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L-Type Ca^{2+} Channels Gaining Respect in Heart Failure

Timothy J. Kamp, Jia-Qiang He

L-type Ca^{2+} channels are essential for the initiation and regulation of excitation-contraction (EC) coupling in adult cardiac muscle.[1] The rapid influx of Ca^{2+} through these channels triggers release of intracellular Ca^{2+} from the sarcoplasmic reticulum (SR) stores, and the resulting Ca^{2+} transient activates the myofilaments and thus contraction. Given that failing ventricular myocytes exhibit impaired contractility and abnormal Ca^{2+} transients, the L-type Ca^{2+} channel makes for a good suspect as a contributor to the derangement of EC coupling. However, most initial studies of whole-cell currents through L-type Ca^{2+} channels (I_{Ca}) in both human heart failure and animal models did not show a significant difference between nonfailing and failing myocytes.[2] In the meantime, important changes in the abundance and/or function of other Ca^{2+} cycling proteins including the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), Na^{+}-Ca^{2+} exchanger (NCX), and ryanodine receptors (RyRs) took the spotlight as the bad actors in heart failure.[3] The L-type Ca^{2+} channel was left behind as a boring, obligatory participant in EC coupling.

Times may be changing for the status of the L-type Ca^{2+} channel in the world of failing hearts. The article by Chen et al,[4] in this issue of *Circulation Research*, unmasks important changes in the density as well as regulation of L-type Ca^{2+} channels in failing human ventricular myocytes. And, perhaps most encouragingly, the authors show that these changes can be partly reversed in patients with left ventricular assist devices (LVADs). This article adds more evidence to recent publications suggesting significant changes in L-type Ca^{2+} channel abundance and function in heart failure.[5,6] To appreciate the story, one needs to step back from the macroscopic or whole-cell currents through L-type Ca^{2+} channels and look behind the scenes. Whole-cell currents are determined by the number of functional channels (N), the probability of a channel being open (p_o), and the current through a single open channel (i) as described by the following simple relationship: $I_{Ca,L}=N{*}p_o{*}i$. Thus, the finding that peak I_{Ca} is comparable in failing and nonfailing myocytes when measured using standard voltage-clamp techniques may in fact be hiding counterbalancing changes in channel density, gating, and single-channel current. It is also important to realize that the altered conformation of the action potential in failing myocytes can have a large effect on the influx of Ca^{2+} through L-type Ca^{2+} channels even if all of the properties of the channels are unchanged.[7,8]

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res.* 2002;91:451-453.)

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***Circulation Research* is available at http://www.circresaha.org**
DOI: 10.1161/01.RES.0000035346.21625.4A

Tracking the Density and Gating of L-Type Ca^{2+} Channels

A number of techniques exist that provide detailed information on the number and gating of L-type Ca^{2+} channels. Biochemical techniques have generally suggested no change or a decrease in protein abundance of channel subunits or binding sites for Ca^{2+} antagonists in failing hearts[2]; however, it is difficult to extrapolate these results to functional channels at the surface membrane. A single-channel electrophysiological study provided the first clue that the story on L-type Ca^{2+} channels may be more complicated than originally suspected. It was demonstrated that the number of functional channels and the open probability of the channels were markedly increased in failing human ventricular myocytes without changes in single-channel current, just as if the channels had been stimulated by protein kinase A (PKA)-dependent phosphorylation.[6] Given that the whole-cell currents were unchanged, the authors speculated that the density of channels is decreased in failing cells with a compensatory increase in the availability and open probability of the remaining channels. The limitation of that study was that by necessity the single-channel recordings were made from channels on the surface sarcolemma, whereas most of Ca^{2+} channels are concentrated in the t-tubules in ventricular myocytes where the majority of EC coupling action occurs.

An alternative approach measuring intramembrane charge movement associated with L-type Ca^{2+} channels was taken in the tachycardia-pacing canine heart failure model.[5] With appropriate voltage protocols, maximal intramembrane charge movement should be directly proportional to L-type Ca^{2+} channel number. Despite finding a comparable density of I_{Ca} in control and failing myocytes as had others,[9] the study showed more than a 50% decrease in maximal amount of intramembrane charge movement again suggesting that the overall density of L-type Ca^{2+} channels is significantly decreased. The limitation of the charge movement study is that it is difficult to isolate charge movement due to L-type Ca^{2+} channels in myocytes that have many different voltage-dependent ion channels.

Given this background, Chen and colleagues[4] decided to take a closer look at the whole-cell I_{Ca} in failing human ventricular myocytes. They similarly found that the peak density of I_{Ca} was not changed in a major way; however, unlike previous studies, they did detect a hyperpolarizing shift in the voltage dependence of activation in failing cells. These investigators reasoned that if the basal gating activity of the channels was increased in the presence of reduced channel density, then maximal stimulation of channels by the

