Editorials
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A New Role for Calmodulin in Ion Channel Biology

Dan M. Roden

Delayed Rectifier Currents in Heart

A time-dependent repolarizing current carried by outward movement of potassium ions was first identified by Hodgkin and Huxley, and has been recognized in heart since the 1960s.1,2 Experiments in the 1980s found that the amplitude of the current, then termed $I_{Ks}$, increased when intracellular calcium was increased.3 The key advance to further understanding of the physiology of this important repolarizing current was the recognition in 1990 that it includes at least two distinct components, now termed $I_{Kc}$ and $I_{Kr}$.4 These two currents have very different voltage-dependent gating behaviors and sensitivities to drugs and to activation of second messenger pathways. The amplitude and gating of $I_{Kc}$ is quite labile in experimental preparations and increases dramatically in response to activation of PKA.5–7 Such $I_{Kc}$ variability has been invoked as a mechanism underlying variability in the extent to which $I_{Kc}$ blockers prolong QT interval, a common problem in clinical medicine and drug development.7–10 At its simplest level, patients with robust $I_{Kc}$ display minimal QT prolongation with $I_{Kr}$ block, whereas those with $I_{Kc}$ reduction may display little QT prolongation at baseline but striking QT prolongation when $I_{Kr}$, the major mechanism supporting normal repolarization in this setting, is blocked by drugs.

Molecular genetic studies in the mid 1990s led to identification of the genes whose expression underlies these currents: $I_{Kc}$ is generated by expression of KCNH2 (initially termed HERG), and $I_{Kr}$ is generated by the coexpression of a pore forming subunit, KCNQ1 (formerly termed KvLQT1), with important function modifying ancillary subunit KCNE1 (or minK).11 Mutations in KCNQ1 are the most common cause of long QT syndrome (LQT1). When mutations in KCNQ1, KCNE1, and other potassium channel genes were identified in the long QT syndrome, an initial assumption was that these would disrupt gating to cause loss of potassium current and thereby QT prolongation. One of the most interesting stories in this field over the last ten years has been the increasingly rich detail of molecular mechanisms responsible for normal channel function that have flowed from studies of mutant channels identified in individual affected patients. These studies have not only advanced our understanding of mechanisms in relatively uncommon diseases such as the long QT syndrome, but also have important implications for an evolving understanding of integrated molecular and cellular electrophysiology. Two articles in the current issue of Circulation Research focus on the question of why calcium alters $I_{Kc}$ amplitude and come to the same, rather unexpected and surprising conclusion.12,13

Why Study Calmodulin Binding to KCNQ1

The idea that KCNQ1 and KCNE1 must coexpress to generate $I_{Kc}$ was one of the earliest examples of what is now evolving into a common theme in ion channel biology: that no channel exists as a single pore-forming subunit, but rather channels exist as multimolecular complexes that include pore-forming subunits, function modifying proteins, anchoring proteins, and other subunits whose function is only now being appreciated. One very interesting molecule that is now increasingly well recognized to bind to ion channels is the calcium ligand calmodulin. Distinct calmodulin binding sites on L-type calcium channels,14,15 sodium channels,16,17 and other members of the KCNQ superfAMILY18,19 have recently been described and are thought to mediate calcium-sensitive gating processes of the encoded currents. Indeed, mutations affecting KCNQ2-calmodulin binding have been described in familial epilepsy.20 Potential calmodulin binding motifs are found in the KCNQ1 C terminus: accordingly, both the Pongs-Attali group12 and the Pitt group13 postulated that calmodulin would bind to the KCNQ1 protein and modulate current amplitude (perhaps even calcium sensitivity). Both groups further hypothesized that LQT1 mutations in or near calmodulin binding sites would disrupt the interaction and thereby alter function.

