The real estate of cardiac signaling: Location, location, location

Meena S. George* and Geoffrey S. Pitt†‡

**Center for Neurobiology and Behavior and †Departments of Medicine and Pharmacology and Center for Molecular Cardiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032*

Determining how cells distinguish between adaptive and maladaptive signals when they appear to share the same molecular pathways has been a vexing biological problem. The ability to identify distinctive features of a pathophysiological response compared with a physiological response would allow for the rational design of approaches to eliminate or diminish undesirable consequences of pathophysiological responses while preserving the beneficial effects of physiological signals. These issues are especially pertinent in the heart and in understanding the development of heart failure, for which >500,000 new cases are diagnosed in the United States each year. Major factors contributing to worsening heart failure include a number of compensatory neurohormonal signals intended to counteract decreased cardiac output, such as hyperadrenergic stimulation (1). In a recent issue of PNAS, Balijepalli *et al.* (2) provide new insight into how adrenergic signaling pathways are organized in the heart.

Adrenergic signaling in the myocardium contributes to the control of heart rate (chronotropy), strength of contraction (inotropy), and rate of relaxation (lusitropy) by changing the levels of intracellular Ca^{2+} or by altering the sensitivity of critical regulatory proteins to Ca^{2+}. Signaling is mediated predominantly by two distinct β-adrenergic receptors, β_1 and β_2, which differ in their abundance, distribution, and downstream signal transducers (3). Approximately 75% of the cardiac β-adrenergic receptors are β_1, which appear to be distributed globally throughout the sarcolemma. β_1 receptors couple to the G_s heterotrimeric G protein. The less-abundant β_2 receptors reside predominantly in caveolae (4), specialized compartments of the plasma membrane organized by caveolins. Caveolae are flask-shaped membrane invaginations rich in cholesterol and glycosphingolipids that house and coordinate multiple signaling components, many of which appear to be dedicated to Ca^{2+} signaling (5). Besides their distinct homes, β_2 receptors also differ from β_1 in that they couple to both G_s and G_i. Nevertheless, stimulation of either β_1 or β_2 activates adenylyl cyclase to increase intracellular cAMP. In turn, cAMP activates protein kinase A, resulting in the phosphorylation of key elements of the contractile apparatus and of proteins that control internal Ca^{2+} levels. Prominent among the PKA targets are the L-type voltage-gated Ca^{2+} channels ($Ca_V1.2$), which open upon membrane depolarization, allowing Ca^{2+} to enter the cell. The "receptors" for this Ca^{2+} signal are the ryanodine receptors (RyR2), Ca^{2+} release channels on the sarcoplasmic reticulum (SR) that flood the cytoplasm with additional Ca^{2+} that then initiates contraction (Fig. 1). PKA phosphorylation of L-type Ca^{2+} channels potentiates inward Ca^{2+} current and thereby augments contraction.

