A molecular formula for heart failure and sudden cardiac death. Focus on “Na\textsubscript{v}1.5-dependent persistent Na\textsuperscript{+} influx activates CaMKII in rat ventricular myocytes and N\textsubscript{1325}S mice”

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Calcium homeostasis is the key element in regulation of cardiac myocyte function, as it is the basis for control of excitation-contraction coupling. Under normal conditions, intracellular Ca levels are precisely regulated on a beat-to-beat basis to allow efficient orchestration of both cardiac rate and synchronous contraction of cardiac muscle comprising the atria and ventricle to drive efficient blood flow through the vascular system. However, under pathophysiological conditions, either congenital or acquired with age, alterations in Ca homeostasis in the diseased heart can lead to electrical and mechanical dysfunction, potentially lethal arrhythmias, cardiomyocyte necrosis, and heart failure. These events manifest themselves as serious medical issues for which new and improved therapies are critically needed. In the interesting study by Yao et al. (2) in this issue of American Journal of Physiology-Cell Physiology, the authors clearly outline a series of mechanisms and molecular events (in Fig. 7 of their article) that are coupled via a cycle and that can cause severe cardiac dysfunction, ultimately resulting in lethality. Importantly, by identifying critical control points affecting the pathway illustrated in Fig. 7 of the article, potential therapeutic targets are plainly identified whose successful modulation would offer new drugs to address the serious unmet medical need of protecting against heart failure and sudden cardiac death.

It is known that the activities of the two proteins shown in Fig. 7 are upregulated in myocytes from diseased heart; the magnitude of late sodium current (I\textsubscript{Na\textsubscript{L}}) mediated by the cardiac Na channel, Na\textsubscript{v}1.5, and the activity of the Ca/calmodulin-dependent protein kinase II (CaMKII) are both increased. Furthermore, it has been shown that phosphorylation of Na\textsubscript{v}1.5 by CaMKII enhances I\textsubscript{Na\textsubscript{L}}, and it is also well recognized that increasing intracellular sodium via I\textsubscript{Na\textsubscript{L}} will result in a rise in intracellular Ca that can trigger activation of CaMKII. Thus, the propensity for a vicious, and potentially lethal, cycle exists, but the molecular events underlying the coupling of these two processes have not previously been well characterized. Using a wide variety of experimental approaches and techniques, Yao et al. clearly present a compelling data set that delineates the factors mediating the Na\textsubscript{v}1.5-CaMKII interaction, and, indeed, demonstrate that the two proteins can directly associate under certain conditions, fostering severe pathological consequences.

Initiation of this deleterious series of cardiac events is triggered by Na loading of myocytes via enhanced I\textsubscript{Na\textsubscript{L}}. This can be initiated experimentally by application of chemical Na\textsubscript{v}1.5 agonists that slow channel inactivation [e.g., anemone toxin II (ATX-II), veratridine], or it can occur naturally through phosphorylation of the channel, by gain-of-function channel mutations that increase persistent Na current, or by reactive oxygen or nitrogen species and other metabolites that are formed during cardiac ischemia that stimulate I\textsubscript{Na\textsubscript{L}}. The elevated intracellular Na, in turn, drives an increase in intracellular Ca through reverse operation of the electrogenic, sodium-calcium exchange transporter (which normally functions to reduce intracellular Ca driven by the usual inwardly directed Na gradient). In addition to having effects on Ca handling, increases in persistent I\textsubscript{Na\textsubscript{L}} will prolong the cardiac action potential and increase the QT interval, which could lead to ventricular tachycardia and fibrillation, and ultimately to a lethal arrhythmia and sudden death.

Once an elevation in intracellular Ca occurs, the serine/threonine CaMKII is activated and begins to phosphorylate a number of substrates, such as phospholamban, the sarcoplasmic reticulum (SR) Ca-ATPase, the ryanodine receptor, and the L-type Ca channel, Ca\textsubscript{v}1.2, as well as autophosphorylation of the enzyme itself, which permits sustained kinase activity in the absence of Ca and calmodulin. Hyperphosphorylation of these targets disrupts normal SR Ca handling, sometimes leading to SR Ca overload, spontaneous SR Ca release, and alterations in both normal patterns of excitation-contraction coupling and electrical waveform propagation over the heart. It is therefore not unexpected that overexpression of the cardiogenic CaMKII \(\delta\)-isoform in the heart produces lethal arrhythmias, cardiac hypertrophy, and heart failure in genetically engineered animal models, while knockout of the enzyme is cardioprotective against structural heart disease. Importantly, one of the other substrates phosphorylated by CaMKII is the cardiac Na channel, which subsequently results in enhancement of I\textsubscript{Na\textsubscript{L}}. Thus it is not surprising that one of the links to the lethal arrhythmias induced by overexpression of CaMKII is via this last series of events. Moreover, Yao et al. now demonstrate that a direct protein-protein interaction occurs between Na\textsubscript{v}1.5 and CaMKII when increases in intracellular Ca levels are sustained, which further facilitates efficient Na\textsubscript{v}1.5 phosphorylation and stimulation of I\textsubscript{Na\textsubscript{L}}.

