Mechanism of Ca\(^{2+}\)-dependent desensitization in TRP channels

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Review

The 30+ members of the family of TRP channels are diverse in their physiological roles, yet the mechanisms that regulate their gating may be conserved. In particular, all TRP channels show an activity-dependent inhibition which is mediated by Ca\(^{2+}\). The mechanism by which Ca\(^{2+}\) inhibits TRP channels is currently a matter of intense debate, with Ca\(^{2+}\)-regulated kinases, phosphatases, phospholipases and calmodulin all proposed to be involved. In this review, we will discuss different mechanisms for Ca\(^{2+}\)-dependent desensitization in TRP channels. We will conclude with a model that focuses on Ca\(^{2+}\)-dependent activation of phospholipase C and Ca\(^{2+}\) binding to calmodulin and propose that the phospholipase C and calmodulin pathways are structurally and functionally coupled.

The TRP Family of Ion Channels

The first TRP channel was identified in 1977 as a phototransduction mutant in Drosophila.2 Since that time the TRP channel family has grown to include 28 mammalian members.3,4 The widespread importance of TRP channels is demonstrated by the great variety of their proposed functions: invertebrate phototransduction; regulation of vascular tone, transduction of painful stimuli; transduction of moderate temperature changes; phototransduction; regulation of vascular tone, transduction of painful stimuli; excitatory modulation of the cell cycle (reviewed in refs. 5–8); and extension of axonal growth cones.9 With intracellular N- and C-terminal domains, six putative membrane-spanning domains (Fig. 1), and sequence similarity in the pore region, TRP channels fall within the superfamily of ion channels that includes voltage-gated Na\(^+\), K\(^+\) and Ca\(^{2+}\) channels and cyclic nucleotide-gated channels.10,11 For those ion channels within this superfamily for which stoichiometry has been directly examined, all have been shown to be composed of four subunits or pseudosubunits, with auxiliary subunits sometimes present as well5 (Fig. 1).

Review

The TRP family has grown to include 28 mammalian members.3,4 The structures of the N-terminal domains of TRPV1, TRPV2 and TRPV5 have been solved with x-ray crystallography, revealing a classic stack of helix-loop-helix motifs similar to that of other ankyrin repeat-rich proteins (Fig. 3).1,12-14 No conservation between the N-terminal region of TRPM channels and those of TRPV and TRPC channels has been noted. In contrast, the C-terminal domains of TRPV, TRPC and TRPM channels show very high conservation in the proximal region (first 40 amino acids; Fig. 4) containing the so-called TRP box and TRP domain. The sequence similarity decreases over the next ≈100 amino acids with little or no similarity in the most distal region.

Lipids as TRP Channel Agonists

From the time the first TRP channel was cloned, the importance of lipids to their gating has been clear. The TRP and TRPL channels cannot be activated by light in Drosophila lacking functional phospholipase C, whereas diacylglycerol kinase mutations result in constitutively active channels.15,16 Even now the lipid activators of TRP and TRPL are a subject of intense debate, with diacylglycerol and poly-unsaturated fatty acids as the primary contenders.17 The three main sub-families of vertebrate TRP channels, the TRPCs, TRPMs and TRPVs, are all believed to be regulated by lipids. Among the TRPCs, diacylglycerol has been shown to activate TRPC3, TRPC6 and TRPC7; and a PLC metabolite is believed to activate TRPC4 and TRPC5.18 TRPC5 can also be activated by lysophosphatidic acid and sphingosine 1-phosphate.19 and TRPC6 has been proposed to be regulated by PI(4,5)P\(_2\) and PIP\(_3\). Lipid activation has also been well-studied in TRPM channels. PI(4,5)P\(_2\) has been shown to activate TRPM4,21,22 TRPM5,23 TRPM7,24 and TRPM8.25,26 TRPV5 can be activated by PI(4,5)P\(_2\), and PI(4,5)P\(_2\) seems to play a role in diminishing its physiological inhibition by Mg\(^{2+}\).27-30 TRPV1 is still an open question.27-30 Lipid modulation by the remaining mammalian TRP channels has not yet been reported, but it is likely that additional examples of lipid modulation will be identified.
Ca\(^{2+}\) regulation of TRP channels

Although the TRPs are all non-selective cation channels, all conduct Ca\(^{2+}\) to some degree and several are orders of magnitude more selective for Ca\(^{2+}\) than for monovalent cations.\(^5\),\(^32\)–\(^34\) Thus, they can be viewed generally as Ca\(^{2+}\) channels, with Ca\(^{2+}\) influx representing one of their common physiological roles. The tight control of intracellular Ca\(^{2+}\) required for proper cellular function dictates that Ca\(^{2+}\) influx pathways be self-limiting. Thus, for Ca\(^{2+}\)-permeable TRP channels, negative feedback by permeating Ca\(^{2+}\) ions is a critical mechanism for maintaining Ca\(^{2+}\) homeostasis. There is not a single exception to this rule among TRPC, TRPM and TRPV channels: all that have been examined show a Ca\(^{2+}\)-dependent inhibition, inactivation or desensitization. Even TRPM4 and TRPM5, which are activated directly by Ca\(^{2+}\), show a slow, Ca\(^{2+}\)-dependent desensitization.\(^35\),\(^36\)

