Calcium channel regulation by RGK proteins

Robyn Flynn and Gerald Zamponi*
Department of Physiology and Pharmacology; Hotchkiss Brain Institute; University of Calgary; Calgary, AB Canada

Key words: RGK protein, calcium channel, β subunit, Rem, Rem2, Rad, Gem

The RGK family of proteins, small GTPases of the Ras superfamily, are known to regulate calcium currents. It is commonly thought that this is due to an interaction with the Ca₃β subunit, however, the mechanism of this inhibition is unclear. There have been conflicting reports of whether RGK proteins can affect channel trafficking or whether they reduce calcium currents by interacting with channels at the membrane. In the last year, several studies have emerged which explore the intricacies of RGK protein interaction with the channel itself and the importance of the Ca₃β subunit for this interaction, in addition to providing some tantalizing suggestions for the mechanism by which RGK proteins reduce or eliminate calcium currents. In this review, we present an overview of these recent advances and suggest a model that may synthesize these latest works.

Introduction

In the last ten years, small GTPases of the RGK protein family have emerged as potent regulators of HVA calcium channels. The RGK family (Rad, Gem, Kir) consists of four related proteins within the Ras GTPase superfamily: Rad, Gem/Kir, Rem and Rem2. While structurally related, they are subject to differential tissue expression and transcriptional regulation. Rad is expressed in muscle and is upregulated in response to insulin, as well as in diabetic muscle. Gem is found in immune cells, endothelial cells, and some neuronal cells, and is upregulated upon an immune challenge, e.g., with mitogens or cytokines, or following experimental conditions used. Less work has been done on regulation of Rem and Rem2. They are known to be expressed in heart muscle (Rem) and kidney, brain and endothelial cells (Rem2) and, similarly to other family members, appear to show transcriptional regulation.

Because RGK proteins have differential tissue expression, each one also exerts differential effects in its own unique milieu. Being expressed in brain, for example Rem2 is important for early synapse development,11 promotes endothelial cell sprouting13 and enhances survival of embryonic stem cells by regulating the p53 and cyclin DI pathways.16 In addition to each protein’s unique effects, there are functions that all RGK proteins have in common: alterations in cytoskeletal architecture, possibly by interaction with the Rho-kinase pathway and, pertinent to this article, a reduction in calcium currents (reviewed in refs. 14 and 15). All RGK proteins have been shown to bind directly to the Ca₃β subunit,10,17,19 an interaction which has been assumed to underlie the effect of RGK proteins on calcium currents. Recent studies, however, have challenged this assumption. Here, we provide an overview of the latest state of knowledge pertaining to regulation of voltage-gated calcium channels by RGK proteins.

Structural Features and Protein Interactions of RGK Proteins

RGK proteins are comprised of a central Ras-homology domain flanked by long unique N- and C-termini (Fig. 1A and B). Homology between the family members varies from 7% in the N-terminal extension to 40% in the C-terminal extension to 52% in the central Ras domain.15 Typical Ras GTPase protein domains contain five conserved GTP-binding motifs (G1-G5), which can exist in two conformational states, an active GTP-binding and an inactive GDP-binding state. Critical substitutions in these five motifs of RGK proteins suggest that, although they bind guanine nucleotides, they may not behave as canonical GTPases (reviewed in ref. 14). Measured rates of GTP hydrolysis of RGK proteins range from zero (Gem20) to low (Rem and Rem2), to almost 30-fold higher than H-Ras (Gem21). Thus there is little consensus in the field as to whether or not RGK proteins act as GTPases—a discrepancy that may be due to different experimental conditions used.

RGK proteins bind calmodulin (CaM) in a calcium dependent manner via a conserved binding site in their C-termini.22,25 A single point mutation Leu–Gly reduces or abolishes this binding.18,22,24,26 This mutation seems to increase the localization of most RGK proteins in the cell nucleus, although overexpression of the molecular chaperone protein 14-3-3 appears to clear them back to the cytoplasm.24,26,27 Thus, in addition to the Ca₃β subunit, CaM is a common binding partner between RGK proteins and calcium channels.

RGK Proteins Reduce or Eliminate Calcium Currents

All RGK proteins negatively regulate calcium currents, but the mechanism(s) by which this happens are still being elucidated. This phenomenon has been shown using both exogenously expressed and endogenous channels, including L-type channels,10,17,19,25,27-35 N-types,36-38 and P/Q types,39 but not T-types.17,36,40 Since all RGK proteins bind the Ca₃β subunit, it was assumed that this interaction was necessary for ablation of calcium currents, especially in

DOI: 10.4161/chan.4.6.12865
www.landesbioscience.com/journals/channels/article/12865

*Correspondence to: Gerald W. Zamponi; Email: zamponi@ucalgary.ca
Submitted: 09/23/10; Accepted: 09/23/10
Previously published online:
www.landesbioscience.com/journals/channels/article/12865
DOI: 10.4161/chan.4.6.12865
light of the fact that T-type channels, which do not associate with accessory subunits, are unaffected by RGK proteins. Indeed, most reports have demonstrated RGK-Ca\(\alpha\)\(\beta\) interaction to be required for modulation of calcium current density (reviewed in ref. 14). Lately, however, studies have emerged that shed new light on the mechanisms of Ca\(\alpha\) regulation by RGK proteins and suggest that complex molecular dynamics are at work between channels, Ca\(\alpha\)\(\beta\) subunits and RGK proteins.

Two general hypotheses have been proposed to explain the mechanism of the effects of RGK proteins on calcium currents: one, that RGK proteins inhibit channel surface expression; and alternatively, that they inhibit the function of channels already inserted into the membrane.

**RGK Proteins may Exert Multiple Simultaneous Effects on Surface Channels**

The reduction in surface expression hypothesis proposes that an RGK protein disrupts Ca\(\alpha\)\(\alpha_1\)-Ca\(\alpha\)\(\beta\) subunit association, thus retaining newly synthesized channels in the ER and reducing the numbers of functional channels in the cell membrane.\(^{18,26,27,41}\) Using an extracellular epitope to probe for surface expression of calcium channels, these studies showed a dramatic reduction of fluorescent signal when channels and their subunits were coexpressed with RGK proteins. An alternate hypothesis proposes that RGK proteins inhibit channel function in situ, and has been tested using a variety of methods. By labeling N-type channels with a fluorescent variant of \(\omega\)-conotoxin, Chen\(^{36}\) showed no change in channel cell surface expression upon coexpression of Rem2. Similarly, Finlin\(^{10,19}\) found no reduction in membrane expression of channels in the presence of Rem or Rem2 when using surface biotinylation assays.

A recent paper from the Colecraft group\(^{32}\) has demonstrated by flow cytometry a reduction in surface expression of Ca\(\alpha_{1.2}\) by Rem in HEK cells (\(-60\%\)) with a concomitant abolition of calcium currents. Rather than Rem sequestering the Ca\(\alpha\)\(\beta\) subunit, however, this reduction seems to occur by enhancing
the dynamin-dependent endocytosis pathway. Interestingly, restoring Ca\textsubscript{4,1.2} surface expression by coexpressing a dominant negative dynamin mutant was not sufficient to restore Ca\textsubscript{4,1.2} currents, suggesting that Rem has at least one other mode of action to inhibit calcium currents. Additionally, they found that Rem could reduce the open probability of the channel, by immobilizing voltage sensors as well as by another mechanism that leaves voltage sensors free. Lately, another study from this group found that expressing Rem in cardiac myocytes leads to decreased channel open probability.\textsuperscript{35} Thus, the modes of action by which RGK proteins exert their effects on calcium currents may be more complex than previously realized, and may involve a combined effect on cell surface expression levels and alterations in channel function.

**The Role of Ca\textsubscript{4,}\textbeta Subunits in RGK Protein Regulation of Calcium Channels**

RGK protein association with the Ca\textsubscript{4,}\textbeta subunit has been a subject of recent intense investigation (reviewed in ref. 14), since it could potentially present a target for therapeutic regulation of calcium currents. The region of the Ca\textsubscript{4,}\textbeta\textsubscript{2a} subunit required to bind Rem was identified to be a 130 amino acid stretch within the GK domain, a region which also contains the major interaction site between the Ca\textsubscript{4,}\textbeta subunit and the channel, a helix in the I-II linker termed the alpha interaction domain (AID). Truncated GK domains that lost the AID binding retained Rem binding, however, showing there are discrete residues responsible for each. The GK domain is sufficient for RGK-mediated calcium current inhibition in Xenopus oocytes,\textsuperscript{35,42,43} and it can also simultaneously bind both the AID region and Rem, suggesting that competing for the Ca\textsubscript{4,}\textbeta subunit is not a mechanism by which RGK proteins work;\textsuperscript{39} these conclusions also hold for Rem2.\textsuperscript{44} Although one study found that an AID peptide could inhibit the interaction between Gem and Ca\textsubscript{4,}\textbeta,\textsuperscript{45} most other studies support the separation of RGK-Ca\textsubscript{4,}AID binding and Ca\textsubscript{4,}\textbeta-AID interaction.\textsuperscript{30,41,44}

How essential is the RGK-protein-Ca\textsubscript{4,}\textbeta subunit interaction to achieve the functional effect of reducing calcium currents, and what other factors are necessary? Ca\textsubscript{4,}\textbeta\textsubscript{2a} currents from channels expressed in HEK cells in the absence of Ca\textsubscript{4,}\textbeta subunits are reduced 64% by Rem,\textsuperscript{33} but the same channels expressed in Xenopus oocytes in the absence of Ca\textsubscript{4,}\textbeta are minimally affected by Gem or Rem2.\textsuperscript{18,30} A fragment of Ca\textsubscript{4,}\textbeta\textsubscript{2a} that interacts with Rem but not the AID region can partially rescue calcium currents by competition when coexpressed with Rem and Ca\textsubscript{4,}\textbeta,\textsuperscript{19} showing that RGK-\textbeta subunit binding is important for the effect. But Ca\textsubscript{4,}\textbeta subunit binding alone can also not exclusively account for the effect. Indeed, Rem and Rem2 must contain the membrane-targeting sequence in the C-terminus as well as bind the Ca\textsubscript{4,}\textbeta subunit to reduce calcium currents.\textsuperscript{38,44} Thus, the necessity of involving the Ca\textsubscript{4,}\textbeta subunit in RGK modulation of calcium currents is variable, certainly between RGK proteins and possibly between expression systems as well.

**Direct RGK-channel Interaction**

To add further complexity to RGK regulation of calcium channels, Pang et al.\textsuperscript{29} recently reported that Rem (as well as Rad and Rem2) can bind the C-terminus of Ca\textsubscript{4,}\textbeta\textsubscript{2a} channels directly, independently of any interaction with the Ca\textsubscript{4,}\textbeta subunit. To achieve both Ca\textsubscript{4,}\textbeta\textsubscript{2a} C-terminus binding and calcium channel knockdown, Rem had to be targeted to the cell membrane. Calcium-bound calmodulin inhibited this interaction and also partially rescued Rem-mediated calcium current inhibition, but not through Ca\textsubscript{4,}CaM interaction with the Rem C-terminus. This suggests that Rem competes with Ca\textsubscript{4,}CaM for the C-terminus of the channel, perhaps near the IQ motif. In these experiments, the Ca\textsubscript{4,}\textbeta subunit was included in all electrophysiological recordings of channels with or without RGK proteins, and as a result, it is not possible to determine from this study if the RGK-channel C-terminus interaction is sufficient for RGK calcium current knockdown, or if the Ca\textsubscript{4,}\textbeta subunit must be present to achieve it. Thus, this study left open the question: what role, if any, does the Ca\textsubscript{4,}\textbeta subunit play in the role in the channel-RGK protein association?

This question was recently addressed in a new report which examined Gem regulation of Ca\textsubscript{4,}\textbeta\textsubscript{2.1} channels.\textsuperscript{42} After demonstrating a direct biochemical interaction between Gem and the channel, the authors showed that the Ca\textsubscript{4,}\textbeta subunit is required for Gem inhibition of channels in situ. This was done by using inside-out membrane patches from Xenopus oocytes expressing exogenous Ca\textsubscript{4,}\textbeta\textsubscript{2.1} and a \textbeta subunit that could be washed off due to point mutations in the AID-binding region that resulted in a weakened Ca\textsubscript{4,}\textbeta-channel interaction. Interestingly, though, disrupting Gem-Ca\textsubscript{4,}\textbeta interaction with point mutations did not rescue calcium currents, suggesting that although Ca\textsubscript{4,}\textbeta is required to be present, a direct binding between Gem and Ca\textsubscript{4,}\textbeta is not essential for Gem to reduce calcium current. To explain this, the authors proposed a model whereby the Ca\textsubscript{4,}\textbeta subunit serves to prime the channel to receive the inhibitory effects of Gem. Even more interestingly, by exchanging loops between P/Q-type channels and T-type channels, they showed that the region comprised of the S1-S3 transmembrane segments of domain II conferred Gem sensitivity (not necessarily Gem binding, which was not tested). This was surprising, because sites of channel interaction with modulatory proteins are typically located in the linkers between domains or the N- or C-termini. Therefore, this raises the question as to whether this may reflect a potential mechanism of RGK protein lowering the open probability of the channel (perhaps by immobilizing the voltage sensor) as found by Colecraft’s group.

**Further Complexity: Rem2 Multimerization**

To add yet another dimension of complexity to calcium channel regulation by RGK-proteins, we found that Rem2 can form dimers and multimers when expressed in HEK cells. When coexpressing two full-length epitope-tagged Rem2 proteins, we discovered that HA-Rem2 can pull down myc-Rem2 (Fig. 2A), and that this interaction was independent of CaM binding (Fig. 2B). Additionally, the short C-terminal fragment comprising Rem2
unanswered. For example, what role does the CaVβ subunit play in RGK protein-mediated calcium current inhibition? Is it an essential part of an RGK protein-CaVβ subunit-channel complex as reported in the early literature? Does it serve a chaperoning function to bring the RGK protein and channel close enough together to interact directly? Is it dispensable? There may be no clear-cut unifying answers that apply equally to all RGK subtypes and all types of voltage gated calcium channels or their ancillary subunits. Nonetheless, when compiling information from recent studies, a model emerges (Fig. 3B) in which the CaVβ subunit may serve to orient or chaperone the RGK protein to the channel (aided perhaps by the membrane-association domain of RGK proteins). Once at the channel, RGK proteins may then bind the proximal C-terminus of the channel (potentially competing with calmodulin) while the CaVβ subunit binds to the domain I-II linker. The RGK protein may then be in position to exert an effect on the voltage sensors, thus reducing the functionality of the channel. Additionally, it may confer some allosteric interference with normal channel function by holding the C-terminus close to the membrane. Third, multimerization of RGK proteins can provide additional points of regulation of a given channel. If one RGK protein can bind the C-terminus of the channel and be linked to another RGK protein, this leaves open the possibility of additional interactions with other

Figure 2. Rem2 forms multimers under non-reducing conditions. HA-Rem2 and myc-Rem2 were coexpressed in HEK cells, and cells were lysed 2 days after transfection in buffer containing 2 mM Ca. Cleared lysates were incubated with either HA-agarose beads or calmodulin-saparose beads, then washed 3x and eluted with non-reducing sample buffer (lacking DTT or β-mercaptoethanol). Lysates and eluates were separated by SDS-PAGE on layered 10%/16% tricine gels, then transferred to nitrocellulose membranes and probed with anti-myc antibody (Roche). Results are representative of at least three different experiments. (A) Pulldown assay showing an interaction between myc-Rem2 and HA-Rem2. Left, cleared cell lysates probed with anti-myc antibody. Approximate molecular weights are given (kD), and contents of each lane are listed at right. Right, pulldown assay from HA beads. The lowest band in each lane represents the monomeric Rem2. (B) Pulldown assay using calmodulin sepharose beads. Contents of each lane are listed at right, and approximate molecular weights are given (kD). Again, the lowest band in each lane represents the monomeric form of that protein.

residues 284–341 expressed alone in HEK cells shows multimers on a non-reducing gel, but the N-terminal fragment composed of residues 1–149 shows a monomer under the same conditions (Fig. 2B), suggesting that the Rem2 domain involved in self-association is in the C-terminal fragment. Whether multimeric Rem2 is a normal cellular form, or if multimers are formed in response to a physiological event such as neuronal stimulation, is unknown. Since Rem2 seems to use its C-terminus to form multimers, it is possible that other RGK proteins may also form higher-order complexes, as the C-terminus is ~40% conserved within the RGK family. The possibility of multimeric RGK protein complexes must be taken into consideration when one interprets experiments involving RGK mutant constructs, especially when expressed in a system that contains endogenous RGK proteins.

Model for RGK Protein Reduction of Calcium Currents

There has been a recent emergence of several new insights into how RGK proteins interact with the calcium channel machinery to regulate currents. Reports from independent groups have demonstrated a direct binding between RGK proteins and the calcium channel (summarized in Fig. 3A), but numerous questions remain unanswered. For example, what role does the CaVβ subunit play in RGK protein-mediated calcium current inhibition? Is it an essential part of an RGK protein-CaVβ subunit-channel complex as reported in the early literature? Does it serve a chaperoning function to bring the RGK protein and channel close enough together to interact directly? Is it dispensable? There may be no clear-cut unifying answers that apply equally to all RGK subtypes and all types of voltage gated calcium channels or their ancillary subunits. Nonetheless, when compiling information from recent studies, a model emerges (Fig. 3B) in which the CaVβ subunit may serve to orient or chaperone the RGK protein to the channel (aided perhaps by the membrane-association domain of RGK proteins). Once at the channel, RGK proteins may then bind the proximal C-terminus of the channel (potentially competing with calmodulin) while the CaVβ subunit binds to the domain I-II linker. The RGK protein may then be in position to exert an effect on the voltage sensors, thus reducing the functionality of the channel. Additionally, it may confer some allosteric interference with normal channel function by holding the C-terminus close to the membrane. Third, multimerization of RGK proteins can provide additional points of regulation of a given channel. If one RGK protein can bind the C-terminus of the channel and be linked to another RGK protein, this leaves open the possibility of additional interactions with other
parts of the channel and/or the Ca\textsubscript{\beta} subunit, or even an unknown third-party regulatory protein.

This model accounts for only a part of the known effects of RGK proteins on channels, thus additional work will be needed to address issues such as how RGK proteins affect voltage sensors, how they enhance channel endocytosis, and how the relationship between the RGK protein and the Ca\textsubscript{\beta} subunit leads to decreased channel open probability. Only then will we truly understand the precise mechanism of action of these multifunctional proteins.

References
1. Reynet C, Kahn C. Rad: a member of the Ras family overexpressed in muscle of type II diabetic humans. Science 1993; 262:1441-4.
2. Laville M, Aubouef D, Khalfallah Y, Vega N, Riou J, Vidal H. Acute regulation by insulin of phosphatidylinositol-3-kinase, Rad, Glut 4 and lipoprotein lipase mRNA levels in human muscle. J Clin Invest 1996; 98:43-9.
3. Maguire J, Santoro T, Jensen P, Siebenlist U, Yewdell J, Kelly K. Gem: an induced, immediate early protein belonging to the Ras family. Science 1994; 265:241-4.
4. Cohen L, Mohr R, Chen Y, Huang M, Kato R, Dorin D, et al. Transcriptional activation of a ras-like gene (Kiri) by oncogenic tyrosine kinases. Proc Natl Acad Sci USA 1994; 91:12448-52.
5. Vanhove B, Hofer-Warbinek R, Kapetanopoulos A, Hofer E, Bach F, de Martin R. Gem, a GTP-binding protein from mitogen-stimulated T cells, is induced in endothelial cells upon activation by inflammatory cytokines. Endothelium 1997; 5:51-61.
6. Leone A, Mitsuades N, Ward Y, Spinelli B, Poulaki V, Tsokos M, et al. The Gem GTP-binding protein promotes morphological differentiation in neuroblastoma. Oncogene 2001; 20:3217-25.
7. Warren K, Foster N, Gold W, Stanley K. A novel gene family induced by acute inflammation in endothelial cells. Gene 2004; 342:85-95.
8. Finlin B, Andres D. Rem is a new member of the Rad- and Gem/Kir Ras-related GTP-binding protein family repressed by lipopolysaccharide stimulation. J Biol Chem 1997; 272:21982-8.
9. Finlin B, Shao H, Kodono-Okuda K, Guo N, Andres D. Rem2, a new member of the Rem/Rad/Gem/Kir family of Ras-related GTPases. Biochem J 2000; 347:223-31.
10. Finlin B, Mosley A, Crump S, Correll R, Oxcan S, Sarin J, et al. Regulation of L-type Ca\textsuperscript{2+} channel activity and insulin secretion by the Rem2 GTPase. J Biol Chem 2005; 280:41864-71.
11. Paradis S, Harrar D, Lin Y, Koon A, Hauser J, Griffith E, et al. An RNAi-based approach identifies molecules required for glutamatergic and GABAergic synapse development. Neuron 2007; 53:217-32.
12. Schroder E, Magyar J, Burgess D, Andres D, Satin J. Chronic verapamil treatment remodells ICa,L in mouse ventricle. Am J Physiol Heart Circ Physiol 2007; 292:1906-16.
13. Biering R, Beato M, Edel M. An endothelial cell genetic screen identifies the GTTPase Rem2 as a suppressor of p19Arf expression that promotes endothelial cell proliferation and angiogenesis. J Biol Chem 2008; 283:4408-16.
14. Correll R, Pang C, Niedowicz D, Finlin B, Andres D. The RGK family of GTP-binding proteins: regulators of voltage-dependent calcium channels and cytoskeleton remodeling. Cell Signal 2008; 20:292-300.
15. Kelly K. The RGK family: a regulatory tail of small GTP-binding proteins. Trends Cell Biol 2005; 15:640-3.
16. Edel M, Menchon C, Menendez S, Consiglio A, Raya A, Iripius Belmonte J. Rem2 GTPase maintains survival of human embryonic stem cells as well as enhancing reprogramming by regulating p53 and cyclin D1. Genes Dev 2010; 24:561-73.
17. Finlin B, Crump S, Satin J, Andres D. Regulation of voltage-gated calcium channel activity by the Rem and Rad GTPases. Proc Natl Acad Sci USA 2003; 100:14469-74.

