

# PIP<sub>2</sub> hydrolysis underlies agonist-induced inhibition and regulates voltage gating of two-pore domain K<sup>+</sup> channels

Coeli M. B. Lopes<sup>1</sup>, Tibor Rohács<sup>1</sup>, Gábor Czirják<sup>2</sup>, Tamás Balla<sup>3</sup>, Péter Enyedi<sup>2,3</sup>  
and Diomedes E. Logothetis<sup>1</sup>

<sup>1</sup>Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029, USA

<sup>2</sup>Department of Physiology, Semmelweis University, Budapest, H-1444, Hungary

<sup>3</sup>Endocrinology and Reproduction Research Branch, NICHD, NIH, Bethesda, MD 20892, USA

Two-pore (2-P) domain potassium channels are implicated in the control of the resting membrane potential, hormonal secretion, and the amplitude, frequency and duration of the action potential. These channels are strongly regulated by hormones and neurotransmitters. Little is known, however, about the mechanism underlying their regulation. Here we show that phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) gating underlies several aspects of 2-P channel regulation. Our results demonstrate that all four 2-P channels tested, TASK1, TASK3, TREK1 and TRAAK are activated by PIP<sub>2</sub>. We show that mechanical stimulation may promote PIP<sub>2</sub> activation of TRAAK channels. For TREK1, TASK1 and TASK3 channels, PIP<sub>2</sub> hydrolysis underlies inhibition by several agonists. The kinetics of inhibition by the PIP<sub>2</sub> scavenger polylysine, and the inhibition by the phosphatidylinositol 4-kinase inhibitor wortmannin correlated with the level of agonist-induced inhibition. This finding suggests that the strength of channel PIP<sub>2</sub> interactions determines the extent of PLC-induced inhibition. Finally, we show that PIP<sub>2</sub> hydrolysis modulates voltage dependence of TREK1 channels and the unrelated voltage-dependent KCNQ1 channels. Our results suggest that PIP<sub>2</sub> is a common gating molecule for K<sup>+</sup> channel families despite their distinct structures and physiological properties.

(Resubmitted 21 December 2004; accepted after revision 24 January 2005; first published online 27 January 2005)

**Corresponding authors** D. E. Logothetis: Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029, USA. Email: diomedes.logothetis@mssm.edu

P. Enyedi: Department of Physiology, Semmelweis University, Budapest, Hungary, H-1444. Email: enyedi@puskin.sote.hu

K<sup>+</sup> channels are the largest and most diverse family of ion channels. They fall into three main groups based on their structural properties: voltage-gated (6 transmembrane or 6TM), inward rectifiers (2TM) and two-pore domain (4TM) channels. While all these channels are highly selective for K<sup>+</sup> over other ions, they exhibit distinct physiological and biophysical properties.

Recently, two-pore (2-P) domain potassium channels have been shown to serve as molecular determinants of several leak K<sup>+</sup> currents. Leak K<sup>+</sup> currents serve to establish the resting membrane potential and modify the duration, frequency and amplitude of action potentials. The activity of 2-P domain channels is strongly regulated by a number of agents such as protons, protein kinases, and temperature (Lopes *et al.* 2000; Kim *et al.* 2001). In addition, three members of this family TREK1 (KCNK2), TREK2 (KCNK10)

and TRAAK (KCNK4) are shown to be mechano-sensitive. They are also targets of receptor-mediated regulation by neurotransmitters and hormones (for review see Lesage & Lazdunski, 2000; Goldstein *et al.* 2001; Patel & Honore, 2001). This modulation appears to be important for a number of physiological processes, including aldosterone secretion (Czirjak *et al.* 2000) and regulation of neuronal activity (Talley *et al.* 2000; Brickley *et al.* 2001). Some members of this ion channel family are activated by volatile anaesthetics (Patel *et al.* 1999; Sirois *et al.* 2000), and contribute to the effect of these pharmacological agents on neural activity (Sirois *et al.* 2000).

