A third mode of ouabain signaling. Focus on “Regulation of ERK1/2 by ouabain and Na-K-ATPase-dependent energy utilization and AMPK activation in parotid acinar cells”

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IN RECENT YEARS evidence has emerged that the Na-K-ATPase, a canonical active ion pump, has parallel roles as a platform for cell signaling. Na-K-ATPase has been shown to associate directly with other proteins in signaling complexes in cardiac myocytes, renal cells, and several other cell types (4). Signals transmitted through the Na-K-ATPase and associated Src have been implicated in hypertrophy, proliferation or stasis, and resistance to apoptotic events. The signaling variously activates phospholipase C, Akt, epidermal growth factor receptor, and reactive oxygen species, and signaling by interaction with inositol-1,4,5-trisphosphate receptor has been implicated in Ca²⁺ oscillations and nuclear factor-κB activation (1). All of these signaling events occur with the specific inhibitor ouabain, a cardiac glycoside. Cardiac glycosides and bufadienolides, the only well-characterized extracellular ligands of the Na-K-ATPase, were originally discovered as plant and amphibian toxins, but a body of evidence shows that some function as endogenous regulators in mammals (8). In many investigations of ouabain-mediated signaling, a principal experimental read-out has been detection of activated (phosphorylated) mitogen-activated protein kinase ERK1/2.

Ouabain elicited signaling has been viewed by some investigators as occurring at concentrations too low to inhibit the Na-K-ATPase, but this needs to be understood in a physiological context. From biochemical studies, ouabain is not known to interact with purified Na-K-ATPase without inhibiting it, but effective signaling may require the occupancy of only a fraction of the Na-K-ATPase present in a cell (6). In intact cells, ouabain at very low concentrations has been shown to activate trafficking of other transporters, and in some cases, it actually increases measured Na-K-ATPase activity (2, 8). There is evidence for separate (and slowly interconvertible) pools of Na-K-ATPase with pumping or signaling functions and different locations or different associated proteins (5). Thus, in some experimental paradigms, the signaling effect of ouabain is amplified relative to its effect on ion transport. In many other studies, however, signaling events have been investigated at high enough concentrations of ouabain to inhibit a substantial fraction of the Na-K-ATPase. The higher the ouabain concentration used, the more intracellular signaling pathways may be called into play.

In a previous paper, Plourde and Soltoff (7) observed in parotid acinar cells that the phosphorylation of ERK1/2 in response to muscarinic cholinergic agonists was potentiated by treatment of the cells with ouabain at concentrations that do inhibit the enzyme. Ouabain treatment by itself, however, without concomitant muscarinic cholinergic receptor activation, caused a much more limited phosphorylation of ERK1/2. In this way parotid acinar cells were different from the tissues and cells where ouabain alone robustly activated ERK1/2. A salient observation was that blocking Na-K-ATPase by eliminating extracellular K⁺ resulted in ERK1/2 phosphorylation similar to that with 10⁻⁵ M carbachol. This suggested that it was inhibition of Na-K-ATPase activity, not necessarily signaling, that affected ERK1/2 activation in parotid cells. Activated ERK1/2 also played a positive role in modulating Na-K-ATPase activity, suggesting bidirectional interactions.

In a new paper published by Soltoff and Hedden (9), carbachol was shown to cause phosphorylation and activation of AMPK, the AMP-activated, energy state-sensing kinase (9) (Fig. 1). Concomitant treatment with ouabain blocked the phosphorylation of AMPK. This is consistent with the idea that the muscarinic cholinergic activation of the Na-K-ATPase...
results in enough hydrolysis of ATP to elevate the AMP-to-ATP ratio (presumably assisted by adenylate kinase to redistribute the ATPase product ADP to ATP and AMP). To test whether activated AMPK regulates ERK1/2 phosphorylation, the investigators used 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), a cell-permeable AMP mimetic, and found that it reduced the ERK1/2 stimulation seen not only in response to carbachol but also in response to another agent that stimulates the Na-K-ATPase, a purinergic agonist. AICAR did not affect ERK1/2 phosphorylation in response to the Src-signaling growth factor EGF, which is independent of Na-K-ATPase activity. AICAR treatment also blocked, or rather bypassed, the effect of ouabain, as predicted since it was used to mimic the elevated hydrolysis of ATP that ouabain inhibits. Consistent with this model, measured ATP levels were markedly reduced by carbachol and protected by ouabain. All of this was seen in freshly dissociated parotid acinar cells but not in a cell line derived from parotid cells. This cell line proved to have lower Na-K-ATPase activity, lower LKB1, the kinase that normally phosphorylates AMPK in energy signaling, and no detectable reduction in ATP concentration in response to carbachol. Instead, the cell line appeared to lack these metabolic features of the highly secretory native parotid acinar cell.

This work reveals a novel alternative pathway through which ouabain can affect signaling events in mammalian cells. It joins a distinguished list. Classically, inhibition of ion transport is known to alter the Na\(^+\) gradient and thus affect intracellular Ca\(^{2+}\) handling through NCX, the Na\(^+\)/Ca\(^{2+}\) exchanger. The Na\(^+\)/Ca\(^{2+}\) exchanger concept has been refined by the description of spatially limited Ca\(^{2+}\) signals controlled by the subcellular locations of Na-K-ATPase (3). Independently, Na-K-ATPase has been shown to be a platform for the assembly of Src and other signaling molecules, in some circumstances generating cellular events even in the apparent absence of alterations of ion homeostasis. The work of Soltoff and Hedden, however, illustrates that in a tissue with a very active Na-K-ATPase, signaling events can occur not through alteration of ion concentrations or through signal complex assembly, but through the AMPK-mediated response to reduction of cellular ATP. They observed that the ouabain-evoked enhancement of ERK1/2 phosphorylation was accompanied by similar effects on the upstream kinases RAF and MEK. The mechanism by which AMPK affects the ERK1/2 signaling pathway will be the next interesting step to define.

REFERENCES