18. Bégün P, Nagashima K, Gonoi T, Shibasaki T, Takahashi K, Kashima Y, et al. Regulation of Ca^2+ channel expression at the cell surface by the small G-protein Kir/Gem. J Biol Chem 2001; 411:701-6.

19. Finlin B, Correll R, Pang C, Crump S, Satin J, Andres D. Analysis of the complex between Ca^2+ channel beta-subunit and the Rem GTPase. J Biol Chem 2006; 281:23557-66.

20. Opatowsky Y, Sasson Y, Shaked I, Ward Y, Chomsky-Hecht O, Litvak Y, et al. Structure-function studies of the G-domain from human gem, a novel small G-protein. FEBS Lett 2006; 580:3959-64.

21. Splingard A, Ménétrey J, Perderiset M, Cicolari J, Regazzi K, Hamoudi E, et al. Biochemical and structural characterization of the gem GTPase. J Biol Chem 2007; 282:1905-15.

22. Fischer R, Wei Y, Anaghi J, Berchtold M. Calmodulin binds to and inhibits GTP binding of the ras-like GTPase Kir/Gem. J Biol Chem 1996; 271:25607-78.

23. Moyers J, Bilan P, Zhu J, Kahn C. Rad and Rad-related GTPases interact with calmodulin and calmodulin-dependent protein kinase II. J Biol Chem 1997; 272:11832-9.

24. Bégün P, Mahalakshmi R, Nagashima K, Cher D, Kuwamura N, Yamada Y, et al. Roles of 14-3-3 and calmodulin binding in subcellular localization and function of the small G-protein Rem2. Biochim J 2005; 390:67-75.

25. Correll R, Pang C, Finlin B, Dailey A, Satin J, Andres D. Plasma membrane targeting is essential for Rem-mediated Ca^2+ channel inhibition. J Biol Chem 2007; 282:28431-40.

26. Bégün P, Mahalakshmi R, Nagashima K, Cher D, Ikeda H, Yamada Y, et al. Nuclear sequestration of beta-subunits by Rad and Rem is controlled by 14-3-3 and calmodulin and reveals a novel mechanism for Ca^2+ channel regulation. J Mol Biol 2006; 355:34-46.

27. Bégün P, Mahalakshmi R, Nagashima K, Cher D, Takahashi A, Yamada Y, et al. 14-3-3 and calmodulin control subcellular distribution of Kir/Gem and its regulation of cell shape and calcium channel activity. J Cell Sci 2005; 118:1923-34.

28. Andres D, Crump S, Correll R, Satin J, Finlin B. Analyses of Rem/RGK signaling and biological activity. Methods Enzymol 2006; 407:484-98.