The formation of the positive feedback loop shown in Fig. 7 that couples Na\textsubscript{v}1.5 and CaMKII activities to yield adverse cardiac effects has been demonstrated by Yao et al. in several ways. First, inhibition of Na\textsubscript{v}1.5 through application of either pharmacological or biological reagents breaks the vicious cycle. As expected, elimination of I\textsubscript{Na\textsubscript{L}} with the Na\textsubscript{v}1 pore blocker tetrodotoxin, or the state-dependent, small-molecule, Na\textsubscript{v}1.X inhibitor ranolazine, which has an affinity for the slow inactivated form of Na\textsubscript{v}1.X channels, abolishes the futile cycle, as shown in both in vitro and in vivo model systems. Suppressing Na\textsubscript{v}1.5 activity with small interfering RNA specifically directed against this channel has similar effects. Secondly, application of a putative inhibitor of the Na/Ca exchange
transporter prevents $I_{\text{NaL}}$, activation of CaMKII, as expected, because the pathway for intracellular Ca elevation in cardiomyocytes is blocked by this probe. Finally, inhibitors of CaMKII diminish the effects caused by enhancing $I_{\text{NaL}}$. Taken together, these results from Yao et al. present a convincing argument for the existence of a synergistic interaction between Na,1.5 and CaMKII that can be highly cardiotoxic in patients with heart disease.

One of the striking features of the present study is that it validates new therapeutic targets as well as reinforces some existing views on how to treat heart disease. For example, demonstrating that the molecular basis for initiation of much cardiac pathophysiology is due to enhancement of the persistent Na current focuses attention on the targeting of a Na,1.5 channel conformation specifically responsible for $I_{\text{NaL}}$. While Na channel blockers have been identified that appear to target the late Na current (e.g., ranolazine), this mechanism might be improved upon by finding drug candidates with a similar mechanism, but which also display Na,1 subtype selectivity. Although traditionally it was thought that small-molecule, state-dependent Na,1 blockers suitable for therapeutic use would not show selectivity among the isoforms of the Na,1 superfamily because of the high sequence homology existing between family members, the recent identification of Na,1.7 and Na,1.8 subtype-selective Na,1 blockers, upon attempts to develop new therapies for treating pain, provide a proof-of-concept that subtype-selective Na,1 channel blockade is feasible. Therefore, it will be of interest to profile clinically a Na,1.5-selective inhibitor that also specifically blocks $I_{\text{NaL}}$. Interestingly, it appears that enhanced $I_{\text{NaL}}$ through either Na,1.7 or Na,1.8 is also associated with the pathophysiology of pain, because the late Na current is involved in mediating robust electrical signaling in the peripheral nervous system (PNS). Perhaps targeting $I_{\text{NaL}}$ in the PNS may thus result in new analgesic drugs, as well.

The Na/Ca exchanger has previously been recognized as a drug target for preventing Ca overload in cardiomyocytes, but attempts to pharmacologically manipulate this mechanism with small-molecule blockers has thus far met with limited success (1). Indeed, Yao et al. correctly note that the Na/Ca exchange inhibitor that they employ has limited selectivity. However, given that there are newly developed high through-put screening technologies available for initiating transporter screens to interrogate large chemical libraries and discover inhibitor lead structures, it might be worth reexamining the feasibility of targeting this protein. Similarly, the Na/Ca exchanger might be a good substrate for identifying monoclonal antibody reagents that block carrier function as a possible biological therapeutic.

Given that the CaMKII family has a wide tissue distribution, it may be difficult to improve the therapeutic index by developing inhibitors against this class of enzymes, although it is an obvious target to treat long QT syndromes, cardiac ischemia, and heart failure. It is helpful that the δ-isooform is the major family member present in heart, but it is not yet certain whether it is possible to achieve kinase specificity and tissue selectivity with this target. Perhaps a parallel approach for drug development on CaMKII might be to identify agents that would interfere with the interaction between the enzyme and Na,1.5 to interrupt Na,1.5 phosphorylation and subsequently the enhancement of $I_{\text{NaL}}$.

At the end of the article by Yao et al., the authors speculate why such a potentially lethal vicious cycle coupling Na,1.5 and CaMKII would evolve in the mammalian heart. The explanation given, that the interaction between the two proteins may play a fundamental role in linking cardiac contractility and heart rate (i.e., the Bowditch-Treppe effect), is highly plausible. Under normal conditions, a positive chronotropic effect causes CaMKII activation, which will enhance $I_{\text{NaL}}$ and increase intracellular Ca levels, thereby producing a positive inotropic effect. However, under pathological conditions, Ca overload can trigger a downward spiral in cardiac function, ultimately having lethal consequences. New therapies preventing this from happening would be a major medical breakthrough.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES