

Gaining Respectability

Membrane-Delimited, Caveolar-Restricted Activation of Ion Channels

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Voltage-gated sodium channels play a key role in the excitability of myocardial cells and in impulse propagation.^{1,2} An increase in Na⁺ current is, for example, responsible for the generation of the rapid upstroke of the action potential (phase 0). Mutations in the gene encoding this channel (*SCN5A*) have been linked to the pathogenesis of several cardiac channelopathies, including the long-QT and the Brugada syndromes,^{1,2} the discoveries of which have helped in the functional characterization of these channels. In addition, the signaling pathways that regulate Na⁺ channel activity, such as the sympathetic nervous system, have been the subject of numerous studies.^{3–6} Indeed, stimulation of β -adrenergic receptors can both enhance conduction in normal ventricular myocardium but also induce arrhythmic events in a number of cardiac disease states.^{1,2}

Several mechanisms that contribute to the regulation of Na⁺ channels by β -adrenergic stimulation have been documented (see Figure). All of them involve G proteins, although diffusible second messengers are not necessarily involved. For example, besides the coupling of G α_s to adenylate cyclase and downstream protein kinase A (PKA)-mediated phosphorylation events,^{3,4} the so-called “membrane-delimited” pathway does not involve diffusible cytosolic factors. The G α_s subunit can, indeed, directly modify Na⁺ channel activity.^{5,6} This type of regulation is not unique to G α_s , as the direct binding of the G $\beta\gamma$ protein subunit to several Ca²⁺, K⁺, and Cl[–] channels has been extensively documented (for review, see Dascal⁷).

Shibata and colleagues have shown previously that the application of peptides derived from the G α_s sequence in inside-out macropatches enhanced isoproterenol-evoked sodium current (I_{Na}), attributable to an apparent increase in the number of functional channels.^{5,6} In this issue of *Circulation Research*, Shibata and colleagues (Yarbrough et al⁸) report that the source of these new channels could be caveolae. Using antibodies directed against caveolin-3, the muscle-specific isoform of caveolin, these investigators were able to block the direct effect of isoproterenol on the Na⁺ channel (ie, the PKA-independent, G α_s membrane-delimited pathway).

Importantly, this effect seems specific, given that antibodies directed against other caveolin isoforms did not affect isoproterenol activation of Na⁺ channel activity. Moreover, Yarbrough et al⁸ also documented, in cardiac myocytes, the apparent preferential enrichment into caveolar microdomains of Na⁺ channels and G α_s . Although these data led the authors to conclude that an increase in sympathetic nervous system activity could lead to the recruitment of Na⁺ channels out of caveolar microdomains to the sarcolemma, thereby increasing I_{Na} , several caveats regarding this hypothesis should be noted.

Definitive evidence for the translocation of Na⁺ channels out of caveolae upon isoproterenol stimulation would require further subcellular fractionation and/or electron microscopic studies. In the absence of such experiments, there is no reason to think that the G α_s -activated Na⁺ channels necessarily would need to exit caveolar microdomains to be active. The use of the anti-caveolin-3 antibody, although specific for this caveolin isoform, may have had effects on other signaling proteins in myocyte caveolae that affected Na⁺ function. Moreover, it is unlikely that the antibody resulted in the disruption of mature caveolae. This specific antibody, which is directed against an epitope in the nonscaffolding N-terminal end of caveolin-3, probably acts via steric hindrance to prevent G α_s translocation to the Na⁺ channels into the caveolar microenvironment. Alternatively (or additionally), because β -adrenergic receptors have been found to be enriched in cardiac myocyte caveolae,^{9,10} accumulation of the antibody could have altered receptor coupling to G α_s (and its consecutive targeting to Na⁺ channel) upon agonist stimulation. In either case, the argument that blockade of Na⁺ channel translocation out of caveolae is required to explain the decrease in I_{Na} does not necessarily hold.

