Focus on “Contrasting effects of cPLA₂ on epithelial Na⁺ transport”

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THE AMILORIDE-SENSITIVE epithelial Na⁺ channel (ENaC) plays a central role in the reabsorption of salt and fluids across Na⁺-reabsorbing epithelia such as the distal nephron, distal colon, lungs, and ducts of the exocrine glands. ENaC is rate limiting for net Na⁺ reabsorption because it mediates entry of Na⁺ from the luminal fluid during the first stage of electrogenic Na⁺ transport (1, 9). Thus ENaC plays a key role in the regulation of fluid and electrolyte homeostasis and blood pressure. Malfunction or mutations of ENaC are associated with a number of human diseases such as Liddle’s syndrome, an inherited form of arterial hypertension, pseudohypoaldosteronism type 1, pulmonary edema, cystic fibrosis, and influenza (1, 11). Because of its central role in transepithelial fluid transport, the activity of ENaC is highly regulated by hormones such as aldosterone, vasopressin, and insulin, extracellular proteases, accessory proteins, and intracellular mediators such as Na⁺, Ca²⁺, pH, and protein kinases A and C (1, 9). The regulation of ENaC activity occurs at two major levels: 1) at the level of the membrane [i.e., modulation of the number of active channels (channel density)]; and 2) at the level of the channel itself [i.e., modulation of single channel open probability (Pₒ)].

Cytosolic phospholipase A₂ (cPLA₂) and arachidonic acid metabolites have also been implicated in regulating transepithelial Na⁺ transport across epithelial tissues. cPLA₂ selectively hydrolyzes phospholipids at the sn-2 position to release free arachidonic acid (4). After its release, arachidonic acid is converted by cyclooxygenases, lipoxygenases, and cytochrome P-450 monooxygenase to prostaglandins, leukotrienes, and eicosanoids, products that function as second messengers in signal transduction pathways (4). In the 1970s, Goodman et al. (10) demonstrated that the lag period between aldosterone exposure and stimulation of Na⁺ transport across the toad bladder was shortened by the presence of serosal phospholipase A₂, and Yorio and Bentley (16) revealed that transepithelial Na⁺ transport across the toad urinary bladder stimulated by the mineralocorticoid hormone aldosterone was markedly attenuated by mepacrine, a phospholipase A inhibitor. Subsequently, Cantiello and coworkers (6) showed that activation of cPLA₂ and lipooxygenase pathways by the G protein, Gₐ₃, induced both an increase in the number (N) and Pₒ of a poorly selective, 9-pS amiloride-sensitive Na⁺ channel expressed in coverslip-grown Xenopus A6 kidney cells. Aldosterone has itself been shown to cause increases in channel N and Pₒ in A6 cells, although the effect on Pₒ predominates (12). Thus considerable circumstantial evidence exists to support a link between the major salt conservation hormone aldosterone, phospholipase A₂ (PLA₂), and Na⁺ channel activity. These data establish that lipid metabolism is an important component in the regulation of transepithelial Na⁺ transport.

In the current article in focus (Ref. 15, see p. C147 in this issue), Worrell and associates have extended the observations of Yorio and Bentley (16) and Cantiello et al. (6) to ENaC. Using aristolochic acid, a relatively selective inhibitor cPLA₂, and a nonmetabolized analog of arachidonic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), Worrell et al. (15) present data indicating that cPLA₂ activity and free arachidonic acid are necessary to support transepithelial Na⁺ transport across confluent monolayers of Xenopus A6 cells, at least under conditions of chronic aldosterone exposure, which mimics the situation seen under conditions of salt deprivation.

Interestingly, activation of short-circuit current as a result of aristolochic acid inhibition of cPLA₂ was only seen after addition of the inhibitor to the apical membrane compartment; addition of aristolochic acid to the basolateral membrane produced the opposite effect, i.e., inhibition of transepithelial current. Addition of ETYA reversed the stimulation due to aristolochic acid at the apical membrane, but was without effect on the inhibition of current seen on the basolateral addition of aristolochic acid. Single channel data revealed that ETYA decreased Pₒ of the highly selective, 4-pS ENaC channel, strongly suggesting that arachidonic acid...
and/or its metabolites regulate transepithelial Na\(^+\) transport by altering ENaC \(P_o\).

