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Can ENaC regulate ICF as well as ECF volume? Focus on “Osmotic pressure regulates $\alpha\beta\gamma$ -rENaC expressed in *Xenopus* oocytes”

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IN THE CURRENT article in focus, Ji et al. (Ref. 5, see p. C1182 in this issue) present evidence that osmotic pressure can acutely regulate activity of the amiloride-sensitive epithelial Na^+ channel (ENaC) expressed in oocytes. Given the restricted tissue distribution of ENaC, is it plausible that ENaC could play an important role in cell volume regulation in response to anisomotic stimuli *in vivo*?

The kidney regulates extracellular fluid (ECF) volume by adjusting Na^+ transport rate along the kidney tubules and ECF osmolarity by adjusting permeability of the renal tubules to water. Close control of ECF volume and osmolarity requires simultaneous regulation of Na^+ and water transporters resident in the cortical collecting tubules and collecting ducts, and homeostasis is accomplished by excretion of urine highly variable in volume and osmolarity. A consequence is that the apical surfaces of the epithelial cells in the distal nephron are bathed in renal tubule fluid that is likewise highly variable (Fig. 1). In other words, as the cells regulate whole body ECF volume and osmolarity, they are presented with the significant challenge of maintaining their own intracellular fluid (ICF) volume and osmolarity. In the presence of antidiuretic hormone (ADH), water permeability is high along collecting tubules and ducts and tubule fluid osmolarity can increase up to 1,200 mosM. In the absence of ADH, this region becomes impermeable to water and osmolarity falls to as low as 50 mosM as salts are reabsorbed. These ADH-stimulated changes in permeability can occur quite rapidly, and the renal cells in this region must quickly adjust to variable tonicity.

At the molecular level, Na^+ is reabsorbed along the nephron by a number of apical transporters expressed in a region-specific pattern (Fig. 1). Up to the distal tubule, the osmotic environment of any given region of the renal tubule cells is fairly constant, and long-term adjustments to the chronic hyperosmolarity of the medullary loop of Henle and chronic hyposmolarity of the ascending loop and distal tubule are in place to maintain cell volume. Beyond the distal tubule, there are acute fluctuations in tubule fluid osmolarity and in the same region there is fine adjustment of the ECF volume by the regulating activity of the apical amiloride-sensitive ENaC in the cortical collecting tubules and

collecting ducts. For example, ENaC activity can be rapidly increased by aldosterone and ADH in this region (Fig. 1), which is associated with increased phosphorylation of ENaC subunits (3, 4, 10) and results in increased ECF volume. Additional evidence that ENaC plays a key role in controlling ECF volume is that mutations in the channel that cause increased activity lead to hypertension, whereas inactivating mutations are associated with hypotension (reviewed in Refs. 3 and 4).

Because of the hand-in-hand association of rapid Na^+ and water transport regulation and consequent exposure to apical anisomotic fluid in the collecting tubules and ducts, it would be propitious if ENaC could play a role in rapidly sensing and maintaining cell volume in its host cell. Besides kidney, ENaC is located in other epithelia (including colon, sweat and salivary glands, amphibian skin, and bladder; Ref. 4) where Na^+ and water transport are highly regulated, and these tissues must also face the challenge of maintaining ICF volume and osmolarity in a fluctuating milieu. Cell shape is critical for optimal function of alveolar type I cells, which also express ENaC (4); cell swelling would lengthen the pathway for oxygen diffusion and gas exchange.

The ENaC α -, β -, and γ -subunits share significant homology and membrane topology features with the mechanosensitive degenerins of *Caenorhabditis elegans* (3, 4). This has stimulated investigators to look for evidence of ENaC mechanosensitivity, i.e., response to membrane stretch (1, 2, 5, 7, 8). Amiloride-sensitive stretch activation has been demonstrated in B lymphocytes (1), bovine α -ENaC or $\alpha\beta\gamma$ rat ENaC (rENaC) in planar lipid bilayers (2), and reconstituted α -ENaC from osteoblasts (7). The work by Ji et al. (5) goes beyond the issue of stretch activation to test the hypothesis that amiloride-sensitive ENaCs play a role in cell volume regulation. Their choice of *Xenopus laevis* oocytes injected with $\alpha\beta\gamma$ -rENaC takes advantage of the apparent lack of background regulatory volume decrease (RVD) in response to hypotonic media and lack of regulatory volume increase (RVI) in response to hypertonic media (5, 6). A similar recent study that also used injected oocytes (6) asked whether expression of an anion exchanger (AE2) could function-

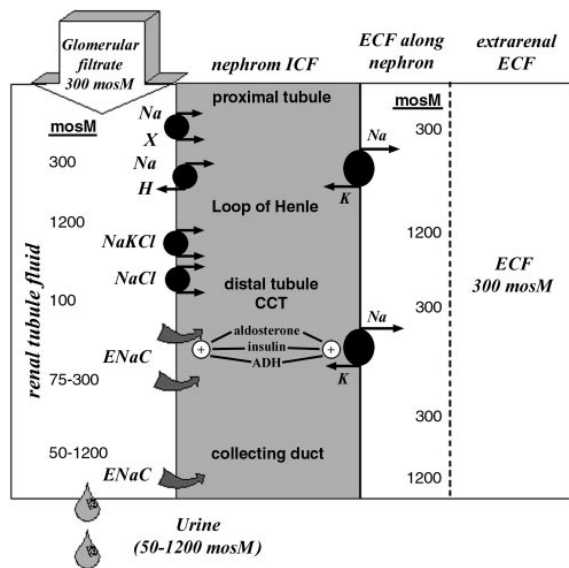


Fig. 1. Compartmental model of nephron intracellular space (shaded) in contact with renal tubular fluid of variable osmolarity on the luminal or apical face of the cell (*left*) and with intrarenal extracellular fluid (ECF) of variable osmolarity on the serosal or basolateral face of the cell (*right*), itself in contact with extrarenal ECF of constant osmolarity. Apical Na^+ -coupled transporters are located in the proximal tubule, and loop of Henle and amiloride-sensitive epithelial Na^+ channels (ENaC) are located in the cortical collecting tubule (CCT) and collecting ducts. Na^+ - K^+ -ATPase is located in the basolateral membranes. Na^+ transport via ENaC and Na^+ - K^+ -ATPase is adjusted by many factors including aldosterone, insulin, and antidiuretic hormone (ADH). X = anion, sugar, or amino acid.

ally complement the endogenous Na^+/H^+ exchanger to allow RVI in response to cell shrinking. AE2 did not confer primary RVI, but secondary RVI was observed when oocytes were primed with hypotonic swelling before the hypertonic stimulus. Ji et al. (5) observed primary RVI in oocytes expressing $\alpha\beta\gamma$ -rENaC. Whole cell currents were reversibly regulated in a fashion directly dependent on external osmolarity: currents rapidly increased after cell shrinkage in hypertonic media and rapidly decreased after cell swelling (4-fold increase in current over the range of 70–450 mosM), with no parallel regulation in water-injected oocytes. Although the increase in current in hypertonic media is a significant demonstration, the investigators established a physiological correlate. Oocyte cell volume was maintained nearly constant in 60% of the ENaC-injected oocytes in hypertonic media, an effect partially blocked by amiloride. In comparison, <20% of the water-injected oocytes exhibited any RVI.

This report by Ji et al. (5) demonstrates that ENaC contributes at least a missing link if not the direct effector necessary for RVI in oocytes. Whether native ENaC can regulate ICF in addition to ECF volume in a physiologically relevant setting like the collecting ducts or frog skin has not yet been addressed, although the literature on cells that possess native ENaC contains pieces of this interesting puzzle. About 10 years ago, Sun and Hebert (12) examined RVI in isolated perfused inner medullary collecting ducts exposed to hypertonic perfusate (apical) and bath (serosal) solutions. RVI was

dependent on the presence of ADH or cAMP, blocked by inhibitors of $\text{Cl}^-/\text{HCO}_3^-$ exchange, required Na^+ in the medium, and was reduced or abolished by 0.1 mM amiloride in perfusate or bath (this dose will block a number of cation transporters, and lower doses were not studied). They concluded that a luminal amiloride-sensitive pathway may contribute to cell volume regulation in the inner medullary collecting duct (12). Stokes and Sigmund (11) have recently provided evidence that α -, β -, and γ -ENaC mRNAs are actually expressed and regulated in the inner medulla. Thus amiloride-sensitive ENaC subunits and RVI are colocalized in the medullary tubules, and a relationship may exist between the two. Wills and colleagues (13) examined the response of native Na^+ channels expressed in cultured *Xenopus* renal cells (A6) to changes in solution osmolarity. In contrast to the findings of Ji et al. (5), short-circuit current increased in hyposmotic solution (over 30 min) and was undetectable in hypertonic solution, an effect seen only when serosal osmolarity was adjusted. They concluded that the apical amiloride-sensitive Na^+ channel is sensitive to small changes in serosal tonicity. This effect could play a role in ECF regulation; ICF volume was not measured in this study. Palmer and Frindt (8) examined cortical collecting tubule Na^+ channel kinetics in a patch pipette during membrane stretch by negative pressures but did not observe a consistent effect; there was a tendency to increase open probability. These latter two studies do not concur with the observation in ENaC-injected oocytes that amiloride-sensitive current increases in cells shrunken in hypertonic medium (5).

Is there a relationship between stretch activation of ENaC reported in planar bilayers and in lymphocytes and the RVI or RVD responses in oocytes? Channel activity increases with both mechanical stretch in the planar bilayer membrane and in oocytes shrunken in hypertonic medium. It is difficult to imagine that a channel would experience stretch in a shrunken oocyte, but it is perhaps possible that crumpling the underlying cytoskeleton could provide a force on the channels analogous to stretch. However, channel properties are modulated differently in the two conditions: amiloride sensitivity of the current increases significantly in shrunken oocytes and decreases in the stretched bilayers and lymphocytes; selectivity for Na^+ over other cations decreases in both cases (1, 2, 5). The decreases in current in oocytes exposed to hypotonic medium observed in this study support the distinction between stretch activation and the response to hypotonic swelling, indicating that factors other than wall tension change channel activity.

The mechanism of channel activation in response to cell shrinking remains to be examined. Acute regulation of ENaC could be mediated by activation of channels resident in the membrane, modulation of channel kinetics, or recruitment of preexisting channels, and there is evidence for all types of regulation (3, 4, 8, 10). RVI undoubtedly requires the participation of other

effector transporters that remain to be identified. Lipid mediators or protein kinase C may be released from the membrane with physical deformation (9) (swelling or shrinking), there is evidence for regulation of channel subunits by phosphorylation of the carboxy terminus (10), and swelling or shrinking may apply a stress to the cytoskeleton, implicated in Na⁺ channel activity regulation (reviewed in Ref. 4). Finally, the existence of three related subunits of ENaC, expressed and regulated differentially in the distal nephron (3, 10), and the fact that insertion of α -subunit alone is sufficient to produce a stretch-activated channel in planar bilayers and transfected cells (2, 7) indicate the likelihood that a variety of subunit combinations may be expressed in epithelia that exhibit combination-specific responses to stretch and anisosmotic stimuli.

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