Indeed, sarcolemmal caveolar microdomains could be an important site of Na⁺ channel activation. Several lines of evidence support this concept. First, a caveat: the term “caveolae” should be used with caution in the context of cardiac muscle. In contrast to skeletal muscle, where caveolin-3 is only observed in association with sarcolemmal caveolae, caveolin-3 in cardiac myocytes has been observed to be associated with both the plasma membrane and the T-tubular system^{11,12} (Prof R.G. Parton, written communication, January 2002). Interestingly, several groups have reported the location of cardiac Na⁺ channels in T-tubules to be in close proximity with the Na⁺-Ca²⁺ exchanger and L-type Ca²⁺ channels (which are in close contact with the sarcoplasmic reticulum [SR]) (for review, see Scriven et al¹³). The data of Yarbrough et al⁸ could therefore be interpreted as complementary evidence that Na⁺ channels are colocalized with other key regulators of excitation-contraction coupling within caveolin-enriched structures in T-tubules.

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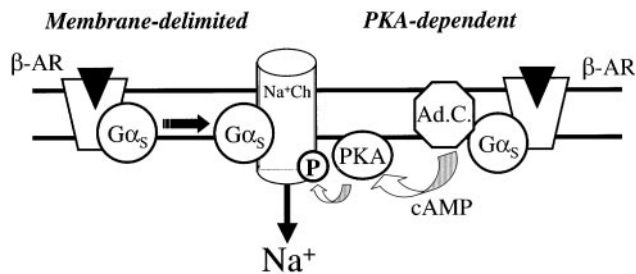
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Schematic representation of the 2 major pathways regulating voltage-dependent Na⁺ channels (Na⁺Ch) in cardiac myocytes. The PKA-dependent pathway (right) involves the coupling of the stimulated β-adrenergic receptor (β-AR) to Gα_s and the activation of adenylate cyclase (Ad.C.). The subsequent phosphorylation of the Na⁺ channel by activated cAMP-dependent protein kinase (PKA) leads to a shift in voltage dependence of channel availability to more negative potentials. Of note, a PKA-dependent process has also been shown to regulate Na⁺ channel trafficking.³ The membrane-delimited pathway (left) is independent of soluble second messengers and involves the direct interaction of the Na⁺ channel with the N-terminal end of the Gα_s protein subunit; this binding produces an increase in I_{Na} without a shift in voltage-dependent current activation.

There are also parallels between the presence of functional Na⁺ channels in caveolae and the targeting to these discrete microdomains of other plasmalemmal channels, including water, Ca²⁺, K⁺, and Cl⁻ channels.^{14–16} For some of these channels, a direct link between their subcellular localization in caveolae and their function has been clearly established. Using a dominant-negative caveolin, Trouet et al¹⁴ demonstrated that the activation of volume-regulated anion channels by cell swelling was dependent on the maintenance of caveolin integrity within caveolae. In smooth muscle and cardiac cells, Löhn et al¹⁵ also have documented that caveolae control the formation of local SR Ca²⁺ release events, thereby playing a role in triggering Ca²⁺ sparks.

Finally, it should be pointed out that Yarbrough and colleagues⁸ used a PKA inhibitor to prevent cAMP-dependent activation of Na⁺ channels, a pathway that represents normally at least 50% of the increase in I_{Na} induced by isoproterenol^{5,6} (see Figure). Interestingly, several of the actors of this parallel Na⁺ channel activation pathway have also been shown to be located within caveolae. β-Adrenergic receptors, adenylate cyclase, and PKA were found closely associated with caveolin in cardiac myocytes.^{9,10} If true, this is additional evidence to suggest that Na⁺ channels might not be required to translocate out of caveolae to be activated. This question could be addressed by exposing patch-clamped cardiac myocytes to caveolin-3 antibodies or (preferably) caveolin-derived peptides and/or caveolae-disrupting drugs and then by evaluating their effects on PKA-dependent activation of Na⁺ channels.

In conclusion, a half century after their discovery, the role of caveolae—and caveolins—in the regulation of intracellular signal transduction cascades has begun to reach maturity. In the cardiovascular system, for example, the recent development of caveolin knockout mice (for review, see Razani and Lisanti¹⁷) has to date confirmed the importance of the caveolar location of eNOS for the regulation of this NO

synthase (see Feron and Kelly¹⁸ for references). Nevertheless, additional studies that build on the observations made by Yarbrough et al⁸ will be required to explore the functional consequences of caveolar compartmentation on the generation of calcium sparks¹⁵ and neuronal and humoral regulation of excitation-contraction coupling.¹⁹ Thus, from the unenviable status of being a “last refuge of scoundrels,” the compartmentation of signal transduction pathways has begun to gain respectability, if not yet nobility, in the molecular pharmacology of the cardiovascular system.

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