Regulation of TRP channels: a voltage–lipid connection

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Abstract

TRP (transient receptor potential) channels respond to a plethora of stimuli in a fine-tuned manner. We show here that both membrane potential and the level of PI (phosphatidylinositol) phosphates are efficient regulators of TRP channel gating. Recent work has shown that this regulation applies to several members of the TRPV (TRP vanilloid) subfamily (TRPV1 and TRPV5) and the TRPM (TRP melastatin) subfamily (TRPM4/TRPM5/TRPM7/TRPM8), whereas regulation of members of the TRPC subfamily is still contradictory. The mechanism whereby PIP₂ (PI 4,5-bisphosphate) acts on TRPM4, a Ca²⁺- and voltage-activated channel, is shown in detail in this paper: (i) PIP₂ may bind directly to the channel, (ii) PIP₂ induces sensitization to activation by Ca²⁺ and (iii) PIP₂ shifts the voltage dependence towards negative and physiologically more meaningful potentials. A PIP₂-binding pocket seems to comprise a part of the TRP domain and especially pleckstrin homology domains in the C-terminus.

Introduction

Phosphoinositides are ubiquitously used signalling molecules in eukaryotic cells, and modulate a plethora of soluble substrate transporters and ion channels. The list of ion channels regulated by PIP₂ [PI (phosphatidylinositol) 4,5-bisphosphate] includes inward-rectifier and voltage-gated K⁺ channels, the two-P domain K⁺ channels, voltage-gated Ca²⁺ channels, cyclic nucleotide-gated channels, intracellular Ca²⁺ release channels, the epithelial Na⁺ and Cl[−] channels and TRP (transient receptor potential) channels (for recent reviews, see [1–3]). This mode of regulation seems to be especially important for TRP channels, which can be activated by many exogenous and endogenous ligands, by chemical stimuli such as protons or reactive oxygen species, and by physical stimuli such as heat or cold, voltage and mechanical stress (for reviews, see [4,4a,5]). One possible explanation for this obvious gating promiscuity, at least for the identified voltage-sensitive TRP channels, seems to depend on the following fact: small changes in Gibbs free energy (ΔG), which is composed of the change of conformational energy upon gating and the sum of energies transduced to the channel from external or internal sources, induce large shifts in the channel's activation curves, which is facilitated by the small gating charges of these voltage-dependent TRP channels [6–9]. It might therefore not be surprising that sequestration of a negatively charged lipid component such as PIP₂ by positively charged domains in channel proteins [3] would sensitively interfere with the gating of TRP channels. PIP₂

comprises approx. 1% of the total acidic membrane lipids and more than 99% of the doubly phosphorylated phosphoinositides in a mammalian cell [10]. The head group of PIP₂ has a very high density of negative charges and may therefore preferentially interfere with proteins that expose positive charges to the plasmalemma. It has also recently been shown that basic peptides sequester multivalent but not univalent PI phosphates [11], which is in agreement with the relatively minor effects of PI monophosphates on TRP channels [12,13].

Regulation of mammalian TRP channels by PIP₂ includes both tonic inhibition of TRPV1 (TRP vanilloid 1) [14,15] and activation of TRPM4 (TRP melastatin 4) [12,13], TRPM5 [16], TRPM7 [17], TRPM8 [18,19] and TRPV5 [18,20]. The mechanisms of TRPM4 activation by PIP₂ discussed below are likely to be also applicable to other TRP channels.

How to measure modulation of TRP channels by PIP₂: the TRPM4 paradigm

We have analysed in detail how PIP₂ modulates the Ca²⁺-activated non-selective TRP channel TRPM4, which involves an increase in the channel's Ca²⁺-sensitivity and a shift in the voltage range for activation towards negative potentials. For this analysis we used different protocols to deplete off PIP₂ from the cell, namely receptor stimulation, incubation of cells with wortmannin (an inhibitor of phosphoinositide 3-kinase, which delays PIP₂ replenishment), application of the PIP₂ scavenger poly(L-lysine) and induction of the PIP₂-depleting enzyme phosphatidylinositol polyphosphate 5-phosphatase IV [21]. All interventions caused a reduction of TRPM4 channel activity [12]. Run-down (desensitization) was prevented or reversed when the non-metabolizable diC8-PIP₂ was applied to the cytosolic side of excised patches. Recovery from desensitization also occurred when MgATP was added to the intracellular side, indicating that PIP₂ levels

Key words: lipid, phosphoinositide, phosphatidylinositol 4,5-bisphosphate (PIP₂), transient receptor potential channel (TRP channel), transient receptor potential melastatin 4 (TRPM4), voltage-dependence.