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cAMP/PKA pathway or by dihydropyridine Ca^{2+} channel activators should unmask a difference in maximally stimulated I_{Ca}. Perhaps it was not surprising that maximally stimulated I_{Ca} by isoproterenol was significantly less in failing myocytes, given the well-documented blunted response of failing hearts to β-adrenergic agonists.[10] Although this may be in part a result of multiple alterations along the β-adrenergic signaling cascade in failing hearts, Chen and colleagues suggested that additional contribution was due to a reduced density of L-type Ca^{2+} channels in the failing myocytes. To support this contention, the authors bypassed the β-adrenergic receptor through adenylate cyclase by directly testing nonhydrolyzable dibutyryl-cAMP. Although dibutyryl-cAMP was more effective than isoproterenol in stimulating I_{Ca}, there was still a 43% smaller maximally stimulated current level in failing myocytes. Therefore, the authors suggest that a major underlying cause for blunted stimulated current levels was the reduction in channel number rather than abnormalities in the β-adrenergic receptor cascade. This interpretation of the results with dibutyryl-cAMP requires that the signaling cascade downstream of cAMP is unaltered, which in fact may not be the case. For example, changes in protein phosphatase activity counterbalancing PKA effects could be important as the authors point out. Alternatively, localization of the PKA to its substrate with the necessary anchoring protein could be impaired in the failing heart, blunting the ability of this pathway to stimulate I_{Ca}. Lastly, the substrate for PKA, presumably one or more subunits of the L-type Ca^{2+} channel, could be altered. To avoid these possible complexities of the PKA signaling cascade, the effect of maximally activating channels via a distinct mechanism using the dihydropyridine BayK 8644 was tested, which acts directly on the channel. Again, in failing myocytes, the maximally stimulated I_{Ca} density was reduced compared with nonfailing myocytes, although the difference was smaller than for the experiments with dibutyryl-cAMP. This result is consistent with a lower density of L-type Ca^{2+} channels in failing myocytes, but the interpretation rests on the assumption that L-type Ca^{2+} channels in control and failing myocytes respond similarly to dihydropyridines. Thus, a number of recent electrophysiological studies have provided complementary evidence that even if I_{Ca} is unchanged in failing myocytes, important differences in channel density and function can be present.

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Subcellular Localization of L-Type Ca^{2+} Channels

Why worry about the number of and gating of L-type Ca^{2+} channels if the final currency of these channels, I_{Ca}, is unchanged in heart failure? One answer lies in the fact that localized, microscopic currents through single L-type Ca^{2+} channel play an important role in determining the functional effect of these channels. In the case of EC coupling, it is the highly localized influx of Ca^{2+} through sarcolemmal L-type Ca^{2+} channels in the restricted space opposite junctional sarcoplasmic reticulum that precisely regulates release of Ca^{2+} from SR.[11] L-type Ca^{2+} channels outside of these junctional domains have a markedly reduced effect on EC coupling. In addition, subcellular localization of L-type Ca^{2+} channels in other subdomains such as in complexes with β_2-adrenergic receptors may be important in regulating cellular signaling.[12,13] Therefore, the present study of Chen et al[4] raises the following questions: where are those channels lost and what subpopulation(s) of channels show the counterbalancing increase in channel gating?

In normal adult ventricular myocytes, most L-type Ca^{2+} channels are concentrated in the t-tubule network and contribute to the synchronized initiation of EC coupling throughout the myocytes.[14] Recent studies in the canine tachycardia-dilated cardiomyopathy model and a doxorubicin-induced cardiomyopathy model have suggested that failing myocytes can have dramatic cellular remodeling with loss of t-tubule membranes.[5,15] Thus, it is possible that the localization of L-type Ca^{2+} channels may be quite different in failing myocytes than control myocytes. Defining the subcellular localization, molecular composition, and function of subpopulations of L-type Ca^{2+} channels represents critical future challenges.

Basal Regulation of L-Type Ca^{2+} Channels

Chen et al[4] hypothesize that there is increased basal phosphorylation of the channel in failing myocytes by cAMP/PKA-mediated pathways based on the shift in the voltage dependence of activation of the channels in failing cells, the effect of PP2a specifically on failing basal I_{Ca}, and the blunted response to isoproterenol and dibutyryl-cAMP. In addition, the previous single-channel study in failing human myocytes suggested a gating pattern typical of PKA-stimulated channels.[6] They suggest that hyperphosphorylated channels are present in failing hearts, analogous to studies of the RyRs.[16] This is an appealing hypothesis, but at this stage no direct evidence has been provided for actual changes in phosphorylation status of the channel measured biochemically. This remains a problem area for L-type Ca^{2+} channels as the molecular details of PKA regulation of the channel in intact cardiac myocytes have remained relatively refractory to biochemical approaches.[17] Furthermore, some of the data by Chen et al[4] are difficult to reconcile with this hypothesis in that H89 and Rp-cAMP failed to alter basal currents in both failing and control myocytes. The recovery of regulation by β-adrenergic stimulation of I_{Ca} in LVAD patients, despite the continued hyperpolarizing shift in the activation of the currents, suggests multiple points of modulation of channel gating. Another critical factor is that other Ser/Thr kinases can regulate L-type Ca^{2+} channels including protein kinase C and Ca^{2+}/CaM kinase and potentially more.[18] One does not even need to invoke differences in the basal phosphorylation state of the channel to explain increased basal open probability. Changes in channel subunit isoforms can also contribute to this behavior. Alternative isoforms of the pore-forming $Ca_v1.2$ channel have been demonstrated in failing hearts,[19] and additionally, auxiliary subunits may have important modulatory roles. For example, overexpression of the β_{2a} subunit in rat ventricular myocytes can clearly increase current levels.[20] Unraveling the mechanism for upregulated basal activity of L-type Ca^{2+} channels in failing myocytes will require future studies.

Summary

The L-type Ca^{2+} channel is gaining respect as a contributor to the pathophysiological changes in Ca^{2+} homeostasis in failing myocytes. Many studies using standard voltage-clamp pulses have not detected significant changes in basal I_{Ca}, but the work by Chen and colleagues[4] highlights significant decreases in density and altered β-adrenergic regulation of L-type Ca^{2+} channels in failing human hearts. Fortunately, these changes are at least partially reversible as demonstrated in LVAD patients. The underlying molecular mechanisms of altered channel density and gating as well as the functional impact of these changes on EC coupling are important topics for future research.

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Key Words: heart ventricle ■ myocardium ■ congestive heart failure ■ L-type calcium channels ■ patch-clamp techniques

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Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Circ Res. 2002;91:451-453
doi: 10.1161/01.RES.0000035346.21625.4A
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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