Effects of Heart Disease on Cardiac Ion Current Density Versus Current Amplitude

Important Conceptual Subtleties in the Language of Arrhythmogenic Ion Channel Remodeling

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It is well established that heart disease can profoundly change cardiac action potentials. Action potential abnormalities are caused by derangements in cardiac ion channel expression and function, often called “ion channel remodeling,” that can cause serious, sometimes lethal, arrhythmias. The literature regarding arrhythmogenic ion channel remodeling is vast and complicated. A PubMed search with the single key word phrase “cardiac channel remodeling” returned 50 publications in 2007 alone, indicating a high level of research activity.

A variety of cardiac disease processes, including myocardial infarction, valvular heart disease, various cardiomyopathies, arrhythmias, and hypertensive heart disease, can cause ion channel remodeling. Many of these cause cardiac hypertrophy, defined as an increase in myocardial cell mass. Because cardiomyocyte number is relatively fixed in adult life, hypertrophy is typified by an increase in cardiomyocyte size, allowing for increased heart mass with the same number of cells. Cell dimension measurements are the most direct means to characterize cardiomyocyte hypertrophy.

In electrophysiological studies, cellular hypertrophy is often assessed by determining cell capacitance. The lipid bilayer (electrically resistive) cell membrane acts as a capacitor separating the electrically conducting intracellular solution from the conductive extracellular solution. Electric current passes across cardiac cell membranes to charge their capacitance, even when no current traverses ion channels. Capacitance is a function of intrinsic capacitive properties (indicated by the “dielectric constant”), the capacitive (in this case, cell membrane) surface area, and the thickness of the capacitor. The thickness and intrinsic capacitive properties of cell membranes are fairly constant, so the dominant factor determining cell capacitance is the total membrane surface area. Cell size increases with cardiac hypertrophy. Augmented cell size is accompanied by increased cell surface area, therefore inevitably increasing cell capacitance.

Ion Channel Remodeling and Corrections for Hypertrophy-Related “Artifacts”

Because hypertrophy increases cell size, increased whole-cell current amplitude is expected: larger cells should have larger total currents. To know whether ion channel function has been changed by the underlying disease process, it is necessary to correct for the effects of cell enlargement per se. The most straightforward way to correct for cell size is to normalize whole-cell current by cell capacitance, providing the density of ionic current relative to membrane surface area (“current density”). Current density measurements have been used for around 20 years as the primary index of disease-induced changes in ionic current function.

Gene Expression Changes and Internal Standards

A corresponding consideration at the molecular level relates to the use of internal standards for the analysis of disease-induced changes in gene expression. Reference genes are usually used as internal standards. It is important to know whether the expression of reference genes is altered in studies of cardiac disease models: if reference gene expression changes, genes whose absolute expression is unaltered may appear to be modified because of variations in the reference gene to which they are normalized. Significant effort is made to be sure that reference gene expression is unaltered in studies of disease effects on the cardiac genome.

The Complexity of Ionic Current Remodeling

Ionic current remodeling is a complex process. In addition to disease-specific changes, alterations may vary with species, myocardial region, and specific channel component. For example, in some disease paradigms, slow delayed-rectifier current (I_{Kr}) may be downregulated with or without concomitant rapid delayed-rectifier current (I_{Kd}) changes. L-type Ca^{2+} current can be downregulated, whereas T-type current is unaltered or even increased, and 1 inward-rectifier K^{+} current component (agonist-induced I_{KAC}) may be downregulated with another (I_{K1}) increased. The molecular basis for these complex patterns of ion channel response is poorly understood, and an improved appreciation of underlying mechanisms is essential for the development of innovative rationally based therapeutic approaches.
New Insights Into the Complex Molecular Basis of K⁺ Current Remodeling in Cardiac Hypertrophy

In this issue of *Circulation Research*, Marionneau et al. report the results of a detailed and elegant study addressing K⁺ current remodeling in mice with cardiac hypertrophy caused by transverse aortic constriction (TAC). They examine TAC-induced changes in a variety of K⁺ currents, including *I*ₖᵥ₄.₃, *I*ₖ, *I*ₖₙ, *I*ₖₛ, and *I*ₖ₁, at the functional and molecular level. They note regionally determined differences in cardiac hypertrophy (none in the right ventricle, more marked in left ventricular endocardium than epicardium) and ion channel changes. As in previous studies, Marionneau et al observe decreased left ventricular current density for a wide range of K⁺ currents; in fact, all of the currents they studied. However, Marionneau et al. make an interesting and novel observation that has not previously been described. Examination of total current amplitudes, rather than current densities, reveals an interesting picture: 2 currents (*I*ₖᵥ₄.₃ and *I*ₖ₁) showed unchanged amplitude, 1 current (*I*ₖₙ) had decreased amplitude and another current (*I*ₖₛ) was increased. Transcript expression was adjusted for hypertrophic changes by multiplying real-time PCR results by left ventricular mass/body weight ratios. For Western blot analyses, proteins were loaded in amounts proportional to left ventricular mass/body weight ratio. With these adjustments, *I*ₖᵥ₄.₃ subunits (Kv4.3, Kv4.2, and KChIP2) and *I*ₖ₁ subunits (Kir2.1 and Kir2.2) were unchanged by TAC, consistent with unchanged ion current amplitude. However, in contrast to ion current data for *I*ₖₙ, which showed decreased current amplitude, hypertrophy-adjusted Kv1.5 and Kv2.1 expression was increased (a result that remains unexplained).