Abbreviations used: DV, decavanadate; PH domain, pleckstrin homology domain; PI, phosphatidylinositol; PIP₂, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; TRP, transient receptor potential; TRPM, TRP melastatin; TRPV, TRP vanilloid.

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can be restored via activation of lipid kinases. Inhibition of PLC (phospholipase C) activity by U73122 induced similar effects as direct application of PIP₂, which can be explained assuming Ca²⁺-dependent activation of PLC (e.g. the most Ca²⁺-dependent PLC isoform PLC δ_1 as shown in supplemental data in [18]). Both PIP₂ and U73122 induce an apparent loss of voltage dependence, e.g. loss of time dependence and slowing of deactivation at negative potentials, due to the dramatic shift of the steady-state open probability towards more negative potentials. All the modulator effects could be also observed in inside-out patches. We therefore conclude that PIP₂-modulating enzymes, such as lipid kinases (phosphatidylinositol 5-phosphate 4-kinase), Ca²⁺-dependent PLC δ_1 or phosphatidylinositol polyphosphate 5-phosphatase IV, form a structural complex together with TRPM4.

A fundamental gating principle and a possible lipid-voltage connection

In excitable cells, voltage-dependent channels are activated by membrane depolarization. Because the voltage sensor has a large charge, approx. 12–14e for the Shaker K⁺ channel [22], activation occurs in a relatively small voltage range. The moved charge for TRP channels is more than ten times smaller than for the classical voltage-dependent channels, causing a very flat activation curve, which is mostly shifted towards very positive potentials, i.e. these channels are almost closed in a physiological range of membrane potentials [7,8,23,24]. If as for the simplest gating model a two open state is considered, from a thermodynamic point of view, the probability of the channel being open under steady-state conditions is

$$P_{\text{open}} = \frac{1}{1 + \exp(\Delta G/RT) \cdot \exp(-zFV/RT)}$$

with

$$\Delta G = (\Delta H - T \cdot \Delta S) - \psi$$

where ΔH is the difference in change of enthalpy for channel opening and channel closing ($\Delta H_{\text{open}} - \Delta H_{\text{close}}$) [8,9], ΔS is the respective difference in entropy, ψ is the sum of energies transduced to the channel, e.g. by binding of a ligand, V is the membrane potential, z represents the gating charge of the channels and R , T and F have the usual meaning. The potential for half-maximal activation of the channel ($P_{\text{open}} = 0.5$), $V_{1/2}$, is defined by

$$V_{1/2} = \frac{\Delta G}{zF}$$

This means that even small changes in the ΔG will induce large shifts in the potentials of half-maximal activation when the moved charge z is small. This is indeed the case for TRP channels (for a detailed discussion see [8]). There is overwhelming evidence that many TRP channels use such a mechanism as a gating tool [7–9,14,24,25].

At least for TRPM8 (see supplemental data in [18]) and TRPM4 [12], PIP₂ has been shown to cause a leftward shift of the voltage dependence of activation. Importantly, depletion

of PIP₂ might be crucially involved in the desensitization of TRP channels to various gating stimuli.

For TRPM4, activation by Ca²⁺ is followed by a fast desensitization. This desensitization is caused by a dramatic rightward shift (towards positive potentials) of the activation curve, i.e. channels become unavailable [12,24,25]. PIP₂ prevents desensitization of TRPM4 in inside-out patches by inducing large voltage shifts of TRPM4, which is part of the crucial mechanism for channel activation by this PI [12]. All measures that sensitize TRPM4 to Ca²⁺ also shift the activation curve towards negative potentials, e.g. phosphorylation by protein kinase C, the vanadate decamer DV (decavanadate; a compound with six negative charges), endogenously or intracellularly applied PIP₂ or the activator BTP2 [3,5-bis-(trifluoromethyl)pyrazole derivative] [8,12,24–26]. Figure 1 shows the dramatic shift of the activation curves of TRPM4 by PIP₂ towards negative values.

Site of interaction

PIP₂ is anchored in the plasma membrane by its fatty acid side chains and exposes its negative charges close to the inner leaflet of the membrane. Because of the high density of negative charges, one possible interaction with proteins occurs via electrostatic interaction with positively charged residues of the targeted proteins [see e.g. for the best-known example the MARCKS (myristoylated alanine-rich C-kinase substrate) protein [3]]. This electrostatic interaction does not show isomer specificity [3]. Another interaction type is binding of PIP₂ in a PI-binding pocket, which is well understood for its interaction with PH domains (pleckstrin homology domains) such as those present in PLC δ_1 [27]. It has been shown for TRPM4 and TRPM8 that PIP₂ is much more efficient for channel activation than phosphatidylinositol 3,4-bisphosphate or phosphatidylinositol 3,5-bisphosphate, although all three compounds probably have a similar electrostatic effect [12,13,18,28]. Therefore these TRP channels might interact with PIP₂ via a more structured binding pocket rather than through pure electrostatics. A similar situation has been found in Kir channels, one of the best-understood examples for the modulation of channel activity by PIP₂ (see e.g. [29–31]).

