Regulation of cardiac contractility: high time for FXYD

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FXYD PROTEINS COMPRISE a family of small integral membrane proteins that are regulators of ion transport (15). FXYD proteins have an extracellular NH2 terminus containing the signature PFXYD motif, a single transmembrane (TM) domain, and a cytoplasmic COOH terminus. They are distributed either in excitable tissues or organs involved in solute and fluid transport. Members in the FXYD family are numbered chronologically according to the dates they are cloned. With the exception of the γ-subunit of Na+/K+-ATPase (FXYD2), all other known FXYD proteins have at least one serine or threonine within the cytoplasmic tail. Alterations in expression/function of FXYD proteins have been implicated in disease states as varied as acute cardiac ischemia [phospholemman (FXYD1)], postinfarction ventricular remodeling (FXYD1), schizophrenia (FXYD3), and malignancies [mammary associated tumor-8 (FXYD3) and dysadherin (FXYD5)].

Phospholemman (PLM) was identified in 1985 as a major substrate for protein kinases (PK) A and C in heart muscle (12). Its 72-amino acid sequence was first determined by classic Edman degradation, and the molecule was subsequently cloned (10). Physiologically, PLM is phosphorylated by PKA at serine 68 and PKC at serine 63 and serine 68 (17). PLM also shares sequence similarity with phospholamban (PLB), a small protein in the sarcoplasmic reticulum (SR) membrane that regulates sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA2) transport activity (R55R1LS69 in PLM and RSAIRRAST17 in PLB). When phosphorylated by PKA at serine 16, PLB dissociates from SERCA2 and thereby relieves its inhibition of SR Ca2+ transport (14).

Although PLM is a major phosphoprotein in cardiac sarcolemma, its function has remained elusive for a long time. Early work based on overexpression of PLM in Xenopus oocytes suggests that PLM is a hyperpolarization-activated anion-selective channel and therefore plausibly involved in regulation of cell volume (9). The breakthrough came in 2002 when Crambert et al. (4) reported that PLM associates with and regulates the activity of α-subunits of Na+/K+-ATPase. When coexpressed with α- and β-subunits of Na+/K+-ATPase in Xenopus oocytes, PLM decreases the Km for Na+ and K+ without affecting Vmax (4). Subsequently, other FXYD family members [FXYD2, channel inducing factor (FXYD4), FXYD5, FXYD7, and a shark homolog of PLM (PLM-S or FXYD10)] have been demonstrated to regulate Na+/K+-ATPase activity. Mutational analysis, coinmunoprecipitation and covalent crosslinking studies, and molecular modeling based on Ca2+-ATPase crystal structure suggest that the single TM segment of FXYD proteins docks into the groove formed between TM segments 2, 6, and 9 of the α-subunit of the Na+/K+-ATPase (8).

In the heart, PLM coimmunoprecipitates with α-subunits of Na+/K+-ATPase (4) and regulates its activity either by modulating Km for Na+ (5) or Vmax (7, 13, 18). Phosphorylation of PLM by either PKA or PKC relieves its inhibition of Na+/K+-ATPase (5, 7, 13). Theoretically, inhibition of Na+/K+-ATPase by PLM is expected to raise intracellular Na+ concentration ([Na+]i), thereby altering the thermodynamic driving force for Na+/Ca2+ exchange and resulting in increased intracellular Ca2+ concentration ([Ca2+]i) and enhanced cardiac contractility. Myocytes isolated from congenic PLM-null mice, however, had similar resting [Na+]i (5) and contraction amplitudes at physiological extracellular Ca2+ concentration (16) compared with wild-type C57BL/6 myocytes. Another complexity is that PLM has been shown to regulate cardiac Na+/Ca2+ exchange activity, independent of its effects on Na+/K+-ATPase (3).

The elegant study by Bell et al. (1) in the American Journal of Physiology-Heart and Circulatory Physiology provides strong support for the hypothesis that the physiological function of PLM is regulation of cardiac contractility. The authors clearly demonstrated that whole heart contractile function, measured both in vivo and in vitro, was depressed in congenic PLM-null mice compared with wild-type mice. Cardiac dysfunction was ascribed to the absence of PLM since there were no differences in protein expression of α1-, α2-, β1-, and β2-subunits of Na+/K+-ATPase, SERCA2, PLB, and Na+/Ca2+ exchanger between wild-type and PLM-null mouse hearts. Supporting this conclusion is that by two-dimensional gel analysis and mass spectroscopy the authors detected minimal changes in ventricular protein expression between wild-type and PLM-null hearts.

So, what is the mechanism by which PLM regulates cardiac contractility? Is it mainly due to inhibition of Na+/K+-ATPase, as the authors proposed, or does regulation of Na+/Ca2+ exchanger by PLM play a role (16)? Since PLM, Na+/K+-ATPase, and Na+/Ca2+ exchanger colocalize in the sarcolemma and associate with each other, the effects of PLM on [Na+]i, [Ca2+]i, and cardiac contractility, under resting and stimulated conditions, are not easy to predict or model. Demonstrating lower bulk [Na+]i, and [Ca2+]i, in a beating, PLM-null heart will go a long way to support the authors’ hypothesis, although the ionic complexities in the submembranous domain (6) make unambiguous interpretation difficult. In addition, published studies so far have only focused on PLM phosphorylation, but equally important are the phosphatases and signaling mechanisms that promote PLM dephosphorylation, of which nothing is known at present. There are other important questions that need further study. What is the change in the phosphorylated fraction of PLM between basal and β-adrenergic-stimulated states? Are there different pools of PLM (and, by association, Na+/K+-ATPase) in the heart that respond separately to PKA or PKC stimulation (5, 7, 13)? What is the role of the TM segment when the cytoplasmic tail of PLM is sufficient to associate with and inhibit Na+/K+-ATPase (11)? Finally, changes in expression of PLM (18) or its phosphorylation state (2) in heart failure and the consequences in cardiac contractility

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need further exploration. Clearly, the time has come to “fix” the role of FXYD proteins in the regulation of cardiac contractility.

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REFERENCES