CHAPTER 7

Regulation of the Mechano-Gated K_{2P} Channel TREK-1 by Membrane Phospholipids

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I. OVERVIEW

TREK-1 (KCNK2 or $K_{2P}2.1$) is a polymodal K^+ channel that is activated by membrane stretch, intracellular acidosis, heat, and cellular lipids such as arachidonic acid (AA). Phospholipids, including PIP_2, exert a dual dose-dependent effect on TREK-1. Low concentrations transform the mechano-gated K^+ channel TREK-1 into a leak K^+ channel. The phospholipid-sensing domain is a positively charged cluster in the proximal C-terminal domain. This region also encompasses the proton sensor E306 that is required for activation of TREK-1 by cytosolic acidosis. Protonation of E306 increases

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channel-phospholipid interaction leading to TREK-1 opening without direct mechanical stimulation. At higher concentrations, intracellular phospholipids inhibit channel activation by stretch, intracellular acidosis, and AA. Binding endogenous negative inner leaflet phospholipids with polylysine reduces the inhibition and reveals channel stimulation by exogenous intracellular phospholipids. Both stimulatory and inhibitory effects are observed with phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidic acid (PA), but not diacylglycerol (DG), suggesting that the phosphate at position 3 is required, although the net charge is not critical. In conclusion, membrane phospholipids, including PIP_2, are major regulators of TREK-1 channel activity.

II. INTRODUCTION

Mammalian K_{2P} channel subunits have 4 transmembrane segments and 2P domains in tandem, and the family has at least 15 members (Patel and Honoré, 2001). The P domains are part of the K^+ conduction pathway. Functional K_{2P} channels are dimers of subunits and heteromultimerization occurs (Lesage *et al.*, 1996a,b, 1997; Czirjak and Enyedi, 2001; Lauritzen *et al.*, 2003). Although the subunits appear to have the same structural motif, they only share moderate sequence homology outside the P regions (Patel and Honoré, 2001).

Human TREK-1 mRNA is highly expressed in the brain, the spinal cord, the stomach, and, to a lesser extent, in the small intestine (Fink *et al.*, 1996; Medhurst *et al.*, 2001). In the central nervous system, TREK-1 shows the greatest expression in the caudate nucleus and the putamen (Medhurst *et al.*, 2001). Besides hippocampal glutamatergic neurons, TREK-1 is localized in GABAergic interneurons (Hervieu *et al.*, 2001) and sensory neurons of dorsal root ganglia (Maingret *et al.*, 2000a; Medhurst *et al.*, 2001). TREK-1 is present at both synaptic and nonsynaptic sites (Maingret *et al.*, 2000a).

TREK channels are activated by mechanical stress (Patel *et al.*, 1998). The single-channel conductance is about 100 pS (positive potentials, 155 mM K^+), and the outward rectification of TREK-1 in symmetric saline is a combination of a mild intrinsic voltage-dependency and external Mg^{2+} block at negative potentials (Bockenhauer *et al.*, 2001; Maingret *et al.*, 2002). The typical resting activity suggests that TREK-1 will influence both resting excitability and the action potential duration (Patel and Honoré, 2001).

At the whole-cell level, TREK-1 is modulated by cellular volume, with hyperosmolarity closing the channel (Patel *et al.*, 1998). Mechanical force may be transmitted directly to the channel via the lipid bilayer, while the cytoskeleton tonically represses channel activity (Maingret *et al.*, 1999a;

Patel *et al.*, 2001). Heat reversibly opens TREK-1 with about a sevenfold increase in current amplitude for a temperature jump of 10°C (Maingret *et al.*, 2000a). Acidic pH_i shifts the pressure–activation relationship toward lower pressures, leading to channel opening without applied stimuli (Maingret *et al.*, 1999b; Lesage *et al.*, 2000; Honoré *et al.*, 2002). Intracellular acidosis converts a TREK mechano-gated channel into a constitutively active channel. Deletional and chimeric analyses demonstrate that the C-terminal domain, but not the N-terminal domain, is critical for TREK-1 activation by stretch, temperature, and intracellular acidosis (Maingret *et al.*, 1999b, 2000a; Honoré *et al.*, 2002). E306 in the proximal C-terminal domain is the key intracellular proton sensor that regulates TREK-1 channel activity (Honoré *et al.*, 2002).

TREK channels are also sensitive to amphipaths. For instance, TREK-1 is reversibly activated by polyunsaturated fatty acids (PUFAs) including AA, but not by saturated fatty acids (Fink *et al.*, 1998; Patel *et al.*, 1998; Kim *et al.*, 2001a,b). Activation of TREK and TRAAK channels by PUFA in excised patches indicates that the effect does not require the intact cell (Patel *et al.*, 2001), but is due to a direct interaction with the channel in its local membrane environment (Patel *et al.*, 2001). Extracellular lysophospholipids (LP), including lysophosphatidylcholine (LPC), open TREK channels (Lesage *et al.*, 2000; Maingret *et al.*, 2000b). Deletional analysis again indicates that the C-terminal domain, but not the N-terminal domain and the extracellular loop M1P1 of TREK-1, is critical for both AA and LPC activation (Patel *et al.*, 1998; Maingret *et al.*, 2000b). TREK-1 is also opened by intracellular lysophosphatidic acid (LPA; Chemin *et al.*, 2005a). The pharmacological properties of TREK-1 have been reviewed elsewhere (Lesage, 2003; Franks and Honore, 2004).