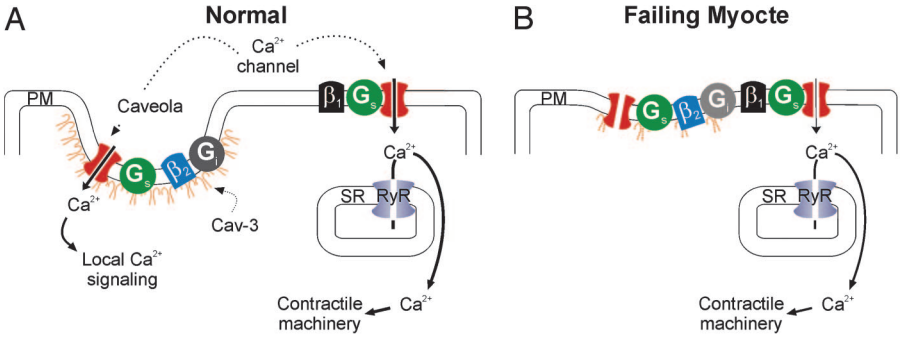

Fig. 1. Caveolar localization of the β_2-adrenergic receptor/L-type Ca^{2+} channel signaling complex. (*A*) The β_2 receptor and its accompanying G_i and G_s proteins are depicted within a caveola, organized by Cav-3. Localized L-type Ca^{2+} channel potentiation in response to β_2 agonists may contribute to Ca^{2+} signaling cascades that are distinct from the larger Ca^{2+} pool involved in excitation–contraction coupling. The β_1 receptors, coupled solely to G_s, are depicted on the plasma membrane (PM) and outside of caveolae. Most L-type Ca^{2+} channels are closely opposed to RyR in the SR. Influx of Ca^{2+} through L-type Ca^{2+} channels triggers opening of RyRs and release of Ca^{2+} from the SR to activate the contractile machinery. (*B*) In heart failure, signaling through β_2 receptors blunts β_1 potentiation of L-type Ca^{2+} channels through a G_i-dependent mechanism (19). This blunted response may result from dysregulation of caveolar organization, thus disturbing the compartmentation of the β_1 and β_2 receptors.

Electrophysiological studies of L-type Ca^{2+} current after adrenergic stimulation revealed important consequences of the localization and G protein-coupling differences between β_1 and β_2 receptor subtypes. By isolating L-type Ca^{2+} channels within a patch pipette, Chen-Izu *et al.* (6) determined that remote stimulation (outside of the pipette) of β_1 increased Ca^{2+} channel current within the pipette, suggesting that β_1 signaling included a diffusive second messenger. In contrast, β_2-specific agonists were effective only when included within the pipette. This membrane-delimited β_2 signaling depended on G_i, because inactivation of G_i with pertussis toxin made β_2 signaling diffusive. Several other studies have provided additional evidence for important functional consequences of differential signaling through the β-adrenergic receptors. For example, sustained signaling through β_1 receptors led to myocyte apoptosis; this β_1-mediated proapoptotic signal depended on Ca^{2+} influx through L-type Ca^{2+} channels and activation of Ca^{2+} calmodulin-dependent kinase II (CaMKII) (7). On the other hand, β_2 activation was protective against apoptotic signals (8–10). Like the membrane-delimited activation of L-type Ca^{2+} currents, coupling of β_2 to G_i was also necessary for prosurvival signaling; G_i inactivation with pertussis toxin blocked protection (8).

Balijepalli *et al.* (2) provide a new wrinkle to this compartmentation story. They demonstrate for the first time that L-type Ca^{2+} channels can be found within caveolae in cardiac myocytes and that β_2 activation of L-type Ca^{2+} channels requires intact caveolae. Electron microscopy showed α_{1C}, the L-type Ca^{2+}

Conflict of interest statement: No conflicts declared.

See companion article on page 7500 in issue 19 of volume 103.

‡To whom correspondence should be addressed. E-mail: gp2004@columbia.edu.

channel pore-forming subunit, within caveolae in neonatal cardiac myocytes. Sucrose density gradients revealed the cosedimentation of α_{1C} with caveolin-3 (Cav-3), the predominant caveolin isoform in the heart. As previously found, the β_2 receptor was enriched in this fraction, and all of the important components of the β_2-adrenergic signaling complex (G_s, G_i, adenylyl cyclase, and PKA) were also present. Interestingly, disruption of caveolae with 10 mM methyl β-cyclodextrin (MβCD) or inhibition of Cav-3 by small interfering RNA prevented β_2 stimulation of L-type Ca^{2+} channel current, suggesting that caveolar localization was necessary. Previously, it was known that Kv1.5 potassium channels are preferentially localized in caveolae (11), and a fraction of cardiac Na^+ channels cosediment with Cav-3 (12), although the functional consequences of the location of either ion channel within caveolae have not yet been determined.