Modulation of channel activity by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub> or PIP<sub>2</sub>) has recently emerged as a crucial aspect of ion channel regulation (Hilgemann & Ball, 1996; Baukrowitz *et al.* 1998; Huang *et al.* 1998; Sui *et al.* 1998; Shyng & Nichols, 1998; Zhang *et al.* 1999; Kobrinsky *et al.* 2000; Chuang *et al.* 2001; Runnels *et al.* 2002; Wu *et al.* 2002; for review see Hilgemann *et al.*

C. M. B. Lopes and T. Rohács contributed equally to this work.

2001). Autonomic receptors ( $\alpha_1$ -adrenergic and  $M_1/M_3$  muscarinic) and angiotensin II receptors (AT1), among others, can couple to G proteins of the  $G_q/G_{11}$  family and, when activated, stimulate phospholipase C (PLC). Since PLC hydrolyses  $PIP_2$ , its stimulation causes local reduction of  $PIP_2$  levels in the plasma membrane (Stauffer *et al.* 1998; Varnai & Balla, 1998; van der Wal *et al.* 2001). For both inwardly rectifying (Kir) and voltage-gated KCNQ potassium channels, agonist-induced inhibition can be observed when the levels of the phospholipid fall below a critical level for maintenance of channel activity (Baukrowitz *et al.* 1998; Xie *et al.* 1999; Kobrinsky *et al.* 2000; Zhang *et al.* 2003). Four members of the 2-P domain family, TASK1 (KCNK3), TASK3 (KCNK9), TREK1 (KCNK2) and TREK2 (KCNK10) have been reported to be inhibited by agonists that activate  $G_q/G_{11}$  proteins both in native and recombinant systems (Talley *et al.* 2000; Millar *et al.* 2000; Czirjak *et al.* 2001; Czirjak & Enyedi, 2002; Talley & Bayliss, 2002; Chemin *et al.* 2003). Here we show that TASK1, TASK3 and TREK1, but not TRAAK, are inhibited by muscarinic  $M_1$  stimulation. Although agonist-induced inhibition of TASK1 channels depended on PLC activation, downstream signals (inositol 1,4,5-trisphosphate, cytoplasmic  $Ca^{2+}$  and diacylglycerol) did not mediate the inhibition (Czirjak *et al.* 2001). Recently it was suggested that agonist-induced inhibition of TASK1 and TASK3 channels was mediated by  $PIP_2$  hydrolysis, but TREK1 and TREK2 current inhibition was mediated by a different mechanism (Chemin *et al.* 2003). In contrast, our results indicate that  $PIP_2$  hydrolysis underlies ACh-induced inhibition of TREK1 as well as TASK1 and TASK3 channels. In addition, we show that  $PIP_2$  activation of TRAAK channels is dependent on mechanical stimulation.

The present study demonstrates that  $PIP_2$  activation is a conserved property of members of the 2-P potassium channel family. We show that all tested members of the 2-P domain potassium channel family were activated by  $PIP_2$ . The extent of agonist-induced inhibition observed by stimulation of  $G_q/G_{11}$  coupled receptors correlated well with both the kinetics of block by the  $PIP_2$  scavenger polylysine (poly Lys) and the extent of inhibition by the phosphatidylinositol 4-kinase (PI 4-kinase) inhibitor wortmannin. These findings suggested a direct role of  $PIP_2$  hydrolysis in agonist-induced inhibition and showed that the extent of inhibition depended on the sensitivity of the channel for  $PIP_2$ . In addition, we show that  $PIP_2$  hydrolysis changed the voltage dependence of TREK1 channels. We also demonstrated the involvement of  $PIP_2$  in the regulation of 2-P channels by mechanical stimulation. Our findings indicate that  $PIP_2$  is critical for both activity and modulation of 2-P domain channels. In addition, we show that  $PIP_2$  modulates the voltage dependence of both 2-P channels and voltage-gated KCNQ channels, despite their distinct structures and physiological properties.

These results suggest a general role for  $PIP_2$  in the regulation of all major families of  $K^+$  channels.

## Methods

### Molecular biology

The human TASK1, rat TASK3, mouse TREK1 and the mouse TRAAK cDNA constructs were subcloned into the pEXO vector (Duprat *et al.* 1997). The human KCNQ1, human KCNE1, human  $M_1$  muscarinic receptor and the human type I  $IP_3$  5-phosphatase were subcloned into the pGEMHE plasmid vector (Liman *et al.* 1992). Both vectors contain the 5'- and 3'-untranslated region of the *Xenopus*  $\beta$ -globin gene to obtain optimal expression in *Xenopus* oocytes. The cDNA was linearized and cRNA was prepared using the Ambion mMESSAGE mMACHINE T7 kit. Point mutants were produced by Pfu based mutagenesis using the QuikChange™ kit (Stratagene Inc., La Jolla, CA, USA).

