Reduced Transient Outward K⁺ Current and Cardiac Hypertrophy
Causal Relationship or Epiphenomenon?

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Action potentials coordinate contraction and relaxation of the heart. The long plateau phase of the action potential ensures sufficient Ca²⁺ entry into the cytoplasm via L-type Ca²⁺ channels to invoke Ca²⁺-induced Ca²⁺ release from the sarcoplasmic reticulum and activation of the contractile filaments. Action potentials are initiated by opening of voltage-gated Na⁺ channels that in turn rapidly depolarize the membrane and open voltage-gated Ca²⁺ and K⁺ channels. Some K⁺ channels activate immediately in response to depolarization, then inactivate within tens of milliseconds later, and mediate a current called the transient outward current (Iₒ). In many mammalian hearts, including human, Iₒ is responsible for the initial rapid phase of action potential repolarization, discernible as a notch preceding the plateau phase, but has little role in terminal repolarization. In contrast, Iₚ in rodents is a major repolarizing current throughout the comparatively shorter cardiac action potential (Figure, panel A) necessary to maintain extremely high heart rates. Iₚ is composed of at least two components in the mammalian ventricle. One component is characterized by a slow recovery from inactivation (Iₚα), another by a relatively fast recovery from inactivation (Iₚβ). Comparison of native currents and heterologously expressed channels indicates that Iₚβ is mediated by Kv4.2 and/or Kv4.3 channels, whereas Iₚα is mediated by Kv1.4 channels.

Prolongation of ventricular action potentials is a common finding in many cardiac disorders, including pathological hypertrophy, heart failure, myocardial infarction, and long-QT syndromes. Two of these diseases, hypertrophy and heart failure, are associated with a decrease of Iₒ in the human heart and in animal models. Without experimental data to address the question, it might seem reasonable that pathologic hypertrophy and heart failure somehow cause a reduction of Iₒ in mice despite significant QT prolongation and frequent ventricular tachycardia.³

This finding was provocative because earlier studies by Barry et al² reported that cardiac-specific functional knockout of Iₒ in mice did not induce hypertrophy. Several differences between the two studies might explain why reduction of Iₒ in one model but not the other resulted in heart failure. In the earlier study, Barry et al² overexpressed a Kv4.2 channel subunit that had a single missense mutation (W362F) located in the pore region. In a heterologous expression system, these mutant subunits can fold, coassemble into tetramers, and insert into the plasma membrane normally, but the channels are nonconducting. However, when coexpressed with wild-type Kv4.2 channel subunits, the mutant subunits have a dominant-negative effect, meaning they coassemble with wild-type subunits to form nonfunctional tetrameric channels. In contrast, Wickenden et al¹ functionally knocked out Iₒ by overexpressing an N-terminal fragment of Kv4.2 channel subunits. This partial channel subunit would, of course, have no channel function on its own but could coassemble with wild-type Kv4.2 subunits and have a dominant-negative effect. It is possible that overexpression of the N-terminal fragment of Kv4.2 has toxic effects leading to heart failure.

Barry et al² also noted that knockout of Iₒ was associated with an increase in Kv1.4 channel expression, perhaps as a compensatory response. However, upregulation of Kv1.4 channel expression was not responsible for the lack of hypertrophy or failure in mice overexpressing Kv4.2W362F because crossbreeding of Kv1.4⁻/⁻ and Kv4.2W362F transgenic mice produced offspring that had structurally normal hearts despite significant QT prolongation and frequent ventricular tachycardia.³

KChIP2 (Kv channel interacting protein 2) is an auxiliary K⁺ channel subunit that binds to the N-terminal of Kv4 channel subunits in the heart and increases the number of channels in the plasma membrane and the rate of Kv4 channel recovery from inactivation.⁴ Regional differences in the expression of KChIP2 are responsible for the transmural gradient in Iₒ density in the canine heart.⁵ Recently, Kuo et al⁶ found that knockout of KChIP in mice nearly eliminated Iₒ and increased their susceptibility to arrhythmia but did not cause any changes in heart volume, ventricular wall thickness, or contractility.