The pleiotrophic effects of Ca\(^{2+}\) on many enzymes makes it difficult to establish which cellular pathway is involved in desensitization.\(^37\) Indeed, Ca\(^{2+}\) may act via several pathways, and perhaps the effectors regulated by Ca\(^{2+}\) vary as a function of stimulus intensity, metabolic state or other factors. For TRP channels, Ca\(^{2+}\) has been proposed to produce desensitization via kinases, phosphatases, phospholipases and calmodulin (CaM). Below we will briefly review the evidence for each of these pathways.

**Mechanism—Ca\(^{2+}\)-Regulated Kinases and Phosphatases**

A number of kinases and phosphatases are reported to regulate the function of TRP channels. For example, phosphorylation by the Ca\(^{2+}\)- and diacylglycerol-dependent protein kinase C has been proposed to cause desensitization of TRPC5.\(^38\) In contrast, phosphorylation by protein kinase A and protein kinase C has been proposed to slow desensitization in TRPV1 channels,\(^39\),\(^40\) and protein kinase C has also been implicated in reversing desensitization of TRPM4 channels.\(^41\) Ca\(^{2+}\)/CaM regulated phosphorylation/dephosphorylation has also been proposed: phosphorylation of TRPC5 by myosin light chain kinase is associated with slowing of desensitization\(^42\) and dephosphorylation of TRPV1 by calcineurin is reported to promote desensitization.\(^40\),\(^43\) Thus, although phosphorylation seems to be involved in activation, and perhaps desensitization, of several TRP channels, no unifying theme has yet emerged.

**Mechanism—Direct Modulation of TRP Channels by Calmodulin**

CaM is a ubiquitous Ca\(^{2+}\)-binding protein with four EF-hand motifs. Ca\(^{2+}\) binds to each of these EF hands and promotes a structural rearrangement that increases the affinity of CaM for other proteins, such as ion channels. The Ca\(^{2+}\)-bound form of CaM (Ca\(^{2+}\)/CaM) regulates many ion channels: cyclic nucleotide-gated channels,\(^44\) KCNQ channels,\(^45\) voltage-gated Ca\(^{2+}\) channels,\(^46\) and small conductance Ca\(^{2+}\)-activated K\(^+\) channels (SK channels),\(^47\) among others. The apo (Ca\(^{2+}\)-free) form of CaM can also be important for regulating protein function. For example, in voltage-gated Ca\(^{2+}\) channels, the interaction between the channels and apo-CaM is required to prevent rapid, voltage-dependent inactivation.\(^48\) In KCNQ channels, apo-CaM is an auxiliary subunit that appears to be required for functional expression.\(^45\) Finally, Ca\(^{2+}\)-independent pre-association between CaM and SK channels allows Ca\(^{2+}\) to gate the channels rapidly.\(^47\)
It is widely believed that a direct binding of Ca\(^{2+}\)/CaM to TRP channels plays a role in Ca\(^{2+}\)-dependent desensitization. Prediction algorithms for Ca\(^{2+}\)/CaM binding sites and in vitro binding screens have led to the identification of a large number of candidate Ca\(^{2+}\)/CaM-binding sites in TRP channels (reviewed in ref. 49). Pharmacological agents, such as CaM inhibitors, anti-CaM antibodies and mutant CaM diminish Ca\(^{2+}\)-dependent desensitization in a number of TRP channels.\(^{49-52}\) However, these tools do not distinguish between direct binding of Ca\(^{2+}\)/CaM to TRP channels and indirect regulation of gating through Ca\(^{2+}\)/CaM-sensitive kinases, such as myosin light chain kinase, and phosphatases, such as calcineurin. Indeed, modulation of TRPC\(^{19,42}\) and TRPV\(^{39,40}\) channels by these enzymes has been proposed. Mutations that disrupt Ca\(^{2+}\)/CaM binding to channel fragments in vitro have been tested for their effects on desensitization in whole cells, but interpretation of such experiments has been confounded by the mutations causing non-functional channels (example in ref. 51), or important changes in channel function (e.g., disrupting ATP binding\(^{13}\)), or partial or ambiguous effects on Ca\(^{2+}\)-dependent desensitization (example in ref. 53). Thus, whether Ca\(^{2+}\)/CaM binding directly to TRP channels is a cause of desensitization remains an open question.