When coexpressed with the 5HT4 receptor, serotonin inhibits TREK-1 (Fink *et al.*, 1996; Patel *et al.*, 1998). This effect is mimicked by a membrane permeant cAMP derivative and the effect is mediated by protein kinase A-mediated phosphorylation of S333 in the C-terminal domain (Patel *et al.*, 1998). In contrast, sodium nitroprusside and 8-Br-cGMP increase TREK-1 currents (Koh *et al.*, 2001) probably through the PKG consensus sequence at S351 (Koh *et al.*, 2001).

Although a minor component of the plasma membrane, PIP_2 is increasingly recognized as a key physiological regulator of ion channel activity (Hilgemann *et al.*, 2001). PIP_2 controls the nucleotide sensitivity of K_{ATP} channels and promotes channel opening (Baukrowitz *et al.*, 1998; Shyng and Nichols, 1998). It directly activates inward rectifier IRK potassium channels and is essential for G$\beta\gamma$-protein activation of GIRK channels (Huang *et al.*, 1998; Mirshahi *et al.*, 2003; Delmas and Brown, 2005); PIP_2 also regulates both activation and inactivation of voltage-gated K^+ channels (Loussouarn, 2003

#1031; Oliver *et al.*, 2004). Genetic alterations of K^+ channel–PIP_2 interactions can lead to channelopathies such as Andersen's and Bartter's syndromes (Lopes *et al.*, 2002). Receptor-mediated PIP_2 hydrolysis plays a key role in the regulation of several ion channel types (Kobrinsky *et al.*, 2000; Chuang *et al.*, 2001; Runnels *et al.*, 2002; Suh and Hille, 2002; Zhang *et al.*, 2003), including the TASK K_{2P} channels (Czirjak *et al.*, 2001; Chemin *et al.*, 2003).

In the present chapter, we review the evidence that membrane phospholipids, including PIP_2, are major regulators of TREK-1 channel activity. Regulation of mechano-gated K_{2P} channels is complex with acute stimulation at low concentration and inhibition at high concentration.

III. TREK-1 STIMULATION BY MEMBRANE PHOSPHOLIPIDS

Both the cloned and endogenous neuronal TREK-1 channels are highly dependent for their activity on membrane phospholipids (Chemin *et al.*, 2005b; Lopes *et al.*, 2005). The cationic molecules polylysine (pL) and spermine, which have a high affinity for phospholipids, inhibit TREK-1 channel activity at rest, but the activity is restored and even stimulated by PIP_2 (Chemin *et al.*, 2005b; Lopes *et al.*, 2005). The inhibitory effect of polycationic molecules is thought to be due to the fact that they interact with the negative charges of essential membrane phospholipids, thus removing them from their electrostatic interaction with specific positively charged segments in the channel protein (Huang *et al.*, 1998; Lopes *et al.*, 2002). In the presence of intracellular polylysine or other polyamines, TREK-1 remains in an inactive state (Chemin *et al.*, 2005b; Lopes *et al.*, 2005; Fig. 1A). We have identified a cluster of five positive charges in the proximal C-terminal domain of TREK-1 that is central to the effect of phospholipids (Chemin *et al.*, 2005b; Fig. 1A). When the positive charges are deleted, TREK-1 becomes more resistant to the activation by PIP_2 (Chemin *et al.*, 2005b).

Using a complementation yeast system designed to assay for membrane–protein interactions (Aronheim, 2001), we have shown that the C-terminal domain of TREK-1 is in close proximity to the plasma membrane (Chemin *et al.*, 2005b). Moreover, the positive charges in this cluster, crucial for PIP_2 stimulation of the channel, are also associated with the bilayer as confirmed by fluorescence experiments (Chemin *et al.*, 2005b). The model presented in Fig. 1 summarizes our results and our mechanistic interpretation concerning the effects of PIP_2. When the positively charged segment cannot interact with the inner leaflet phospholipids, as for instance in the presence of polyamines, TREK-1 is inactive and stretch or internal acidification cannot promote channel activity (Fig. 1A). When PIP_2 neutralizes the positively charged