These data imply a role for arachidonic acid itself in the “tune-down” response at the apical membrane, while suggesting that a downstream product of arachidonic acid metabolism could be responsible for the “tune-up” effect exerted at the basolateral membrane. The authors (15) postulate that the reduction in ENaC \(P_o\) by ETYA reflects the tonic production of arachidonic acid via a tonically active cPLA\(_2\) associated with the apical membrane.

The most likely downstream effector molecule is the arachidonic acid metabolite, prostaglandin E\(_2\) (PGE\(_2\)). Because synthesis of PGE\(_2\) can be regulated at the level of cyclooxygenase, the role of this metabolite in ENaC regulation was confirmed when the addition of ibuprofen (a cyclooxygenase inhibitor) had identical effects at the basolateral membrane as did aristolochic acid, i.e., inhibition of transepithelial current. Although inhibition of transepithelial Na\(^+\) transport could not be reversed by basolateral application of ETYA, it was reversed by basolateral addition of the arachidonic acid metabolite, PGE\(_2\).

On the basis of these data, the authors postulate that arachidonic acid from a perinuclear pool of cPLA\(_2\) is metabolized to PGE\(_2\), and this PGE\(_2\), in turn, is necessary to support transepithelial transport, presumably through autocrine and paracrine actions. It should be noted, however, that PGE\(_2\) has been shown to stimulate transepithelial Na\(^+\) transport across A6 cell monolayers by promoting a cAMP/protein kinase A (PKA)-mediated increase in ENaC density in the apical membrane (13). Thus the PGE\(_2\)-mediated reversal of aristolochic acid and ibuprofen effects on transepithelial transport observed by Worrell et al. (15) may not be entirely related to the cPLA\(_2\)/arachidonic acid signaling pathway, but rather they may also reflect activation of the cAMP/PKA pathway via PGE\(_2\).

Worrell and coworkers (15) propose that the apical downregulation of ENaC via arachidonic acid and the necessity of PGE\(_2\) to support transepithelial Na\(^+\) transport across A6 cell monolayers provide a signaling mechanism to fine-tune Na\(^+\) transport based on the relative activity of the apical and perinuclear pools of cPLA\(_2\). Coupling of a luminal receptor with apically restricted cPLA\(_2\) would provide a novel transduction mechanism for the regulation of ENaC by luminal signaling molecules. Although these signaling molecules and their receptors remain to be identified, candidates include bradykinin, adenosine, ATP, and endothelin, all of which are present in the luminal fluid (5). Alternatively, the apical cPLA\(_2\) pool may be coupled to a basolateral receptor through a second messenger pathway. Potential candidates for basolateral signaling molecules, coupled to either the apical or perinuclear pool of cPLA\(_2\), include epidermal growth factor and endothelin, both of which have been shown to activate cPLA\(_2\) and arachidonic acid release in the distal nephron (5).

