Cardiac Cytoskeleton and Arrhythmia
An Unexpected Role for Protein 4.1R in Cardiac Excitability

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The cellular activity of both excitable and nonexcitable cells depends on the coordinate activities of membrane proteins in metazoan cells. Underlying these integral membrane proteins is an elaborate cytoskeletal network that defines cell shape and local membrane architecture and provides strength and stability for cell-to-cell and cell/matrix interactions. Moreover, the metazoan cytoskeleton serves a critical role in the organization, maintenance, and function of specialized membrane domains.1

In the cardiovascular system, the essential role of the submembrane cytoskeleton for membrane protein and cellular function is clearly illustrated by dysfunction in cytoskeletal elements in human disease. In erythrocytes, ankyrin-R and β-spectrin directly link membrane proteins to the actin cytoskeleton.2 Human loss-of-function mutations in either ANK1 (encodes ankyrin-R) or SPTN1 (encodes β-spectrin) result in loss of membrane/cytoskeletal coupling, membrane protein instability, and spheroctosis/anemia.3,4 In heart, dysfunction in cytoskeletal proteins including ankyrin-B, ankyrin-G, α-syntrophin, and emerin are linked with potentially fatal human arrhythmias caused by loss of proper coupling between plasma or nuclear membrane proteins and the cytoskeletal network.5–7 In this issue of Circulation Research, Stagg et al extend this paradigm by demonstrating an unexpected role for the cytoskeletal protein 4.1R (red cells) in cardiac excitability.8 Specifically, Stagg et al demonstrate that mice lacking protein 4.1R display multiple cardiac phenotypes (bradycardia, prolonged QTc) and aberrant cardiac myocyte electrical activity.8

Two decades ago, a role for protein 4.1 in the regulation of excitable myocyte function would have seemed unlikely. Similar to spectrin and ankyrin,2 protein 4.1R was initially identified as a critical structural component of the erythrocyte spectrin- and actin-based membrane cytoskeleton.9 Specifically, F-actin and 4.1R interact with heterotetramers of spectrin at network junctions2 to coordinate membrane architecture and mechanical stability.10 In addition, protein 4.1R forms a ternary complex with glycoporphin-C and p55.11

Similar to ankyrin- and spectrin-based human erythrocyte disease phenotypes, human gene mutations in 4.1R have been linked with hereditary elliptocytosis, a condition in which red blood cells lose their characteristic discoid shape and are more prone to shearing in the peripheral circulation.12,13 In fact, severe hemolytic anemia is found in ~10% of patients with hereditary elliptocytosis.13

In 1999, Conboy and colleagues generated mice lacking 4.1R to further investigate the role of 4.1R in the erythrocyte.14 Consistent with the observed 4.1R-associated human red blood cell phenotypes,15 4.1R−/− mice display mild hemolytic anemia as a result of hereditary elliptocytosis.14 4.1R−/− erythrocytes also display decreased membrane associated glycoporphin-C and p55.14 Beyond the erythrocyte, 4.1R has also been identified in brain, kidney, lung, and heart.16 In fact, 4.1R−/− mice display defects in movement, coordination, balance, and learning associated with loss of 4.1R expression in key central nervous system regions, including cerebellar granule cells and the dentate gyrus of the hippocampus.17

In this issue of Circulation Research, Stagg et al present the first report of a cardiac phenotype associated with the loss of the cytoskeletal protein 4.1R.8 Previous work suggested a potential role for protein 4.1R in cardiac function based on its strategic localization at the myocyte intercalated disc, Z-line, and peripheral sarcolemma.18 In this study, Stagg et al report that 4.1R−/− mice display reduced heart rates and prolonged QT, intervals. Isolated 4.1R−/− ventricular cardiomyocytes display prolonged action potentials, aberrant Ca2+ transients (30% increase in amplitude, 16% delay in decay time), increased sarcoplasmic reticulum Ca2+ stores (increased 28%), and increased spark frequency. Electrophysiological analysis of 4.1R−/− cardiomyocytes reveals dysfunction in specific myocyte currents including Na/Ca exchanger (NCX) current, transient-outward K+ current (Ito), and persistent sodium channel current (Ips). Specifically, the authors observed a significant decrease in NCX current density, a striking increase in Ito, and faster kinetics of Ito, inactivation in protein 4.1R−/− myocytes. On the contrary, there was no effect on I Ca, Na/K ATPase, and plasma membrane Ca2+ ATPase (PMCA) currents. Finally, reduction of protein 4.1R expression in mice affects the myocyte expression of Na1.5 (cardiac Na channel, decreased 40%) and protein 4.1G (increased 1.9-fold); however, their membrane localizations by immunofluorescence were unaffected. The authors observed no changes in PMCA2, NCX1, spectrin, α-actinin, and tropomyosin expression and/or immunolocalization. 4.1R−/− hearts revealed no defects in the structural organization (sarcomere organization, transverse-
tubule, sarcoplasmic reticulum, intercalated disc, and mitochondrial membrane structures). Together, these data provide an initial glimpse into the importance of protein 4.1R expression for normal cardiac excitable function.