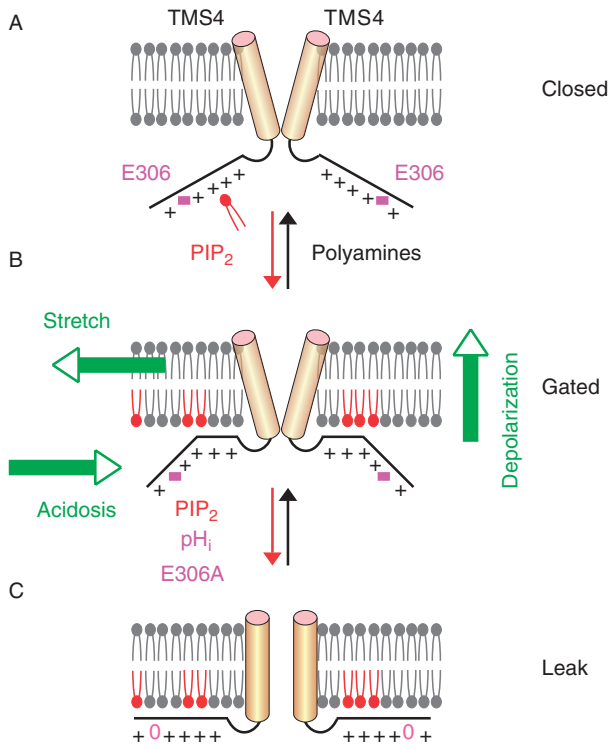

FIGURE 1 Model of TREK-1 gating. (A) Positive charges in the C-terminal domain of TREK-1 are critically required for PIP_2 stimulation. In the 5 + A mutant, R297, K301, K302, K304, and R311 are substituted by an alanine. In the E306A mutant, glutamate at position 306 is substituted by an alanine. In the presence of polylysine (pL) or endogenous polyamines, TREK-1 is in the closed state and not activable (closed state). (B) Phospholipids, including PIP_2, electrostatically interact with the positively charged cluster (5+) in the cytosolic proximal C-terminal domain of TREK-1. Insertion of PIP_2 in the inner leaflet of the bilayer controls coupling of the C-terminal domain of TREK-1 with the plasma membrane. When partially coupled, TREK-1 is in the closed state but activable by membrane stretch, depolarization, and cytosolic acidosis (gated state). (C) This membrane interaction is favored when the negative charge of the proton sensor E306 is masked by either protonation at acidic pH_i or by substitution with an alanine (E306A). The E306A mutant is locked open and behaves as a leak K^+ channel. Similarly, in the presence of exogenous phospholipids, cytosolic acidosis irreversibly locks TREK-1 open (leak state).

C-terminal segment, it inserts in the membrane near the channel making it activable by stretch, depolarization, or cytosolic acidification (Fig. 1B). This model proposes that E306 plays an important regulatory role in the interaction between membrane phospholipids and the C-terminal domain of

TREK-1 (Fig. 1C). Protonation of E306 makes the region more positive, and probably increases the electrostatic interaction with the negative charges of inner leaflet phospholipids. The interaction is then sufficiently strong that dissociation rate is slower than the fluid exchange time when the cytosolic pH is brought back to normal. The TREK-1 channel becomes constitutively open (Chemin *et al.*, 2005b). Removal of the negative charge of E306 by substituting an alanine mimics the effect of acidic pH_i (Fig. 1C).

PI that lacks the phosphates on the inositol group stimulates TREK-1 channel activity. Furthermore, PS and PE (Fig. 6A) that do not belong to the phosphoinositide family, but are also inner leaflet phospholipids, stimulate TREK-1 (Chemin *et al.*, 2005b). Similar to PIP_2, these phospholipids lock TREK-1 in the open conformation during cytosolic acidosis (Chemin *et al.*, 2005b). The presence of a large polar head is not an absolute requirement since PA (Fig. 6A) also stimulates TREK-1 after a previous polylysine treatment (Chemin *et al.*, 2005b). However, the negative phosphate group at position 3 of the glycerol is critical as DG (Fig. 6A) does not stimulate channel activity. Thus, although PIP_2 is generally considered as the key phospholipid for regulation of many ion channels, other inner leaflet phospholipids such as PI, PE, or PS can fulfill the same role (Chemin *et al.*, 2005b; Fig. 6).

TREK-1 conduction has an intrinsic voltage-dependency (Bockenhauer *et al.*, 2001; Maylie and Adelman, 2001; Maingret *et al.*, 2002; Chemin *et al.*, 2005b; Lopes *et al.*, 2005). When TREK-1 is locked open by PIP_2, the outward rectification disappears. This may reflect modification of the local electric field by the negative charge on PIP_2.

Glutamate is a major excitatory neurotransmitter in the central nervous system via ionotropic receptors. Glutamate also activates metabotropic receptors (mGluRs) that modulate neuronal excitability and synaptic transmission, resulting in persistent depolarization and increased cell firing. mGluR1 and mGluR5 are located primarily in postsynaptic areas where they can tonically modify cellular excitability. Group I mGluRs are coupled to Gq that in turn stimulates phospholipase C (PLC), increasing phosphoinositide hydrolysis and the generation of inositol trisphosphate ($InsP_3$) and DG. TREK-1 channel activity, recorded in the presence of AA, is reversibly inhibited by DG and this mechanism may contribute to the down-modulation of TREK-1 by stimulation of mGluR1 and mGluR5 (Chemin *et al.*, 2003). Furthermore, wortmannin (that inhibits PI 4-kinase, depleting PIP_2) inhibits TREK-1 and slows recovery of TREK-1 from Gq-coupled receptor-induced inhibition (Lopes *et al.*, 2005). PIP_2 hydrolysis may thus also contribute to Gq-coupled receptor-mediated TREK-1 inhibition (Lopes *et al.*, 2005).

