is necessary for full expression of aldosterone-dependent transport is difficult to ascertain since basal αENaC expression is required for channel assembly and function.

SGK1

The serum- and glucocorticoid-dependent kinase SGK1 was identified as an aldosterone-induced protein in screens of expressed genes in renal cell lines.[44,45] Furthermore, PCR analysis of isolated CCDs showed that SGK1 mRNA levels increase with a physiological dose of aldosterone *in vivo*.[46] Co-expression of SGK1 with ENaC subunits increases channel conductance in heterologous expression systems[44,45] strongly suggesting a role for this in upregulation of Na transport. On the other hand, as discussed above for αENaC, induction of SGK1 is not sufficient to drive channel activity in the kidney as dexamethasone, which does not increase transport, had a similar effect on SGK1 expression in the rat CCD.[46] In mice in which the sgk1 gene was deleted, the amount of cleaved γENaC was reduced, indicating that the kinase is necessary for complete processing and/or trafficking of the channels. However treatment of animals with aldosterone increased ENaC conductance of CCD cells to the same extent as it did in those of wild-type animals.[47] Thus the kinase is not essential for the physiological response to the steroid. The reason for the discrepancy between effects on processing and function remains unresolved.

GILZ

The glucocorticoid-induced leucine-zipper protein GILZ is also an aldosterone-induced protein. As was the case for SGK1, the gene was identified using screens for mRNA expression.[48,49] Physiological doses of aldosterone increased the expression of GILZ in the rat CCD.[46] Again as in SGK1, coexpression of GILZ increased the activity of ENaC in heterologous expression systems.[49] However mice lacking GILZ retained Na normally in response to a low-salt diet,[50] suggesting that any defect in ENaC was either subtle or could be compensated by other factors. Direct analysis of ENaC activity in animals lacking this gene has not been carried out.

USP2-45

The de-ubiquitinating enzyme USP2-45 was identified as an aldosterone-induced protein in a screen of gene expression in isolated distal nephron segments.[51] This is an intriguing candidate in light of the finding that mutations producing Liddle's syndrome in which ENaC is hyperactive the binding of the ubiquitinase Nedd4-2 is impaired.[52,53] Increased de-ubiquitinase activity could have the same effect, thereby increasing channel activity. However, mice lacking USP2-45 responded normally to dietary Na restriction.[54] The authors of that study concluded that this protein was not a critical element in the response of the kidney to aldosterone.

In summary, no aldosterone-induced protein has been found to be either necessary or sufficient to account for increased ENaC activity in the kidney when aldosterone levels are elevated. This might mean that the crucial gene product is yet to be identified. Alternatively, many

different proteins could contribute to the effects of the steroid, and the loss of any one of them can be compensated by changes in the others. New approaches will be required to test either of these possibilities.

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