Although the findings of Stagg et al provide exciting new insight into the role of protein 4.1R in cardiac excitability, the cellular role(s) for 4.1R in the myocyte are not completely clear. Based on its role in the erythrocyte, 4.1R could serve as a protein scaffold to link membrane ion channels and transporters to the spectrin/actin-based cytoskeleton. In support of this hypothesis, NCX current and Na,1.5 expression are reduced in 4.1R−/− myocytes. However, NCX1 expression and localization are unaffected in 4.1R−/− myocytes, and 4.1R−/− myocytes actually display increased I_{Na}. Thus, whereas the increased QT, intervals and prolonged action potentials of 4.1R−/− animals may be explained by the observed increase in persistent sodium current, faster rate of I_{Na} inactivation, and decreased I_{Na,CEN} density in 4.1R−/− cardiomyocytes, the molecular role for protein 4.1R clearly entails more than simply targeting or scaffolding functions. More likely, protein 4.1R plays multiple roles in organizing myocyte membrane signaling pathways by coordinating membrane domain formation, membrane protein targeting, and regulation of local signaling pathways. Consistent with this potential multifunctional role for cytoskeletal protein 4.1R, our group has documented the role of cytoskeletal-associated ankryins in the proper targeting, stabilization, and biophysical regulation of key proteins involved in excitation–contraction coupling. For example, ankyrin-G binding is required for both the targeting and biophysical regulation of cardiac Na,1.5, and dysfunction in the ankyrin-G-based pathway for Na,1.5 regulation is associated with Brugada syndrome arrhythmias. Likewise, ankyrin-B directly interacts with a protein complex consisting of β-spectrin, NCX1, Na/K ATPase, inositol(1,4,5)trisphosphate receptor, and the signaling molecule protein phosphatase 2A in heart. The proper targeting and stabilization of this complex are required for normal Ca2+ dynamics in ventricular cardiomyocytes. Ankynin-B gene missense mutations in humans or reduced ankynin-B expression in mice that affect these interactions have been implicated in potentially fatal arrythmias in humans and mice. Finally, recent findings from Bennett and colleagues reveal the importance of ankynin-, spectrin-, and adducin-based pathways for membrane biogenesis and maintenance in other complex vertebrate cell types. Together, these findings suggest that 4.1R may play unexpected roles in the regulation of cardiomyocyte membrane excitability and present a number of interesting questions. For example, is the loss of NCX current the result of disrupting a direct interaction between 4.1R and NCX1? Moreover, does 4.1R directly interact with Na,1.5? What are the roles of other 4.1 genes in heart? What are the implica-
tions of protein 4.1R loss for dysfunction in cardiac excitability and arrhythmias? No sustained arrhythmias were observed in 4.1R-deficient mice. However, based on I_{Na} and Ca2+ overload phenotypes observed in 4.1R−/− myocytes, it is likely that these cells will be susceptible to afterdepolarizations in response to adrenergic agonists. Similarly, 4.1R−/− mice may be susceptible to polymorphic ventricular arrhyth-
mia in response to catecholamines. Moreover, based on the observed bradycardia in 4.1R−/− mice, future experiments should also focus on the role of 4.1R in sinoatrial node myocytes. In summary, the new findings of Stagg et al provide further evidence to support an emerging theme that cytoskeletal proteins play important roles not only for establishing membrane architecture and integrity, but also for regulating the membrane protein constituents that influence the electrical properties of excitable cells.

Sources of Funding

P.J.M. is supported by NIH grants R01HL084583 and R01HL083422 and the Pew Scholars Trust (P.J.M.).

Disclosures

None.

References


**Key Words:** protein 4.1R arrhythmia cytoskeleton spectrin Na/Ca exchanger
Cardiac Cytoskeleton and Arrhythmia: An Unexpected Role for Protein 4.1R in Cardiac Excitability
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Circ Res. 2008;103:779-781
doi: 10.1161/CIRCRESAHA.108.186460

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

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