IV. TREK-1 INHIBITION BY MEMBRANE PHOSPHOLIPIDS

Intracellular PIP_2 stimulates TREK-1 in 46% of patches (Chemin *et al.*, 2005b; Fig. 2A and C), while it inhibits the other 54% (Fig. 2B and C). This dual behavior is observed with excised patches from cells of the same culture dish and is independent of the level of channel expression. A transient stimulation sometimes occurs before complete inhibition (Fig. 2B). The stimulation

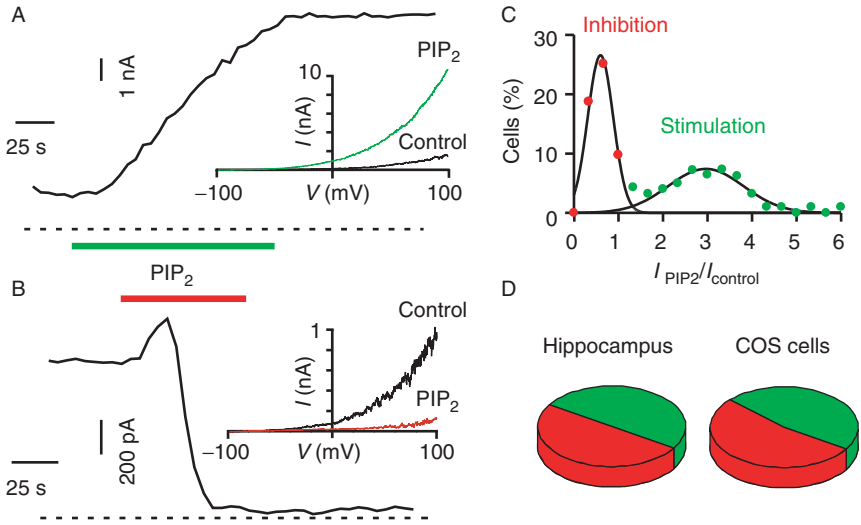

FIGURE 2 Dual regulation of TREK-1 by PIP_2. (A) Inside-out patch excised from a transiently transfected COS cell expressing TREK-1. The holding potential was −80 mV and the patch was stimulated every 5 s by a voltage ramp of 600 ms in duration from −100 to 100 mV. The current amplitude was measured at 90 mV. 5 μM PIP_2 added intracellularly as indicated by a horizontal green line stimulates TREK-1 channel activity. The horizontal dashed line indicates the zero current. The inset illustrates the stimulatory effect of PIP_2 on the I–V curve of TREK-1 recorded in a physiological K^+ gradient. (B) Same in a patch where TREK-1 is inhibited by PIP_2. The application of PIP_2 (5 μM) is indicated by a red line. (C) Relative distribution of the differential effect of PIP_2 (5 μM) on TREK-1 channel activity (bins represent 33% of variation, $n = 81$). Two populations of patches were identified showing inhibition and stimulation. Both populations were fitted with Gaussian curves. The wheel representation illustrates the percentage of patches showing stimulation (green) and inhibition (red) ($n = 81$). In stimulated and inhibited patches $I_{PIP2}/I_{control}$ was 3.4 ± 0.7 ($n = 37$) and 0.3 ± 0.03 ($n = 44$), respectively. (D) Effect of PIP_2 on TREK-1 expressed in infected cultured hippocampal neurons ($n = 8$) and transfected COS cells. The inhibitory and stimulatory effects of PIP_2 are illustrated in red and green, respectively. In stimulated and inhibited patches from hippocampal neurons $I_{PIP2}/I_{control}$ was 3.3 ± 0.6 ($n = 4$) and 0.08 ± 0.05 ($n = 4$), respectively.

or inhibition persists after washout (Fig. 2A and B), suggesting that the channel-associated PIP_2 has a very slow washout rate. The bimodal effect is not specific to COS7 cells, since we found similar results in hippocampal neurons that express TREK-1 (Fig. 2D). Inhibition by PIP_2 decreases both basal activity and mechanically stimulated activity (Fig. 3A–C). TREK-1 activation by intracellular acidosis (Fig. 4A and B) or AA (Fig. 4C and D) is similarly inhibited by PIP_2. However, it is only when TREK-1 was first activated by AA that the inhibitory effect of PIP_2 was completely reversible ($n = 6$; Fig. 4C). Competition of the negative AA with PIP_2 may increase the dissociation rate.

Polylysine converts the inhibitory effect of PIP_2 to stimulation (Fig. 5A–D). Remarkably, the dose–effect curve of PIP_2 is biphasic, with net stimulation at lower concentration and inhibition at higher concentrations (Fig. 5D).

