Defining the MO’s of RGK proteins

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Members of the RGK (Rad-Rem-Rem2-Gem/Kir) family of small GTP binding proteins profoundly inhibit high voltage-activated CaV1.X and CaV2.X Ca2+ channels. The ability of RGK proteins to interact with these channels was revealed when Gem was flushed out of a yeast-2-hybrid screen which used the channel β subunit as bait.1 The functional significance of this interaction was immediately clear as Ca2+ currents were more-or-less absent in cells coexpressing Gem with either CaV1.2, CaV1.3 or CaV2.1. Since the ablation of Ca2+ currents by Gem, and later Rad, Rem and Rem2, required coexpression of a β subunit isoform, two reasonable assumptions arose regarding the nature of RGK protein-mediated inhibition. The first assumption was that RGK proteins blocked channel membrane expression by interfering with the β subunit’s well-known ability to promote channel trafficking. The second assumption was that RGK proteins interacted only with the β subunit.

The first indication that first assumption might be a bit naïve became apparent when Chen et al.2 reported that HEK 293 coexpressing CaV2.2 channels and Rem2 displayed virtually no difference in maximal ω-conotoxin GVIA binding relative to cells expressing just the channel. However, other evidence, such as the reduction of intramembrane gating charge movement in cardiomyocytes by virally-overexpressed Gem,3 still supported the idea that RGK proteins reduce channel membrane expression. These conflicting viewpoints were reconciled by Yang and colleagues4 who, using HEK293 cells coexpressing CaV1.2, β2a and Rem, found that Rem can support three distinct modes of inhibition: 1) reduction of channel membrane expression, 2) immobilization of the voltage-sensors, and 3) reduction of channel Po without impaired voltage-sensor movement (see Figure 1).

Two relatively recent studies have challenged the second assumption that RGK proteins exert their influence on channel activity solely via interactions with the β subunit. Fan et al. washed away β subunits from inside-out membrane patches to demonstrate that application of purified Gem can directly inhibit CaV2.1 channels,5 while Yang et al. showed a substantial component of the total inhibition of CaV1.2 effected by Rem or Rad, but not by Gem or Rem2, remains intact in HEK293 cells coexpressing a mutant β2a subunit that does not bind RGK proteins in vitro.6 The latter study also showed that this newly uncovered form of inhibition, was reliant on the proximal amino-terminus of the principal α1C subunit of the CaV1.2 heteromultimer.

In the highlighted study, the Colecraft laboratory set out to identify the cognate elements of Rem that are essential for α1C subunit-binding-dependent (ABD) inhibition.7 To accomplish this goal, they employed 3-cube FRET and co-immunoprecipitations to demonstrate that the α1C amino-terminus interacts with residues 265–285 in the distal carboxyl-terminus of Rem. ABD inhibition of L-type current was blunted in HEK293 cells expressing Rem constructs lacking this portion of the carboxyl-terminus in manner consistent with the aforementioned binding assays, but expression of the Rem carboxyl-terminus (residues 224–297) alone was insufficient for ABD inhibition. However, ABD inhibition was fully restored with the addition of the G
Domain (Ras-like core) of Rem. By contrast, the Rem carboxyl-terminus did not support ABD inhibition when fused to the Gem G Domain, despite the high level of conservation between the G domains of Gem and Rem. The authors integrated their observations in a model where voltage-sensor translocation is partially impeded by the binding of the Rem carboxyl-terminus to the amino-terminus of CaV1.2, which in turn is dependent on the specific anchoring of the Rem G domain to an unknown moiety. Interestingly, the linear sequences required for ABD inhibition overlap with those required for β subunit-binding-dependent (BBD) inhibition, whereby the G domain and carboxyl-terminus interact with the β subunit and the plasma membrane, respectively, to generate a steric tension that prevents channel opening without great effect on movement of the voltage sensors.8

In addition to defining the structural elements of Rem that are important for ABD inhibition of CaV1.2, Puckerin and colleagues showed that this form of inhibition is physiologically relevant in cardiomyocytes as adenoviral expression of the α1C amino-terminus significantly reduced inhibition of native L-type currents by coexpressed Rem.7 Since ABD inhibition has only been demonstrated to be a property of CaV1.2 channels so far, one can envision that specific therapeutic strategies based on the Rem-CaV1.2 interaction could be developed.7,8 Still, more investigation of these fundamental mechanisms is necessary since it is now clear that RGK protein-mediated inhibition of CaV

Figure 1. Three in one: inhibition of L-type CaV1.2 channels by Rem. Rem utilizes three distinct, but overlapping, mechanisms to inhibit CaV1.2 channel heterologously-expressed in HEK293 cells.4 Two of these modes, a reduction of channel membrane expression (N; red circle) and inhibition of pore opening (Po; blue circle), are dependent on an interaction with the channel β subunit.6 The third mode, in which the voltage-sensing elements of the channel are immobilized (Qmax; green circle), is facilitated by an interaction between the proximal amino-terminus (encompassing residues 93–153) of the α1C subunit of CaV1.2 and the distal carboxyl-terminus of Rem (residues 265–285).7 This latter form of inhibition specifically requires the Ras-like G domain of Rem, although the Rad G domain is likely capable of substituting for the Rem G domain. Gem and Rem2 operate only through β-dependent mechanisms.6,7
channels is far, far more complex than we first had thought.

Abbreviations
ABD  $\alpha_{1C}$ subunit-binding-dependent
BBD  $\beta$ subunit-binding-dependent
RGK  Rad-Rem-Rem2-Gem/Kir

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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