29. Pang C, Crump S, Jin L, Correll R, Finlin B, Satin J, et al. Rem GTPase interacts with the proximal Ca^2+ C-terminus and modulates calcium-dependent channel inactivation. Channels (Austin) 2010; 4:192-202.

30. Seu L, Pitt G. Dose-dependent and isoform-specific modulation of Ca^2+ channels by RGK GTPases. J Gen Physiol 2006; 128:605-13.

31. Yada H, Murata M, Shimoda K, Yuasa S, Kawaguchi H, Ieda M, et al. Dominant negative suppression of Rad leads to QT prolongation and causes ventricular arrhythmias via modulation of L-type Ca^2+ channels in the heart. Circ Res 2007; 101:69-77.

32. Yang T, Xu X, Kerman T, Wu V, Colecraft H. Rem, a member of the RGK GTPases, inhibits recombinant Ca^2+^2^ channels using multiple mechanisms that require distinct conformations of the GTPase. J Physiol 2010; 588:1665-81.

33. Crump S, Correll R, Schroder E, Lester W, Finlin B, Andres D, et al. L-type calcium channel alpha-subunit and protein kinase inhibitors modulate Rem-mediated regulation of current. Am J Physiol Heart Circ Physiol 2006; 291:1959-71.

34. Bannister R, Colecraft H, Beam K. Rem inhibits skeletal muscle EC coupling by reducing the number of functional L-type Ca^2+ channels. Biophys J 2008; 94:2631-8.

35. Xu X, Marx S, Colecraft H. Molecular mechanisms, and selective pharmacological rescue, of Rem-inhibited Ca^2+^2^ channels in heart. Circ Res 2010; 107:620-30.

36. Chen H, Puhl Hr, Niu S, Mitchell D, Ikeda S. Expression of Rem2, an RGK family small GTPase, reduces N-type calcium current without affecting channel surface density. J Neurosci 2005; 25:9762-72.

37. Ward Y, Spinelli B, Quon M, Chen H, Ikeda S, Kelly K. Phosphorylation of critical serine residues in Gem separates cytoskeletal reorganization from downregulation of calcium channel activity. Mol Cell Biol 2004; 24:651-61.

38. Flynn R, Chen L, Hamed S, Stafford J, Zamponi G. Molecular determinants of Rem2 regulation of N-type calcium channels. Biochem Biophys Res Commun 2008; 368:827-31.

39. Leyris J, Gondeau C, Charnet A, Delatte C, Rouset M, Cens T, et al. RGK GTPase-dependent Ca^2+^2^ channel inhibition is independent of Ca^2+^ beta-subunit-induced current potentiation. FASEB J 2009; 23:2627-38.

40. Fan M, Yang J. A new paradigm for Gem regulation of voltage-gated Ca^2+ channels. Biophysical Society 54th Annual Meeting. San Francisco CA 2010.

41. Bégün P, Ng Y, Krause C, Mahalakshmi R, Ng M, Hunziker W. RGK small GTP-binding proteins interact with the nucleotide kinase domain of Ca^2+^ channel beta-subunits via an uncommon effector binding domain. J Biol Chem 2007; 282:11509-20.

42. Fan M, Butrai Z, Luo H, Levenson-Palmer R, Yang J. Direct inhibition of P/Q-type voltage-gated Ca^2+ channels by Gem does not require a direct Gem/Ca^2+_beta interaction. Proc Natl Acad Sci USA 2010.

43. Cens T, Restituito S, Vallentin A, Charnet P. Promotion and inhibition of L-type Ca^2+ channel facilitation by distinct domains of the subunit. J Biol Chem 1998; 273:18308-15.

44. Correll R, Botzet G, Satin J, Andres D, Finlin B. Analysis of the Rem2—voltage dependent calcium channel beta subunit interaction and Rem2 interaction with phosphorylated phosphatidylinositol lipids. Cell Signal 2008; 20:400-8.

45. Sasaki T, Shibasaki T, Bégün P, Nagashima K, Miyazaki M, Seino S. Direct inhibition of the interaction between alpha-interaction domain and beta-interaction domain of voltage-dependent Ca^2+ channels by Gem. J Biol Chem 2005; 280:9308-12.