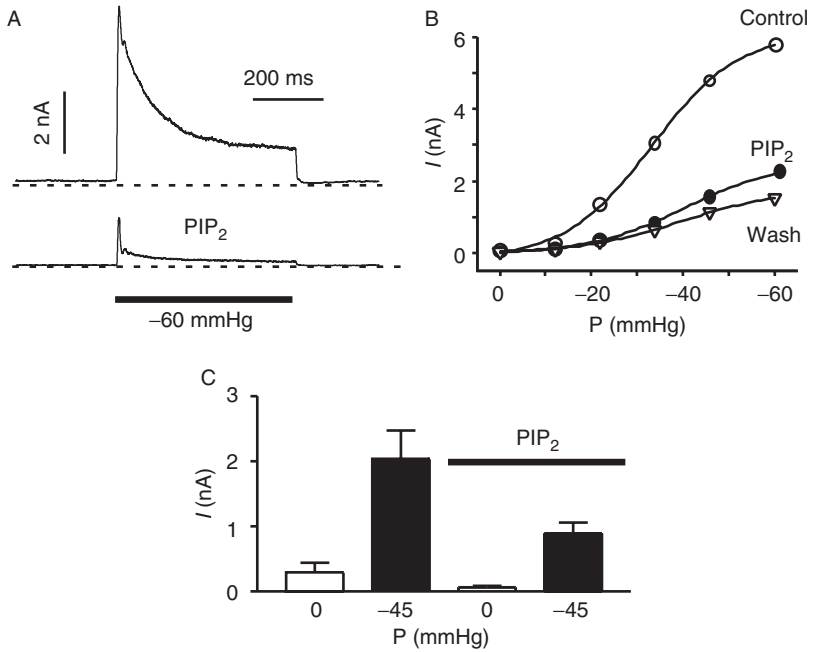

FIGURE 3 PIP_2 inhibits TREK-1 activation by membrane stretch. (A) Effect of PIP_2 (5 μM) in an inside-out patch excised from a transfected COS cell at a holding potential of 0 mV. A membrane stretch of −60 mmHg was applied as indicated by a horizontal line. (B) Pressure–effect curve of TREK-1 in control ($P_{0.5}$: −33.2 mmHg, k: 9.5), in the presence of intracellular PIP_2 (5 μM; $P_{0.5}$: −40.8 mmHg, k: 10.6), and after washout ($P_{0.5}$: −39.7 mmHg, k:11.4). $P_{0.5}$ is the midpoint of the pressure-effect curve and k is the slope factor. Same patch as A. (C) Effect of PIP_2 (5 μM) on TREK-1 current amplitude measured at rest and during a stimulation of −45 mmHg ($n = 10$).

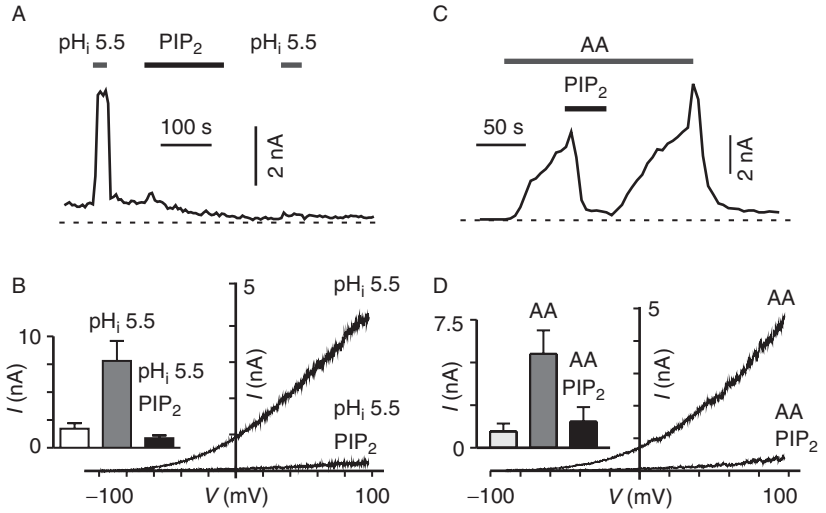

FIGURE 4 PIP_2 inhibits TREK-1 activation by acidic intracellular pH and the polyunsaturated fatty acid AA. (A) Effect of PIP_2 (5 μM) on the acidic (pH_i 5.5) activation of TREK-1. Currents were measured at 90 mV during voltage ramps. (B) TREK-1 current at pH_i 5.5 in the absence and in the presence of PIP_2 (5 μM). The holding potential was −80 mV and the patch was stimulated every 5 s by a voltage ramp of 600 ms in duration from −100 to 100 mV. The current amplitude was measured at 90 mV ($n = 18$). (C) Effect of PIP_2 (5 μM) in the presence of 10-μM AA applied intracellularly. (D) TREK-1 currents stimulated by AA in the presence and in the absence of PIP_2 (5 μM). The holding potential was −80 mV and the patch was stimulated every 5 s by a voltage ramp of 600 ms in duration from −100 to 100 mV. The current amplitude was measured at 90 mV ($n = 6$).

Either there are heterogeneous sites of interaction or there is drug accumulation at a multioccupancy "site." Inhibition is not specific for PIP_2 with PI being the most potent inhibitor (Fig. 6A–D).