The C terminus of KCNQ1 is a large structure, encompassing about half of the molecular weight of the protein. The Pongs-Attali group’s in silico predictions suggested that the C terminus includes four α-helices, and two of these are highly homologous to those previously identified as calmodulin-binding sites in other members of the KCNQ1 family. Accordingly, they focused their attention on mutations in or near these two “proximal” α-helices. By contrast, the Pitt group focused on two calmodulin consensus binding sites, termed IQ domains, located in the C terminus. One of these is within the region studied by the first group, and the other is much more distal. Using somewhat different techniques, both groups demonstrated that calmodulin binds to C-terminal peptide fragments. One group identified a calmodulin binding region near the proximal 2 α-helices, whereas the Pitt group suggested that both the proximal and distal IQ domains were required. The surprising result from both groups was that C-terminal peptide fragments expressed alone were insoluble, whereas coexpression with calmodulin rendered the complexes soluble. This result, in turn, strongly

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supports the idea that an interaction between calmodulin and this region of this channel protein produces a change in conformation that allows the protein to be soluble. A particularly intriguing result was that a calmodulin mutant that is deficient in calcium-binding activity nevertheless restored solubility. A next step for both groups was to study DNA variants located in or near the calmodulin binding sites. Indeed, mutations that disrupted the interaction of calmodulin with the channel protein resulted in striking reductions in current amplitude and this seemed attributable to reduced cell surface expression.

Another obvious question is whether calmodulin binding to the channel protein mediates the long-recognized calcium-sensitivity of the current. This turned out to be a more difficult problem to approach experimentally. Probably the most direct evidence was the finding that calcium buffering in Xenopus oocytes reduced current, whereas direct injection of increasing concentrations of calcium led to increased .

Therefore, both groups come to several common conclusions. First, calmodulin does indeed bind to the C terminus of KCNQ1, not a big surprise given the precedent with many other ion channels to date. The second is the not terribly unexpected result, again given precedents with other channels, that calmodulin binding to KCNQ1 seems important for calcium sensitivity of the channel, although the details are yet to be worked out. The final conclusion, however, seems unexpected: that calmodulin binding to KCNQ1 is required for delivery of the channel protein to the cell surface, and that this chaperone effect is not dependent on calcium binding to calmodulin.

The articles differ in their approaches and in some of the details of some of their results. One detail that our group has also studied is the extent to which the distal C terminus can be considered a tetramerization domain. When C-terminal fragment proteins are expressed, only those that include the distal helix coassemble as tetramers. By contrast, we and one of the other groups reported here find that full length channels can assemble even in the absence of this distal α-helix. It seems likely that full length channels have multiple regions that can serve to bind subunits to each other.

**Misprocessing Is a Common Mechanism for Loss of Membrane Protein Function**

The interaction between calmodulin and KCNQ1 that enables the channel protein to be assembled correctly, folded correctly, and delivered to the cell surface, further reinforces the concept that ion channels are multimolecular complexes. An emerging idea is that misfolding is a characteristic of mutant ion channel proteins and that such misfolded proteins are often not delivered to the cell surface, resulting in loss of phenotypes. This is the case not only with mutations in KCNQ1, but also in other forms of the long QT syndrome, in the Brugada syndrome attributable to loss of sodium channel expression, and other diseases of membrane proteins, such as cystic fibrosis or nephrogenic diabetes insipidus. A recent survey of the mechanisms whereby mutations in KCNH2 generate LQT2 revealed that misprocessing was, in fact, probably the most common disease mechanism.

It has been 10 years since KCNQ1 was cloned. In that time, important features of its physiology and pathophysiology have emerged, and the current articles add a new and somewhat unexpected twist to this by demonstrating that calmodulin binding to the channel is required for channel processing to the cell surface, and that this is not calcium-dependent. This is a beautiful example of how studying mechanisms in individual channel mutations is yielding a surprising richness of detail in channel physiology. Although this approach could eventually lead to mutation-specific therapies, only very few mutations have been subjected to the sort of detailed and sophisticated analysis reported here. One vision in this young field is that in vitro information may eventually prove useful in patient management, such as stratifying prognosis or therapy by mutation severity or mechanism. Thus, the present work also serves to emphasize the emerging problem that while the clinical community accumulates larger and larger numbers of variants in ion channels and other genes, the extent to which these are individually characterized, and the characteristics actually made available to the larger clinical and basic communities, is lagging behind. This is not a problem that is unique to the world of ion channel biology, but one which genome science will have to face as the personalized medicine revolution evolves.

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**References**


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