Where, then, do the present data fit into the big picture of normal and dysfunctional ENaC regulation? These new findings seem to contradict earlier observations that provided evidence for inhibition of Na\(^+\) current via inhibition of PLA\(_2\) (16). However, the present studies are the first to dissect PLA\(_2\) signaling pathways in well-polarized monolayers of an established Na\(^+\)-transporting epithelial cell line, and, therefore, may more closely reflect the situation in the kidney under conditions of salt deprivation. The observation that ETYA inhibited ENaC \(P_o\) suggests that at least one level of regulation exists either directly at the channel or with a closely associated regulatory protein. Downstream targets of arachidonic acid include regulatory enzymes such as calmodulin kinase II and protein kinase C (PKC); PKC has also been implicated in the inhibition of amiloride-sensitive Na\(^+\) currents in *Xenopus* oocytes heterologously expressing ENaC (3) and so may account for the mechanism underlying the inhibitory effects of PLA\(_2\). What of the stimulatory effects associated with PGE\(_2\) at the basolateral membrane? The situation here is much less clear, although experiments performed in other systems have suggested that PGE\(_2\) can activate adenylate cyclase and thus formation of cAMP with subsequent activation of PKA. Kokko et al. (13) have shown that chronic exposure to PGE\(_2\) at the basolateral membrane of A6 cells resulted in both an increase in the total number of 4-pS Na\(^+\) channels (presumably synonymous with ENaC) at the apical surface and accumulation of cellular cAMP, although direct PKA-mediated phosphorylation of ENaC has been harder to demonstrate (14).

Certainly, increased Na\(^+\) absorption in response to aldosterone is more in keeping with the role of this mineralocorticoid in salt conservation than is an aldosterone-mediated inhibition of ENaC current. It seems likely that the net effects of aldosterone to increase transport are the result of competing inhibitory and stimulatory influences exerted at opposite membrane domains and exerted both on intrinsic channel activity, as determined by channel \(P_o\), and on the total number of active channels present at the cell surface (N). Other regulatory molecules may well exert similar inhibitory influences on PLA\(_2\) at the apical membrane, as does aristolochic acid in the experiments described by Worrell et al. (15) in the present report. As described above, the renal tubule expresses luminal receptors for a variety of ligands that are found in tubular fluid after filtration at the glomerulus (2, 7, 8). Thus Na\(^+\) reabsorption, as handled by the distal convoluted tubule and collecting duct, is by necessity highly responsive to even slight changes in the internal environment as transmitted through the glomerulus. The exquisite sensitivity exhibited by the kidney in terms of Na\(^+\) handling is simply reflective of the importance of this ion in whole body volume homeostasis.

Thus the work of Worrell et al. (15) is important for reasons that are broader than the specific focus of the paper. These findings show that ion channels in general, and ENaC in particular, interact with their environment in a complex way, a way that is often times overlooked when considering only the biophysical characteristics of the channel in a single model system. The
findings also show directly that the same treatment can have different effects, depending on which side of the cell is exposed to the treatment. Whether or not arachidonic acid metabolites actually play any significant cellular physiological role in the regulation of ENaC is perhaps not the main point of these experiments. Rather, the direct demonstration that these compounds (and probably a variety of other cellular constituents and metabolites) can alter channel function means that interpretation of ion channel function and regulation in intact cells must take into account the environment in which the experiments are performed. Although this may be stating the obvious, the obvious is sometimes overlooked, misunderstood, or worse, even ignored. Does ENaC expressed by mammalian cells respond similarly? Are the same enzymes present in the plasma membrane of mammalian cells? Are these enzymes present in the plasma membrane of oocytes, and do cloned ENaC subunits, when heterologously expressed, respond similarly to regulation by cPLA2? It is reasonable to assume that mammalian renal principal cell membranes are different from Xenopus cell membranes. One broad question that these experiments trigger is: If arachidonic acid metabolites and cPLA2 provide some “background” regulation of ENaC, how is the intrinsic function and regulation of ENaC altered when these elements are not present? What portion of ENaC, or the surrounding membrane, is affected by arachidonic acid metabolites? How do these metabolites prevent the channels from opening and reduce the time the channel spends in the open state? Is the thermal responsiveness of the channel altered? Do the metabolites directly interact with the channels, or do they alter the membrane surrounding the channels? Thus experiments such as those reported by Worrell et al. (15) need not be directly related to actual cellular events. Their intrinsic value may be to remind us that ion channel function and regulation are very complex and depend on many factors. The environment in which biophysical observations are made may have a profound influence on what is actually observed. Only by experiments such as described by Worrell et al. (15) and an open mind about the interpretation of such findings will we come closer to a more realistic understanding of ion channel function and regulation.

REFERENCES