When the first application of phospholipids (shown in red) does not produce a significant effect on current amplitude (see for instance PE and PS; Fig. 6B–D), a brief treatment with polylysine leads to dramatic stimulation during a second application (Fig. 6B–D, shown in green). If one remembers the earlier finding that PIP_2 stimulates at low concentration and inhibits at high concentration, then an immediate conclusion is that polylysine is probably reducing the number of PIP_2 molecules interacting with the channel, particularly at sites responsible for the inhibitory effect. As pointed out above, the polar head of the phospholipid is not critical for channel inhibition as PA also inhibits TREK-1 (Fig. 6D). However, the phosphate at position 3 of the glycerol moiety apparently is required (as observed for

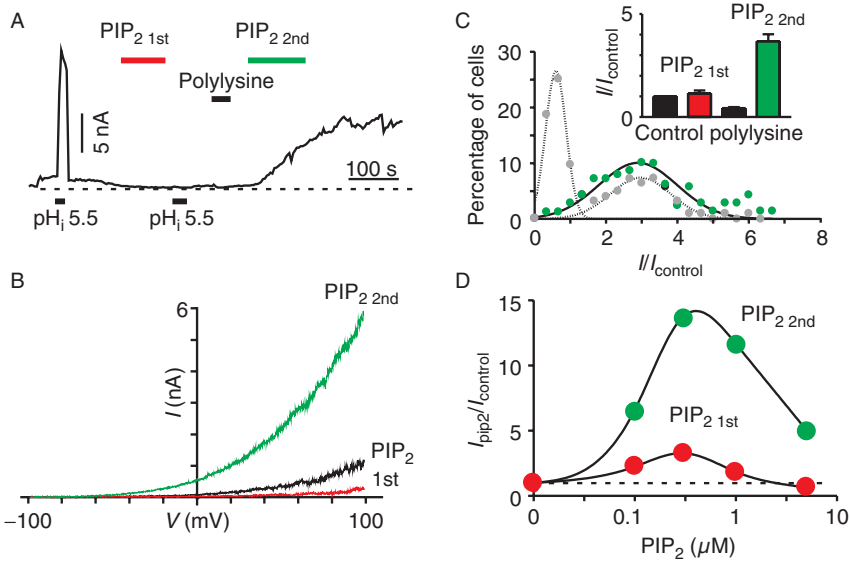

FIGURE 5 Polylysine lowers PIP_2 inhibition. (A) Effect of PIP_2 (5 μM) before (1st) (shown in red) and after (2nd) polylysine pretreatment (30 μg/ml; shown in green). The patch was stimulated by intracellular acidosis to pH_i 5.5 before and after the first application of PIP_2. The current amplitude was measured at 90 mV. (B) *I-V* curves corresponding to the experiment illustrated in A. (C) Distribution of the differential effect of PIP_2 (5 μM) on TREK-1. Before polylysine pretreatment, two populations of patches were identified showing inhibition and stimulation (gray circles). Both populations were fitted with Gaussian curves. The green circles indicate the distribution of the patches after polylysine pretreatment (30 μg/ml for 1 min). The inset shows the effect of polylysine pretreatment on the magnitude of the effect of PIP_2 (5 μM) on inside-out patches excised from TREK-1 transfected COS cells ($n = 68$). Patches with both stimulatory and inhibitory PIP_2 responses were averaged. (D) Dose–effect curve of PIP_2 on TREK-1 expressed in COS cells before (red) and after (green) pretreatment with polylysine (30 μg/ml for 1 min) on the same inside-out patch excised from a TREK-1 transfected COS cell. In these experiments, inside-out patches were held at −80 mV and stimulated by voltage ramps of 600 ms in duration from −100 to 100 mV every 5 s.

TREK-1 stimulation), since DG fails to affect the basal TREK-1 current (Fig. 6D shown in red).

When all five basic residues required for the stimulatory effect of PIP2 (Fig. 1) are substituted by alanines, inhibition by PIP_2 is not affected (Fig. 7A–D). Similarly, deletion of the N-terminal domain fails to affect either the inhibitory or stimulatory effect of PIP_2 (Fig. 7A and B). The C-terminal domain can be progressively truncated (leaving intact the polycationic cluster), removing PIP_2 inhibition but maintaining PIP_2 stimulation (Fig. 7A and B and 7C inset). The deletion of the same distal C-terminal domain (deletion of the last

