J Physiol 588.11 (2010) pp 1801–1802

Rem-induced inhibition of Ca^{2+} channels – a three-pronged assault

Bernhard E. Flucher
Department of Physiology & Medical Physics, Medical University Innsbruck, Innsbruck, Austria

Email: bernhard.e.flucher@i-med.ac.at

Rem is a member of the RGK family of Ras-related monomeric G-proteins. Like other members of the Ras superfamily, RGK proteins regulate cytoskeletal remodelling through the Rho kinase pathway. In addition, all members of the RGK family – Rad, Rem, Rem2 and Gem/Kir – are potent inhibitors of high voltage-activated Ca^{2+} channels (Correll *et al.* 2008). However, the physiological stimuli and the mechanisms of Ca^{2+} channel inhibition are largely unknown. The study by Yang and coworkers published in a recent issue of *The Journal of Physiology* (Yang *et al.* 2010) addresses several controversial issues of RGK-mediated Ca^{2+} channel inhibition and finds a surprising multiplicity of inhibitory mechanisms at work.

Principally Ca^{2+} currents can be regulated by two distinct mechanisms: by modulating the number of channels in the membrane or by modulating the channel open probability, which in turn depends on the gating properties and on the single channel conductance. There is general agreement that inhibition by RGK proteins depends on the auxiliary Ca^{2+} channel β subunit and actually requires direct binding of an RGK protein to a β subunit. But this is also where the agreement ends. Some studies support an inhibitory effect of RGK proteins on membrane expression of Ca^{2+} channels. Others provide evidence that RGK proteins modulate the biophysical properties of Ca^{2+} channels in the plasma membrane. Interestingly, this controversy is reminiscent of a similar debate about the mode of action of the β subunit. This cytoplasmic channel subunit is essential for membrane expression of Ca^{2+} channels in heterologous cells and in neurons but not in muscle (Dolphin, 2003; Obermair *et al.* 2008). Moreover, the β subunit can modulate the gating properties in a subunit-specific manner. In light of these parallels, it is plausible that RGK proteins exert their effect on Ca^{2+} channels simply by inhibiting the β subunit functions. Whereas the majority of the earlier studies suggested that GRK proteins compete for binding of the β subunit to the channel or even sequester the β subunit in the nucleus and thus inhibit β-dependent membrane insertion or modulation of the channel, more recent work suggests that RGK proteins accomplish their inhibitory effects by forming a tripartite complex with the β subunit and the pore-forming α_1 subunit (Correll *et al.* 2008). Furthermore, increasing evidence indicates that RGK proteins can acutely inhibit surface-expressed Ca^{2+} channels. However, a conclusive resolution of this controversy is not yet in sight.

On the side of the RGK proteins the situation is similarly complex. Although their nucleotide binding site differs from that of other Ras proteins and has a lower affinity, RGK proteins also function as molecular switches which cycle between a GTP-bound active and a GDP-bound inactive state. Whether GTP binding is required for RGK-mediated inhibition of Ca^{2+} channels is controversial. In addition, the C-terminus of RGK proteins, which contains phosphorylation sites, protein- and lipid-binding domains and is responsible for plasma membrane targeting, appears to be critically involved in Ca^{2+} channel inhibition.

Thus, multiple possible regulatory mechanisms of the RGK proteins exist *vis-à-vis* multiple possible mechanisms of Ca^{2+} channel inhibition. Yang *et al.* (2010) examined these regulatory mechanisms using heterologous expression in HEK cells of L-type Ca^{2+} channel $Ca_V1.2$ and β_{2a} subunits together with a range of Rem mutants, truncations and fusion proteins. As expected from previous studies, coexpressed wild type Rem drastically reduced Ca^{2+} currents, whereas a C-terminally truncated Rem did not. In order to quantitatively measure surface expression of Ca^{2+} channels, they tagged the channel with an extracellular bungarotoxin binding site, stained it with fluorescent quantum dots and analysed surface expression in living cells using flow cytometry. Combined with the electrophysiological analysis of gating charge movements, this new surface expression assay for the first time allowed differentiating between a reduced surface expression of the channels and the immobilization of gating charges; this innovative approach yielded several surprising results.

First the authors could confirm an effect of Rem on surface expression and that this required both an intact nucleotide binding domain and C-terminus. Remarkably, however, reduced surface expression was not due to reduced membrane insertion, but to increased dynamin-dependent endocytosis of the channels. The second surprise was that, when turnover of channels was blocked by coexpression of dominant negative dynamin, the channels in the membrane were still inhibited by Rem – apparently by the immobilization of the voltage sensors. This capacity of Rem was lost when its nucleotide binding domain was mutated. Finally, they discovered an inhibitory action of Rem on the effective open probability that did not result from the immobilization of gating charges. This effect required membrane targeting of Rem. Nevertheless, for intact Rem to inactivate Ca^{2+} currents, membrane targeting was dispensable as long as sufficient amounts were expressed in the cytoplasm, thus, indicating a role of the C-terminus of Rem in channel modulation beyond that of a simple membrane targeting domain.

By demonstrating that multiple molecular mechanisms can contribute to Rem-induced inhibition of Ca^{2+} channels in a single experimental system, the scientists in the Colecraft lab (Yang *et al.* 2010) clearly showed that these mechanisms are not mutually exclusive. In differentiated cells, specific combinations of Ca^{2+} channel isoforms and members of the RGK GTPase family may selectively utilize one or the other of these mechanisms, thus explaining, at least in part, the conflicting results of previous studies and possibly providing a strategy for cell-specific modulation of Ca^{2+} channels. Together with independent evidence demonstrating the significance of RGK-mediated inhibition of Ca^{2+} channels for the physiology and pathology of excitable cells (Yada *et al.* 2007), a better understanding of the multiple underlying mechanisms may therefore lead to the development of new and more specific drugs targeting Ca^{2+} channel functions.

DOI: 10.1113/jphysiol.2010.191247

References

Correll RN, Pang C, Niedowicz DM, Finlin BS & Andres DA (2008). *Cell Signal* **20**, 292–300.

Dolphin AC (2003). *J Bioenerg Biomembr* **35**, 599–620.

Obermair GJ, Tuluc P & Flucher BE (2008). *Curr Opin Pharmacol* **8**, 311–318.

Yada H, Murata M, Shimoda K, Yuasa S, Kawaguchi H, Ieda M, Adachi T, Murata M, Ogawa S & Fukuda K (2007). *Circ Res* **101**, 69–77.

Yang T, Xu X, Kernan T, Wu V & Colecraft H (2010). *J Physiol* **588**, 1665–1681.