The demonstration that β_2-mediated potentiation of L-type Ca^{2+} currents was caveolae-dependent may have important consequences for the understanding and treatment of cardiac hypertrophy and heart failure. Approximately 90% of L-type Ca^{2+} channels in adult cardiac myocytes are found within T tubules (13), a specialized architecture of tubular invaginations of the sarcolemma, where they face RyRs in the juxtaposed SR. This organization ensures instantaneous release of SR Ca^{2+} stores throughout the cytoplasm after membrane depolarization. Although a small population of RyRs have been found in regions of the SR that are not associated with T tubules or the plasmalemma (14), definitive demonstration and localization of "orphan" L-type Ca^{2+} channels have been more elusive. The presence of L-type Ca^{2+} channels not necessarily associated with the SR, as implied in this new study by Balijepalli *et al.* (2), raises the possibility that Ca^{2+} signaling through this subpopulation of channels may provide a specialized function different from excitation–contraction coupling, such as contributing to the signaling cascades that initiate cardiac hypertrophy. Although several lines of evidence have implicated Ca^{2+} signaling in the development of hypertrophy, it has been difficult to understand how a myocyte can distinguish between hypertrophy-inducing signals and the much larger pool of internal Ca^{2+} that rapidly rises and falls over a 10-fold concentration range with each heart beat (15). A recent report suggests that the hypertrophic signal endothelin-1 increases nuclear Ca^{2+} by an inositol 1,4,5-trisphosphate (IP_3)-triggered release from IP_3 receptors in the nuclear membrane (16), the first convincing demonstration of a separable Ca^{2+} signal in myocytes. Balijepalli *et al.* (2) present another example supporting the idea of cardiac Ca^{2+}-signaling microdomains, this one being in the cytoplasm rather than the nucleus. The emerging picture here is that focal Ca^{2+} changes, whether modest or large, at the site of Ca^{2+} entry can signal downstream pathways, and that this signaling is restricted because of fast termination and rapid diffusion or extrusion, with little change in overall Ca^{2+} concentration. Such a mechanism would allow for specific signaling despite the normal "background" Ca^{2+} fluctuations that are a part of excitation–contraction coupling. It is interesting to speculate that protective adrenergic signaling through β_2 receptors may be related to caveolar localization because CaMKII, necessary for the contrasting β_1-mediated apoptotic signal, has not been reported in caveolae.

The dependence on caveolar localization for β_2-mediated activation of L-type Ca^{2+} channels and the role of both β_2 and Ca^{2+} signaling in cardiovascular physiology also place a new focus on caveolae and Cav-3 in particular. Could dysregulation of caveolar organization contribute to heart failure or cardiac hypertrophy by affecting this signaling complex? The development of heart failure in Cav-$3^{-/-}$ mice (17) makes this an intriguing possibility and suggests that pharmacological modulation of caveolae may be a fruitful avenue for drug development. Further, in at least one report (18), heart failure was accompanied by Cav-3 down-regulation. If destabilization or loss of caveolae contributed to heart failure such that β_2 signaling was no longer segregated (Fig. 1), it might provide a cogent explanation for the observation that adrenergic activation of L-type Ca^{2+} currents in the failing heart is blunted because of β_2 stimulation of a G_i-dependent pathway (19).

These observations also raise many new questions. For example, how is a subpopulation of L-type Ca^{2+} channels targeted to caveolae? Because a postsynaptic density protein 95/discs large/ZO-1 (PDZ)-binding motif in the C terminus of β_2 is important for receptor trafficking and coupling to G_i (20), it is interesting to consider that a PDZ motif in the C terminus of α_{1C}, previously shown to be important for excitation–transcription coupling in neurons (21), might provide the caveolar localization signal to L-type Ca^{2+} channels. Also, certain key experiments in the Balijepalli *et al.* (2) study were performed in neonatal myocytes, which lack the elegant T tubule architecture important for excitation–contraction coupling in adult myocytes. It will be essential to determine whether caveolar localization of L-type Ca^{2+} channels is critical for β_2-regulated L-type Ca^{2+} currents in the adult and whether this arrangement is perturbed in heart failure. Regardless, this study by Balijepalli *et al.* (2) opens new possibilities for the modulation of pathophysiological signaling of the β-adrenergic system that may lead to novel therapies for heart failure.