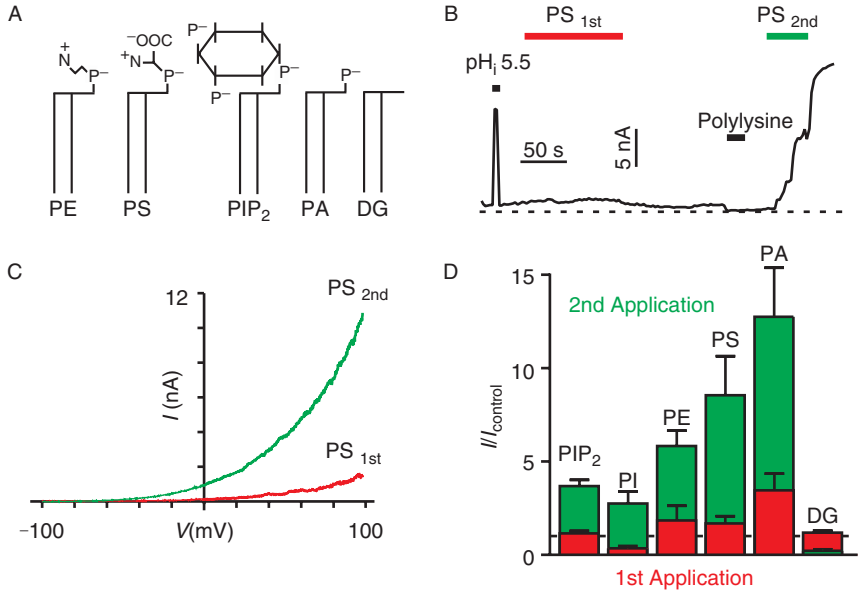

FIGURE 6 Specificity of the effect of membrane phospholipids on TREK-1. (A) Diagram of the different phospholipid molecules. (B) Effect of PS (5 μM) on TREK-1 channel activity. PS was applied before and after polylysine (30 μg/ml) treatment. The patch was stimulated by pH_i 5.5 before PS application. In this experiment, the inside-out patch was held at −80 mV and stimulated by voltage ramps of 600 ms in duration from −100 to 100 mV every 5 s. The current amplitude was measured at 90 mV. (C) *I–V* curves recorded with voltage ramps showing the effect of PS (5 μM) before (shown in red) and after (shown in green) polylysine treatment (30 μg/ml). Same patch as B. (D) Effect of phospholipids (5 μM) on TREK-1. PIP_2 ($n = 81$), PI ($n = 14$), PE ($n = 12$), PS ($n = 9$), PA ($n = 18$), DG ($n = 11$) before (red bars) and PIP_2 ($n = 68$), PI ($n = 11$), PE ($n = 5$), PS ($n = 14$), PA ($n = 6$), DG ($n = 5$) after polylysine treatment (30 μg/ml for 1 min; green bars). Patches with both stimulatory and inhibitory PIP_2 responses were averaged.

100 amino acids) shifts the pressure–effect curve of TREK-1 toward more negative pressures (Maingret *et al.*, 1999b). In other words, truncation of TREK-1 gradually makes channels more resistant to stretch and leads to a decrease in inhibition by PIP_2.

A possible mechanism to explain PIP_2 inhibition is membrane insertion. Amphipaths can alter membrane curvature by inserting asymmetrically into one monolayer or another (Sheetz and Singer, 1974). This partitioning has two effects: compression of the lipids in that monolayer (since the opposing monolayer has not expanded) and the lateral expansion pressure causing curvature toward the other monolayer. If either of these effects favors the