The authors suggest that 2 distinct processes are involved in ion current changes: cellular (cardiomyocyte enlargement) and molecular (alterations in ion channel subunit quantity). The Figure is a schematic intended to clarify this notion. Cardiomyocytes are depicted with gray fill and ion channels by small cylinders in the cell membrane. Nonhypertrophied conditions are shown in the top left area of the figure; hypertrophied conditions are depicted in the lower right stippled area. Total current amplitude is determined by the total number of membrane ion channels per cell, whereas current density is determined by the number of channels per unit of membrane (roughly indicated in the Figure by the length of membrane between channels). A normal cardiomyocyte is depicted in the Figure (A). Ion channel downregulation can occur in the absence of hypertrophy, decreasing both current amplitude and density (Figure, B). A cardiomyocyte that is hypertrophied, but with unaltered overall channel expression per se, is shown in the Figure (C). The total number of channels is not altered; therefore, current amplitude is unchanged. However, because of increased membrane surface area, channels are more widely separated in the membrane and current density decreases. D in the Figure shows a cell with both hypertrophy and decreased ion channel expression, causing both decreased current amplitude and greatly decreased current density.
Which Reflects “Reality”: Changes in Ion Current Density or Changes in Ion Current Amplitude?

Most previous investigations of ion channel remodeling have used current density changes as the primary index of remodeling, but the study by Marionneau et al emphasizes the importance of ion current amplitude. Which is a better reflection of reality, and which index should be used for future studies of ion current remodeling? The answer is that both indices are important: they provide different types of information and need to be used appropriately depending on the question(s) being asked.

The ion current density reflects the amount of current that passes across a given area of membrane, i.e., the rate of ion flow acting on local transmembrane potentials, and is therefore the most relevant index to understanding changes in cellular electrophysiology. This concept is nicely reflected by the results of the Marionneau et al study: current amplitude increased for 1 current ($I_a$), decreased for another ($I_{K,slow}$), and remained unchanged for 2 others ($I_{Na}$ and $I_K$), suggesting little net $K^+$ current amplitude change. However, current density was reduced for all 4 $K^+$ currents studied, and repolarization indices (both QT interval and action potential duration) were significantly prolonged by ≈40%.

On the other hand, consideration of current amplitude is important for understanding the molecular mechanisms underlying disease-induced alterations in ionic current function. As illustrated in the Figure and pointed out by Marionneau et al, decreased ion current density does not necessarily reflect an absolute reduction in the number of ion channel subunits produced by the cell and trafficked to the membrane. Decreased current density may result even when ion channel subunit production and trafficking are unaffected by hypertrophy, because the presence of a normal number of channels in a hypertrophied cell leads to decreased numbers of channels per unit of membrane area (Figure, C).

Important Conceptual Consequences of the Findings by Marionneau et al

Like all important studies, the work of Marionneau et al should change how we do research, leading us to think differently and ask more insightful questions. The key concept that emerges from the study by Marionneau et al is that remodeling (functional and gene expression) changes need to be understood relative to any global hypertrophic response. A gene need not be downregulated to produce transcriptionally determined reductions in the function of its product: it suffices for its product to be in relatively reduced abundance in the enlarged cardiomyocyte. Reduced ion channel density can thus result from a failure of ion channel subunit transcription to be unregulated to the same extent as the transcription of proteins that determine cell size and membrane surface area. Theoretically, an ion channel subunit gene could even be transcriptionally upregulated, but if its upregulation is less than the overall hypertrophic response the membrane surface density of ion channels could still be reduced. Therefore, in trying to understand the molecular regulatory mechanisms responsible for changes in ion current density produced by arrhythmogenic remodeling, we must now consider alterations in total current amplitude as a reflection of total cellular ion channel expression and correspondingly the change in ion channel subunit mRNA and protein expression relative to that of other genes reflecting the hypertrophic response. This important nuance must be considered as investigators study the alterations determining ion channel expression in a wide range of clinically important disease-associated cardiac arrhythmia paradigms.

Acknowledgments

The author thanks France Theriault for secretarial assistance with the manuscript.

Sources of Funding

Funded by an operating grant from the Canadian Institutes of Health Research (MOP 68929) and by the European-North American Atrial Fibrillation Research Alliance (ENAFRA) network award from Fondation Leducq.

Disclosures

None.

References


Key Words: arrhythmia mechanisms transcription genome arrhythmias
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Circ Res. 2008;102:1298-1300
doi: 10.1161/CIRCRESAHA.108.178087
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
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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