Phospholemman and the Cardiac Sodium Pump
Protein Kinase C, Take a Bow

William Fuller, Michael J. Shattock

In excitable tissues, the activity of the plasmalemmal sodium/potassium ATPase (Na/K pump) is vital for the maintenance of normal electrical activity and ion gradients. In cardiac muscle, the transsarcolemmal sodium (Na) gradient established by the Na/K activity is essential not only for generating the rapid upstroke of the action potential but also for driving a number of ion exchange and transport processes that are crucial for normal cellular function, excitation contraction coupling, ion homeostasis and the control of cell volume. These Na-dependent membrane transporters include those responsible for the regulation of other ions (such as the sodium calcium exchanger (NCX), Na/H exchanger and Na-HCO₃ cotransporter), as well as those involved in the movement of substrates and amino acids. PKA phosphorylates serine 68 and PKC phospho-

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From the Institute for Cardiovascular Research (W.F.), Ninewells Hospital, University of Dundee, UK; and the Cardiovascular Division (M.J.S.), King’s College London, UK.

Correspondence to Dr William Fuller, University of Dundee, Vascular Diseases Unit, Institute for Cardiovascular Research, Ninewells Hospital & Medical School, Dundee DD1 9SY, United Kingdom. E-mail w.fuller@dundee.ac.uk

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Progress in defining the physiological function of phospholemman. In cardiac myocytes, phospholemman (FXYD1) associates with the Na/K pump. Evidence from several laboratories supports the notion that phospholemman provides the link between cardiac kinases and the Na/K pump. Phosphorylation of phospholemman by PKA at serine 68 is associated with Na/K pump activation, and it has been proposed that phospholemman regulates the Na/K pump in a manner analogous to regulation of the calcium ATPase SERCA 2a by phospholamban: phosphorylation leading to disinhibition through elevated substrate affinity. In phospholemman deficient mice, β-adrenergic stimulation of the Na/K pump is absent.

Sympathetic stimulation of cardiac myocytes involves activation of PKA, via β-adrenergic receptors, and PKC, via α-adrenergic receptors. Whereas a considerable amount of effort has been dedicated to investigating the effect of PKA agonists on phospholemman and the cardiac Na/K pump, the effect of PKC phosphorylation of phospholemman has remained undefined. In this issue of Circulation Research, Han et al have investigated the effect of PKC activation on Na/K pump function, and the role played in this by phospholemman.

Every laboratory has their favorite method of measuring Na/K pump activity. The power of the research presented by Han et al lies in their use of 3 different techniques to investigate Na/K pump in freshly isolated mouse cardiomyocytes. Measurement of intracellular Na using the Na sensitive dye SBFI assesses the Na/K pump-dependent extrusion of Na after a period of pump inhibition. Simultaneous measurement of intracellular Na and Na/K pump current using 3 to 5 MΩ patch electrodes defines Na/K pump currents over a range of intracellular sodium. Low resistance patch electrodes measure Na/K pump currents at a single intracellular Na. So, not only is the transporting function of the Na/K pump assessed as the generation of an outward current (the exchange of 3Na for 2K), it is also assessed as the extrusion of its intracellular substrate (Na) as a function of its substrate concentration. All 3 techniques agree that PKC activation with phorbol ester increases Na/K pump Vmax, but is without effect on the enzyme’s Na affinity. Crucially, PKC-dependent stimulation of Na/K pump is absent in phospholemman deficient mice, indicating that, as for PKA, phospholemman provides the functional link between kinase and Na/K pump.

Sympathetic stimulation of the heart activates both PKA and PKC. Han et al have gone on to investigate the interaction of these 2 pathways in regulating the Na/K pump. Interestingly, there appears to be little overlap between the 2. PKA activation reduces K₉₅ for Na and PKC activation increases Vmax regardless of the prior state of the myocyte.
Likewise, PLM is further phosphorylated by PKA at serine 68 following PKC activation and vice versa. Hence in vivo, sympathetic stimulation of the mouse heart activates separate complementary pathways leading to Na/K pump activation at the level of both substrate affinity and Vmax. Physiologically, increased Na extrusion during sympathetic stimulation may limit the Na load induced by increased heart rate. While this may limit the associated positive inotropy, by favoring Ca extrusion by NCX it may also play a role in limiting Na and Ca overload, diastolic dysfunction and arrhythmias. Researchers have previously suggested that different isoforms of the catalytic subunit of the cardiac Na/K pump are subject to different regulatory mechanisms. An intriguing possibility raised by Han et al\textsuperscript{11} is that PKA and PKC target different Na/K pump isoforms through their phosphorylation of phospholemman. This highlights a key and hitherto unexplored issue: which proteins direct activated kinases to phospholemman, and what subpopulations of phospholemman are present in cardiac myocytes? The present article suggests that phospholemman exists in at least two subpopulations: one accessible only to PKA and one only to PKC. This implies that only a proportion of the Na/K pump is available to PKA, and a proportion to PKC. The authors speculate that these subpopulations of the Na/K pump represent the two isoforms of the catalytic subunit expressed in cardiac myocytes: \( \alpha 1 \) and \( \alpha 2 \).

The data presented by Han et al\textsuperscript{11} fit with a general scheme proposed by several laboratories, that phospholemman when unphosphorylated inhibits and when phosphorylated activates the Na/K pump. However the specifics of this scheme appear intricate. In reporting that serine 68 phosphorylated phospholemman increases \( K_{0.5} \) for Na whereas serine 63 and 68 phosphorylated phospholemman increases Vmax without effect on \( K_{0.5} \), the authors raise a complex biophysical question. Despite the presence of phosphate on serine 68 following PKC phosphorylation of phospholemman, no effect is observed on the \( K_{0.5} \) for Na. This implies that phosphorylation of serine 63 abrogates the effect of serine 68 phosphorylation, as well as increasing Na/K pump Vmax. This does not sit well with the concept of phosphorylation of phospholemman simply reducing its interaction with the Na/K pump. The relationship between phospholemman and Na/K pump is clearly more complex than that between phospholamban and SERCA2a in the sarcoplasmic reticulum.

To complicate matters further, in purified membrane fractions from phospholemman knockout and wild type hearts, other researchers have described reduced Na/K pump activity in the knockout compared with wild type\textsuperscript{13}: the opposite to that seen in the present study, and to what you would predict if phospholemman is simply a Na/K pump inhibitor. What this probably emphasizes is that comparing wild type with knockout between different laboratories is difficult. The wild type is a mixed population of phosphorylated and unphosphorylated phospholemman, and the relative proportions of these are determined by the adrenergic state of the heart on preparation. In other words, the more your mice are scared, the more phospholemman is phosphorylated.

The observation that phospholemman forms ion channels in lipid membranes led to the proposal that it regulates cell volume,\textsuperscript{14} and recent work has supported the idea that phospholemman forms complexes with itself,\textsuperscript{15,16} although no laboratory has yet produced evidence that phospholemman regulates cardiomyocyte cell volume. Intriguingly, phospholemman is also reported to regulate cardiac NCX: phosphorylation of phospholemman by PKA is associated with NCX inhibition,\textsuperscript{17} and there is convincing evidence that phospholemman interacts physically with NCX.\textsuperscript{18} Whether phospholemman may really moonlight in so many different physiological roles remains to be seen. It is noteworthy and surely relevant that NCX and the Na/K pump are found together in a functional complex in cardiac myocytes.\textsuperscript{19,20} The position of phospholemman in this complex will doubtless be the subject of further investigation.

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### Disclosures

None.

### References


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