This work was supported by National Institutes of Health Grant HL 71165 (to G.S.P.) and American Heart Association Grant 0555875T. G.S.P. is a recipient of the Irma T. Hirschl Career Scientist Award and is the Esther Aboodi Assistant Professor of Medicine. M.S.G. is supported by the Columbia University M.D.–Ph.D. Program.

1. Packer, M. (1988) *Circulation* **77,** 721–730.
2. Balijepalli, R. C., Foell, J. D., Hall, D. D., Hell, J. W. & Kamp, T. J. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 7500–7505.
3. Steinberg, S. F. (2004) *J. Mol. Cell. Cardiol.* **37,** 407–415.
4. Rybin, V. O., Xu, X., Lisanti, M. P. & Steinberg, S. F. (2000) *J. Biol. Chem.* **275,** 41447–41457.
5. Cohen, A. W., Hnasko, R., Schubert, W. & Lisanti, M. P. (2004) *Physiol. Rev.* **84,** 1341–1379.
6. Chen-Izu, Y., Xiao, R.-P., Izu, L. T., Cheng, H., Kuschel, M., Spurgeon, H. & Lakatta, E. G. (2000) *Biophys. J.* **79,** 2547–2556.
7. Zhu, W. Z., Wang, S. Q., Chakir, K., Yang, D., Zhang, T., Brown, J. H., Devic, E., Kobilka, B. K., Cheng, H. & Xiao, R. P. (2003) *J. Clin. Invest.* **111,** 617–625.
8. Communal, C., Singh, K., Sawyer, D. B. & Colucci, W. S. (1999) *Circulation* **100,** 2210–2212.
9. Zaugg, M., Xu, W., Lucchinetti, E., Shafiq, S. A., Jamali, N. Z. & Siddiqui, M. A. Q. (2000) *Circulation* **102,** 344–350.
10. Chesley, A., Lundberg, M. S., Asai, T., Xiao, R.-P., Ohtani, S., Lakatta, E. G. & Crow, M. T. (2000) *Circ. Res.* **87,** 1172–1179.
11. Martens, J. R., Sakamoto, N., Sullivan, S. A., Grobaski, T. D. & Tamkun, M. M. (2001) *J. Biol. Chem.* **276,** 8409–8414.
12. Yarbrough, T. L., Lu, T., Lee, H.-C. & Shibata, E. F. (2002) *Circ. Res.* **90,** 443–449.
13. Shannon, T. R., Wang, F., Puglisi, J., Weber, C. & Bers, D. M. (2004) *Biophys. J.* **87,** 3351–3371.
14. Franzini-Armstrong, C., Protasi, F. & Tijskens, P. (2005) *Ann. N.Y. Acad. Sci.* **1047,** 76–85.
15. Molkentin, J. D. (2006) *J. Clin. Invest.* **116,** 623–626.
16. Wu, X., Zhang, T., Bossuyt, J., Li, X., McKinsey, T. A., Dedman, J. R., Olson, E. N., Chen, J., Brown, J. H. & Bers, D. M. (2006) *J. Clin. Invest.* **116,** 675–682.
17. Woodman, S. E., Park, D. S., Cohen, A. W., Cheung, M. W. C., Chandra, M., Shirani, J., Tang, B., Jelicks, L. A., Kitsis, R. N., Christ, G. J., *et al.*. (2002) *J. Biol. Chem.* **277,** 38988–38997.
18. Petrashevskaya, N. N., Bodi, I., Koch, S. E., Akhter, S. A. & Schwartz, A. (2004) *Cardiovasc. Res.* **63,** 561–572.
19. He, J.-Q., Balijepalli, R. C., Haworth, R. A. & Kamp, T. J. (2005) *Circ. Res.* **97,** 566–573.
20. Xiang, Y. & Kobilka, B. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 10776–10781.
21. Weick, J. P., Groth, R. D., Isaksen, A. L. & Mermelstein, P. G. (2003) *J. Neurosci.* **23,** 3446–3456.