Mechanism—Decrease in Plasma Membrane PI(4,5)P\(_2\) by Phospholipase C

PI(4,5)P\(_2\) levels in the plasma membrane are regulated by a number of enzymes, including receptor-coupled phospholipase C. The pathway from G-protein coupled receptors, phospholipase C, PI(4,5)P\(_2\) and TRP channels allows TRP channel function to be controlled by extracellular signaling molecules. In addition, phospholipase C can be activated by Ca\(^{2+}\) in a receptor-independent manner, and it has been shown for TRPV1 channels that Ca\(^{2+}\) influx through the channels leads to a dramatic decrease in plasma membrane PI(4,5)P\(_2\) levels.\(^{28}\) In addition, resynthesis of PI(4,5)P\(_2\) has been shown to be required for TRPV1 channels to recover from desensitization.\(^{54}\) Given the large number of TRP channels regulated by PI(4,5)P\(_2\), the Ca\(^{2+}\) permeability of this channel class, and the ubiquitous Ca\(^{2+}\)-dependent desensitization of TRP channels, it seems likely that a reduction in PI(4,5)P\(_2\) levels play at least some role in desensitization of many TRP channels. Desensitization would be initiated by channel activation and followed by: Ca\(^{2+}\) influx through channels; a rise intracellular Ca\(^{2+}\); activation of phospholipase C; and, finally, phospholipase C degradation of PI(4,5)P\(_2\) would produce inhibition by disrupting the interaction of the channels with the positive regulator PI(4,5)P\(_2\). Involvement of the C-terminal Domain with PI(4,5)P\(_2\) and Ca\(^{2+}\)/CaM Regulation

The sites of action of PI(4,5)P\(_2\) on TRP channels are not known. However, many of the candidate sites are located in the C-terminal domain. PI(4,5)P\(_2\) is reported to interact with the C-terminal domains of TRPC6, TRPM4, TRPM5, TRPM8 and TRPV1, and TRPV5 (reviewed in ref. 25). Among several proposed sites within the C-terminal regions of TRP channels, a candidate PI(4,5)P\(_2\)-binding site in the proximal C-terminal, near the TRP box, is particularly attractive. The evidence that lipid regulation utilizes the proposed binding sites in functional channels, however, is indirect or absent.

For CaM, the story is more complex. CaM binding to the C-terminal domain has been proposed for TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM4, TRPV1, TRPV4, TRPV5 and TRPV6 (sometimes to multiple sites on the same channel) (reviewed in ref. 49). Many TRP channels have two or more candidate CaM binding sites within their C-terminal region. As shown for the TRPV family in Figure 5, one CaM binding site lies in the highly-conserved proximal C-terminal region—the same region proposed to constitute the PI(4,5)P\(_2\) binding site—and a second localizes to the less-conserved distal C-terminal region. CaM binding sites have been proposed for the N-terminal region of TRPM2 and TRPV1 as well; however there is no sequence conservation between the N-terminal domains of these families.

Competition between Membrane Lipids and Ca\(^{2+}\)/CaM

The mechanism for binding of PI(4,5)P\(_2\) to the C-terminal domain of ion channels has been proposed to involve nonspecific electrostatic interactions between the anionic PI(4,5)P\(_2\) and regions of the
The head group of PIP2 is depicted in red. Ca2+/CaM can disrupt the protein-lipid interaction. In clusters, including MARCKS, GAP43, GRK5, EGFR and the ErbB family, Ca2+/CaM has a net charge of -16 at neutral pH, making it a good candidate to result from its binding to the same site as the anionic lipids. The Ca2+/CaM complex has a net charge of -16 at neutral pH, making it an effective competitor. The opposing effects of PI(4,5)P2 and Ca2+/CaM on TRP channel function raise the possibility that the two modulators compete for the same polybasic/hydrophobic site in the C-terminal domain of TRP channels.

We propose that Ca2+-dependent desensitization via a PLC-induced reduction in PI(4,5)P2 levels and via CaM are functionally and structurally coupled. The cartoon in Figure 6 depicts the essential features of the coupled PLC and CaM pathways hypothesis. Two TRP subunits are shown, with their C-terminal domains shown as positively charged bars. At rest, the positively charged C-terminal domain binds to acidic PI(4,5)P2 (head groups shows as red circles) in the plasma membrane. This membrane-bound conformation of the channels is permissive of activation. Activation allows Ca2+ to enter the cells, and Ca2+ produces desensitization by releasing the C-terminal domain from the plasma membrane. The release occurs either when Ca2+ activates PLC and PLC degrades PI(4,5)P2 (Fig. 6, right) or when Ca2+ binds CaM and Ca2+/CaM displaces PI(4,5)P2 (Fig. 6, left). TRP channel desensitization could thus occur through either or both mechanisms, depending on the amplitude and spatial distribution of the Ca2+ increase, the abundance/activity of PI(4,5)P2, PLC and CaM in proximity to the channels, and their relative sensitivity to Ca2+.

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