## Regulation of the Na,K-ATPase

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$Na^+$,$K^+$-ATPase, the enzymatic equivalent of the membrane Na-K pump, maintains transmembrane electrochemical gradients for $Na^+$ and $K^+$. These gradients serve in secondary active transport processes that regulate cellular ions as well as organic compounds. The $Na^+$-$K^+$ pump therefore has a central role in cell function, and its activity is tightly regulated by a variety of hormones, acting on the pump *via* cell surface receptors coupled to intracellular messenger pathways.

Regulation of the $Na^+$-$K^+$ pump is of particular interest in the heart because of the role intracellular $Na^+$ plays in excitation-contraction coupling and in the "electro-mechanical phenotype" of heart failure. Effects of hormones are controversial. Most controversies arise from inappropriate experimental methods, not taking into account the pump's dependence on both transported ligands at intracellular and extracellular sites and on membrane voltage.

We use the whole-cell patch clamp technique to measure electrogenic $Na^+$-$K^+$ pump current ($I_p$, arising from the 3:2 $Na^+$:$K^+$ exchange ratio) in ventricular myocytes. Provided wide-tipped patch pipettes are used, the technique allows accurate control of pump ligands on both sides of the cell membrane and control of membrane voltage. We have examined effects of hormones coupled to protein kinases A, C and G (PKA, PKC, PKG). Direct phosphorylation of the $Na^+$-$K^+$ pump by protein kinases have been implicated in its regulation for many years. However, such phosphorylation is difficult to demonstrate *in vitro* unless the pump molecule is denatured.

We examined the effect of the catecholamine noradrenaline (NA), typically believed coupled to PKA activation *via* $\beta_1$ and $\beta_2$ adrenergic receptors. NA induced an increase in $I_p$. However, the increase persisted after blockade of $\beta_1/\beta_2$ or inhibition of PKA. In contrast, NA-induced pump stimulation was abolished by ODQ-induced inhibition of nitric oxide-activated guanylyl cyclase, an enzyme coupled to the $\beta_3$ receptor. Stimulation was reproduced by the selective $\beta_3$ agonist BRL 37344.

Angiotensin II induced a decrease in $I_p$ that was abolished by inhibition of PKC, a kinase often implicated in pump phosphorylation/regulation. However, PKC-mediated phosphorylation of the pump molecule itself seemed unlikely because additional experiments indicated that the effect of PKC was was dependent on NAD(P)H oxidase activation; the $Na^+$-$K^+$ pump may be regulated by a direct effect of reactive oxygen species on the pump molecule itself.

Atrial natriuretic peptide (ANP) induced an increase in $I_p$. Most effects of ANP are mediated by the NPRA receptor, a 'membrane guanylyl cyclase' that is insensitive to ODQ. However, ODQ abolished ANP-induced pump stimulation implicating nitric oxide-activated guanylyl cyclase. In agreement with this inhibition of cGMP-activated protein kinase (PKG) also abolished stimulation.

It is concluded that hormone, receptor and protein kinase-mediated $Na^+$-$K^+$ pump regulation is intricately related to nitric oxide and reactive oxygen species metabolism and that direct phosphorylation of the pump molecule is probably not involved.