Rohacs et al. [18] proposed a general role for the proximal C-terminal TRP domain in PIP₂ regulation of TRPM8 and other PIP₂-activated TRP channels, whereas Prescott and Julius [15] identified a more distal C-terminal region as a crucial determinant of PIP₂ inhibition. According to Rohacs [28], a conserved region in the C-terminus, known as the TRP-box and TRP-domain, functions as a general part of the PIP₂-binding pocket in TRP channels. Figure 2(A) shows an alignment for TRPM4 and TRPM5 of the TRP-box and the TRP-domain, which is very homologous for all the mammalian TRP members (see supplemental data in [28]). Mutations of basic residues in this domain induced large changes in PIP₂-sensitivity, which were most pronounced for the mutations in the TRP-domain downstream of the TRP-box (i.e. Arg¹⁰⁰⁸ for rat TRPM8, which is equivalent to Arg¹⁰⁷² in human TRPM4) (Figure 2). The C-terminus of TRPM4 contains two regions

Figure 1 | PIP₂ induces voltage shifts of the activation of TRPM4 in inside-out patches

(A) Current measured in response to the indicated voltage protocol in the absence of PIP₂. Current-voltage relationships (IV curves) are from the steady state (end of the pulse, circles) and tail current (triangles) (middle panel). Voltage dependence of the open probability obtained from normalization of the tail current values to maximal current obtained from fitting the tail current IV. Note activation at very positive potentials in the absence of PIP₂ (for details see [12]). (B) As (A), but now in the presence of 10 μ M PIP₂. (C) Activation curves for TRPM4 under control conditions and in the presence of 10 μ M PIP₂. Note the dramatic shift towards negative potentials. Solid lines represent fits using the Boltzmann equation. Adapted from [12], with permission.

with the consensus sequence for a PH domain, [R/K]-X₃₋₁₁-[R/K]-X-[R/K]-[R/K] (see <http://us.expasy.org/prosite/> and e.g. [32]). The two clusters of basic residues in TRPM4 are formed by amino acid stretches R¹¹³⁶ARDKR¹¹⁴¹ (mutated in the Δ R/K mutant) and R¹¹⁴⁷LKR¹¹⁵⁰ (mutated in the RKR mutant) [12]. The most dramatic reduction of PIP₂ activation of TRPM4 was measured in the Δ R/K mutant. However, also mutations in the TRP-domain (K1059Q, R1062Q, R1072Q, homologous with K995Q, R998Q and R1008Q in TRPM8 [18,28]) and in the second PH domain caused a substantial or even dramatic acceleration of desensitization of TRPM4 after activation by Ca²⁺. However, currents through these mutant TRPM4 channels could be completely rescued by application of PIP₂ [12]. Changes in desensitization may hint at a decreased sensitivity to PIP₂. In conclusion, we favour the view that the first PH domain in TRPM4 is the most crucial part of a PIP₂-binding site and that the clusters of positive changes in the TRP-domain and the second PH domain may contribute to the whole PIP₂-binding pocket. Interestingly, the binding site for DV was also identified as the first PH domain in TRPM4 [24]. So far, all TRPM members and also members of the TRPV subfamily, TRPV5 and TRPV6 [18,20,28], are activated by PIP₂. Only TRPV1 seems to be constitutively inhibited by PIP₂ and released from inhibition

Figure 2 | Structural representation of a putative C-terminal PIP₂-binding regions of TRPM4

(A) Alignment of TRP-box and TRP-domains of TRPM4 and TRPM5 (identical residues in white on black boxes; homologous residues in black in grey boxes). The positively charged residues are conserved. Asterisks mark mutants used for changes in PIP₂ affinity (see text). (B) Two putative PH domains in TRPM4 (dotted lines) which are missing in TRPM5. Asterisks indicate mutants used for narrowing the PIP₂-binding domain (see the text and [12]). Adapted from [12], with permission.

by PIP₂ hydrolysis. For TRPV1, deletion of a very short region in the C-terminus (residues 777–792, including four basic residues, in rat) eliminated this inhibition [15]. It is unclear whether such a short region forms a PIP₂-binding pocket. Other data, however, show that PIP₂ may also activate TRPV1 (see for a meeting report [33]).

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