The KChIP study and most of the Iₒ knockout mice studies demonstrate that a decrease in Iₒ does not necessarily result in cellular hypertrophy or heart failure. Conversely, and contrary to most reports, hypertrophy is also not always associ-
Reduction of \( h_{\text{lof}} \) prolongs APD and may induce hypertrophy via a calcineurin-dependent signaling pathway. A. At physiologically relevant heart rates, \( h_{\text{lof}} \) is the major repolarizing current of the ventricular action potential in rodents. Pharmacological block of \( h_{\text{lof}} \) or functional knockout of Kv4.2 channel subunits lengthens the plateau phase of the action potential. B. Reduction of \( h_{\text{lof}} \) (Kv4.2 channels) but not \( I_{\text{tos}} \) (Kv1.4 channels) prolongs action potentials and increases Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels. Calcium activates calcineurin phosphatase activity in the cytosol. Calcineurin dephosphorylates NFAT, allowing it to translocate into the nucleus, where, together with other factors such as GATA4, it activates transcription of hypertrophic response (HR) genes.

The mechanism by which calcineurin mediates cardiac hypertrophy in mice was first demonstrated in 1999. \(^1\) NFAT3 (nuclear factors in activated T cells) is normally heavily phosphorylated when in the cytoplasm. When NFAT3 is dephosphorylated by calcineurin, it translocates into the nucleus where it can interact with GATA4 to activate transcription of hypertrophic response genes. The importance of the calcineurin pathway in pressure-overload hypertrophy has also been demonstrated using transgenic mice overexpressing a dominant-negative mutant of calcineurin. \(^10\) It is important to note that other signaling pathways such as the protein kinase C pathway or the mitogen-activated protein kinase (MAPK) cascade have also been shown to mediate the hypertrophic response in the heart. \(^11,12\)

Taken together, the majority of published evidence suggests that activation of the calcineurin signaling pathway, but not a reduction in \( I_{\text{lof}} \) density, is causally related to cardiac hypertrophy. Just when it seemed the matter was settled, Kassiri et al.\(^{13}\) report in this issue of Circulation Research that reduction of \( I_{\text{lof}} \) in cultured neonatal rat ventricular myocytes causes hypertrophy via a calcineurin-dependent pathway. Two approaches were used. First, \( I_{\text{lof}} \) was blocked by hetero-podatoxin, a spider toxin that specifically blocks Kv4.2 channels without any effect on Kv1.4 channels. \(^{14}\) Treatment with toxin for 30 to 70 hours of spontaneously contracting cultured myocytes reduced \( I_{\text{lof}} \) by \( \approx 50\% \), increased cell capacitance (an electrical measure of cell size) by 30\%, and increased protein synthesis (measured by \(^{3}H\)-leucine incorporation) by 23\%. For unknown reasons, reduction of \( I_{\text{lof}} \) by the spider toxin also reduced the expression of Kv4.2 channels. The authors speculated that reduction of \( I_{\text{lof}} \) caused more than a doubling of APD\(_{\text{lof}}\), a 47\% increase in cell capacitance, and a 38\% increase in \(^{3}H\)-leucine incorporation, changes that were suppressed by simultaneous overexpression of wild-type Kv4.2 channel subunits. In contrast, elimination of Kv1.4 channel current by overexpression of a dominant-negative transgene did not alter these measures of cell size or growth. This was an expected finding given the slow rate of \( I_{\text{lof}} \) recovery from inactivation.

Overexpression of the dominant-negative Kv4.2 subunits also caused about a 70\% increase in NFAT activity and a similar increase in calcineurin phosphatase activity. \(^15\) Coinfection with a specific noncompetitive inhibitor of calcineurin prevented the increase in cell capacitance and \(^{3}H\)-leucine uptake but did not prevent the decrease in \( I_{\text{lof}} \). The authors concluded that prolongation of APD caused by suppression of \( I_{\text{lof}} \) increases [Ca\(^{2+}\)]\(_i\), which activates calcineurin and subsequent transcription of hypertrophic response genes (Figure, panel B). In support of this hypothesis, prevention of spontaneous contractions and the associated increase in [Ca\(^{2+}\)]\(_i\), by treatment of cells with the L-type Ca\(^{2+}\) channel blocker verapamil or depolarization of cells with high [K\(^{-}\)], eliminated calcineurin activation and the measures of cell growth but did not prevent the reduction of \( I_{\text{lof}} \) or prolongation of APD in electrically paced myocytes.

There is clearly a need to understand why some rodent models show an association between prolonged action potentials and hypertrophy or heart failure and others do not. Equally important is the question of whether studies in rodents provide significant insight into human disorders of cardiac repolarization. For example, despite the presence of markedly prolonged action potentials in human long-QT syndrome, there is no evidence of hypertrophy. \(^15\)

References


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