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Na^+-K^+ ATPase: Regulation by Signal Transduction Pathways in Cardiac Myocytes

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Plasma membrane Na^+-K^+ ATPase (pump) is an essential component to maintain asymmetrical ion distribution across cell membrane. The Na^+-K^+ ATPase was discovered by Jens C. Skou in 1957 and since then physiological and biochemical properties of the enzyme have been extensively studied. Jens C. Skou was awarded the 1997 Nobel Prize in chemistry for his discovery of the Na^+-K^+ ATPase. In heart muscle cells, the Na^+-K^+ pump plays an important role in the regulation of intracellular Ca ion concentration. For an example, an inhibition of the Na^+-K^+ ATPase by cardiac steroids increases intracellular Na ion concentration which increases intracellular Ca ion concentration via Na^+-Ca^{2+} exchange. This is the mechanism by which digitalis produces a positive inotropic action in cardiac muscle.

Intracellular Na ion concentrations are much lower than extracellular concentrations. This lower Na ion concentration is maintained by a Na^+-K^+ pump in the cell membrane. The large Na ion gradient across the cell membrane is an important driving force for several membrane transporters, such as Na^+-Ca^{2+} exchanger, the Na^+-H^+ exchanger, the Na^+-HCO_3^- cotransporter, the Na^+-glucose carrier, and the inward Na ion current during action potentials. Changes in the transmembrane Na ion gradient could affect intracellular Ca ion concentration, intracellular pH, cardiac contractile force, and action potential. Intracellular Na ion overload could cause cardiac arrhythmia. Therefore, it is of fundamental importance to understand the regulation of Na^+-K^+ ATPase when assessing physiological and pathophysiological processes in the heart. Our study has been focused on the regulation of Na^+-K^+ ATPase by signal transduction pathways in cardiac myocytes. The Na^+-K^+ ATPase activities was accessed by measuring

intracellular Na ion concentration and Na^+-K^+ pump current when PLC- or adenylyl cyclase-mediated signal pathway was activated.

The PLC-mediated signal transduction pathway was activated by application of protein kinase C activator, PMA while intracellular Na ion concentration and transmembrane voltage were measured in single guinea pig ventricular myocytes. The PKC activator, PMA decreased intracellular Na ion concentration in dose-dependent manner. 100 nM PMA produced a maximal decrease in Na ion concentration of 1.5 mM from 6.5 ± 0.4 to 5.0 ± 0.4 (mean $\pm$ SE, n=12, $P < 0.01$). The PMA concentration required for a half-maximal decrease in Na ion concentration was 0.46 ± 0.13 nM. The decrease caused by PMA could be blocked by the PKC inhibitors, staurosporine and bisindolylmaleimide I. Stimulation of the α-adrenoceptor with 50 μM phenylephrine decreased intracellular Na ion concentration from 6.1 ± 0.34 to 4.6 ± 0.3 mM (n=11, $P < 0.01$). The decrease in intracellular Na ion concentration produced by phenyephrine was blocked by pretreatment with staurosporine or PMA. The results suggest that activation of PLC-mediated signal pathway results in a decrease in intracellular Na ion concentration via PKC-induced stimulation of the Na^+-K^+ ATPase in cardiac myocytes.

Effect of c-AMP-dependent protein kinase A on the Na^+-K^+ pump current-voltage (I-V) relationship was investigated in isolated guinea pig ventricular myocytes by using wide-tipped, perfused pippetes to record whole cell currents. In those ventricular myocytes in which stable membrane currents were maintained without rundown, the steady state Na^+-K^+ pump I-V relationship was obtained by subtracting the I-V relationship determined in the presence of 0.5 mM strophanthidin to inhibit the pump from that determined just before the application of strophanthidin. In twelve myocytes examined, micromolar forskolin, the adenylyl cyclase activator increased the Na^+-K^+ pump current over the entire membrane voltage range examined from −100 to +30 mV. At 0 mV membrane potential, forskolin increased the pump current by $34.5 \pm 7.2\%$ (mean $\pm$ SE, n=6) at 100 nM $[Ca^{2+}]_i$ and $30.4 \pm 8.0\%$ (n=6) at 50 nM $[Ca^{2+}]_i$, indicating that this increase does not depend steeply on $[Ca^{2+}]_i$ in physiological range. The voltage dependence of the increase in Na^+-K^+ pump current caused by forskolin was

identical to that of Na^+-K^+ pump current under control condition, i.e., the ratio of the pump current amplitude in the presence of forskolin to that in its absence did not change with voltage. Similar results were obtained when myocytes were superfused with Tyrode's solution containing low $[Cl^-]_o$. In a fraction of the myocytes that showed stable membrane currents, forskolin produced little effect on the Na^+-K^+ pump current over the full voltage range tested. Similarly, some myocytes displayed a progressive slow rundown of the Na^+-K^+ pump current, which seemed unaffected by the presence or absence of forskolin..The variability of these effects might be related to varying degrees of loss of soluble intracellular factors during dialysis of the myocytes. Possible explanations for the voltage-dependent scaling up Na^+-K^+ pump current magnitude by forskolin are an acceleration of a voltage-independent and rate-limiting step in the Na^+-K^+ transport cycle or an increase in the number of functional Na^+-K^+ pumps.