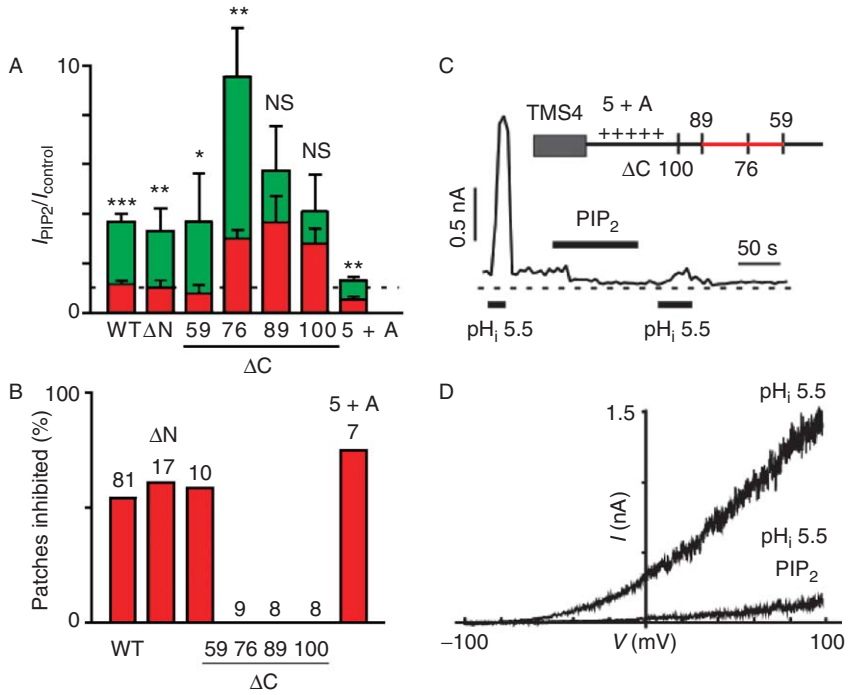

FIGURE 7 The inhibitory effect of PIP_2 is abolished by truncation of the C-terminal domain. (A) Effect of PIP_2 (5 μM) on TREK-1 WT and N- and C-terminal mutants before (red) and after (green) polylysine treatment. Patches with both stimulatory and inhibitory PIP_2 responses were averaged. WT ($n = 81$), ΔN ($n = 17$), ΔC59 ($n = 10$), ΔC76 ($n = 9$), ΔC89 ($n = 8$), ΔC100 ($n = 8$), 5 + A ($n = 7$) before and WT ($n = 68$), ΔN ($n = 8$), ΔC59 ($n = 3$), ΔC76 ($n = 9$), ΔC89 ($n = 4$), ΔC100 ($n = 4$), 5 + A ($n = 18$) after polylysine treatment (30 μg/ml for 1 min). Statistical differences between PIP_2 effects before and after polylysine treatment are indicated by stars (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (B) Percentage of patches inhibited by PIP_2 (5 μM) when applied in the absence of polylysine treatment (first application). The number of experiments is indicated. (C) Effect of PIP_2 (5 μM) on the 5 + A mutant. The patch was stimulated by pH_i 5.5 before and after PIP_2 application. In this experiment, the inside-out patch was held at −80 mV and stimulated by voltage ramps of 600 ms in duration from −100 to 100 mV every 5 s. The current amplitude was measured at 90 mV. The inset shows an illustration of the mutants in the C-terminal domain. In the 5 + A mutant, residues R297, K101, K102, K104, and R111 were substituted with alanines. This cluster of positive charges is required for the stimulatory effect of PIP_2 (shown in green), while the region between Ala352 and Thr322 (between ΔC59 and ΔC89) in the C-terminal domain is required for inhibition (shown in red). (D) *I–V* curves showing the effect of intracellular pH 5.5 before and after PIP_2 (5 μM) stimulation. Same patch as C.

open state, amphipaths will activate the channels. Anionic amphipaths such as AA preferentially insert in the external leaflet of the bilayer because of the electrostatic repulsion with the inner leaflet anionic PS (Sheetz and Singer, 1974; Patel *et al*., 1998). AA and other anionic amphipaths including trinitrophenol are potent openers of TREK-1 (Patel *et al*., 1998, 2001). Conversely, binding of amphipaths to the inner monolayer can explain inhibition by the cationic amphipaths including chlorpromazine and tetracaine (Patel *et al*., 1998). Intracellular PIP_2 inhibits TREK with patches precurved by positive or negative hydrostatic pressures (Honoré, unpublished data). Thus, if amphipath-induced curvature is important to TREK activity, it must be local to the channels.

Why PIP_2 stimulates some patches and inhibits others remains unclear. There are hundreds of active channels in a patch, so the variability cannot be a result of sampling. If there are heterogeneous domains in the membrane with different lipid compositions or different TREK-1 assemblies including with the cytoskeleton, the domains must be of patch dimensions. Possibly, the heterogeneity is the result of differences in global stress, resulting from different balances in cytoskeletal stress and adhesion energy of the seal that cause whole patches to vary in their properties. Activation and inhibition by PIP_2 occur at different PIP_2 concentrations, requiring that two or more PIP_2 molecules/channels are involved. This complex behavior can arise from the existence of two different types of binding sites: a specific one, the proximal cationic cluster, and less specific "sites" such as the boundary lipid domain (Suchyna *et al*., 2004) that can absorb multiple PIP_2 molecules. Alternatively, it may be that binding of PIP_2 to one of the monolayer may lead to saturation and then favor PIP_2 flipping to the other side of the bilayer with opposite curvature effects.

Is the dual modulation of TREK-1 activity observed by direct application of PIP_2 related to TREK-1 regulation by neurotransmitters? PIP_2 is dynamically regulated by kinases and phospholipases, and Gq-coupled receptors stimulate PLC, causing hydrolysis of PIP_2 and generating second messengers DG and $InsP_3$. PIP_2 depletion has been proposed as the mechanism of Gq-related inhibitory effects on TREK-1 (Lopes *et al*., 2005). Since PIP_2 can cause inhibition or stimulation of TREK-1 activity, *in situ* hydrolysis under the influence of hormones or neurotransmitters could create very complex and diverse responses in neurons. Different types of regulation by a given neurotransmitter type could be spatially distributed among the different types of neuronal compartments such as the soma, dendrites, growth cones, or nerve terminals. This has not yet been systematically investigated. At present, activation of Gq-coupled receptors, such as mGluR1 and mGluR5 receptors, has only been seen to cause consistent inhibition of TREK-1 (Chemin *et al*., 2003). It should also be kept in mind that other pathways have also been

proposed to be involved in the regulation of K_{2P} channels by membrane receptors to hormones and neurotransmitters (Chemin *et al.*, 2003; Murbartian *et al.*, 2005; Chen *et al.*, 2006).

In conclusion, gating of TREK-1 is tightly linked to the regulation by membrane phospholipids. Similar types of regulation exist in other families of ion channels, particularly TRPV, which like the TREK channels may serve as sensors of stretch, osmotic pressure, pH, and temperature (Voets and Nilius, 2003).

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