### Electrophysiology

*Xenopus* oocytes were surgically removed from adult females under anaesthesia (0.4% 3-aminobenzoic acid ethyl ester). Frogs were humanely killed after the final oocyte collection. The experiments were carried out with the approval of the local animal care committee (IACUC). Oocytes were isolated using collagenase digestion and injected with 0.5–15 ng of cRNA in 50 nl of sterile water. Macropatch measurements in the inside-out configuration were performed as described (Rohacs *et al.* 2002). Electrodes for oocyte experiments contained (mM): 96 NaCl, 2 KCl, 1  $CaCl_2$ , 1  $MgCl_2$ , 5 Hepes, pH 7.4. For assessment of kinetics of block, inside-out patches were perfused with poly Lys and  $PIP_2$  antibody ( $PIP_2$ Ab) in FVPP solution (mM: 96 KCl, 5 EDTA, 10 Hepes, 5 NaF, 3  $Na_3VO_4$ , 10  $Na_2PO_7$ , pH 7.4 with NaOH), which retarded hydrolysis of  $PIP_2$  and thus stabilized currents (Huang *et al.* 1998). For the  $PIP_2$  activation measurements the perfusion solution contained (mM): 96 KCl, 5 EGTA, 10 Hepes, pH 7.40 (bath solution). Unless otherwise specified, a ramp protocol from  $-100$  to  $+100$  mV was used ( $1$  mV  $ms^{-1}$ ) with a holding potential of  $-80$  mV. Negative pressure applied to the inside-out patches was measured using a BIO-TEK (Winooski, VT, USA) pneumatic transducer tester. Whole-oocyte currents were measured by conventional two-microelectrode voltage-clamp as described (Czirjak *et al.* 2001) unless specified otherwise.

Poly Lys (Sigma, St Louis, MO, USA) with an average molecular weight of 8 kDa was used. Poly Lys has a limited solubility in FVPP solutions; a saturated solution was made by adding  $30$   $\mu g$   $ml^{-1}$  poly Lys and centrifuging the solution (Rohacs *et al.* 2002).  $DiC_8$   $PI(4,5)P_2$  was

purchased from Echelon Research Laboratories Inc. (Salt Lake City, UT, USA) and from Cayman Chemical (Ann Arbor, MI, USA). DiC<sub>8</sub> PIP<sub>2</sub> was dissolved in the bath solution to the indicated concentrations. PIP<sub>2</sub> antibody (Assay Designs, Ann Arbor, MI, USA) was diluted 1:50 in FVPP solutions. Arachidonyl stearyl (AASt) PI(4,5)P<sub>2</sub> was purchased from Roche Molecular Biochemicals and was dissolved in water and sonicated as described (Rohacs *et al.* 2002). Diacylglycerol analogues 1-stearoyl-2-arachidonoyl-sn-glycerol (SAG) and 1-stearoyl-2-linoleoyl-sn-glycerol (SLG) were purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA, USA). They were dissolved in DMSO (20 mM) and stored at -80°C. Before each experiment SAG and SLG were dissolved in bath solution described earlier, and sonicated for 30 min. DMSO was included in all other solutions for these experiments to avoid any artifact caused by the solvent.

Error bars in the figures represent s.e.m. Each experiment shown or described was performed on a minimum of five oocytes. Student's unpaired *t* test was used to assess statistical significance. In Fig. 4D and E, inhibition was calculated by extrapolating the current levels before and after SAG or SLG to the period during the drug application, and comparing this level to the actual current level.

## Results

### TASK1, TASK3, TREK1 and TRAAK channels are activated by PIP<sub>2</sub>

We investigated the effect of PIP<sub>2</sub> on four 2-P domain K<sup>+</sup> channels expressed in *Xenopus* oocytes (Fig. 1). For TASK1, following excision of macropatches in the inside-out mode, K<sup>+</sup> currents substantially decreased (ran down). Channel run-down has been correlated to PIP<sub>2</sub> hydrolysis for Kir channels (Hilgemann & Ball, 1996; Huang *et al.* 1998; Zhang *et al.* 1999). Application of 25 μM arachidonyl stearyl (AASt) PIP<sub>2</sub> activated these channels (Fig. 1A). Subsequent application of polylysine (poly Lys), which acts as a PIP<sub>2</sub> scavenger (Huang *et al.* 1998; Lopes *et al.* 2002; Rohacs *et al.* 2002), caused rapid and persistent current inhibition. TASK3 currents showed less pronounced run-down after excision, were persistently blocked by poly Lys application and were activated by AASt PIP<sub>2</sub> (5 μM) (Fig. 1B).

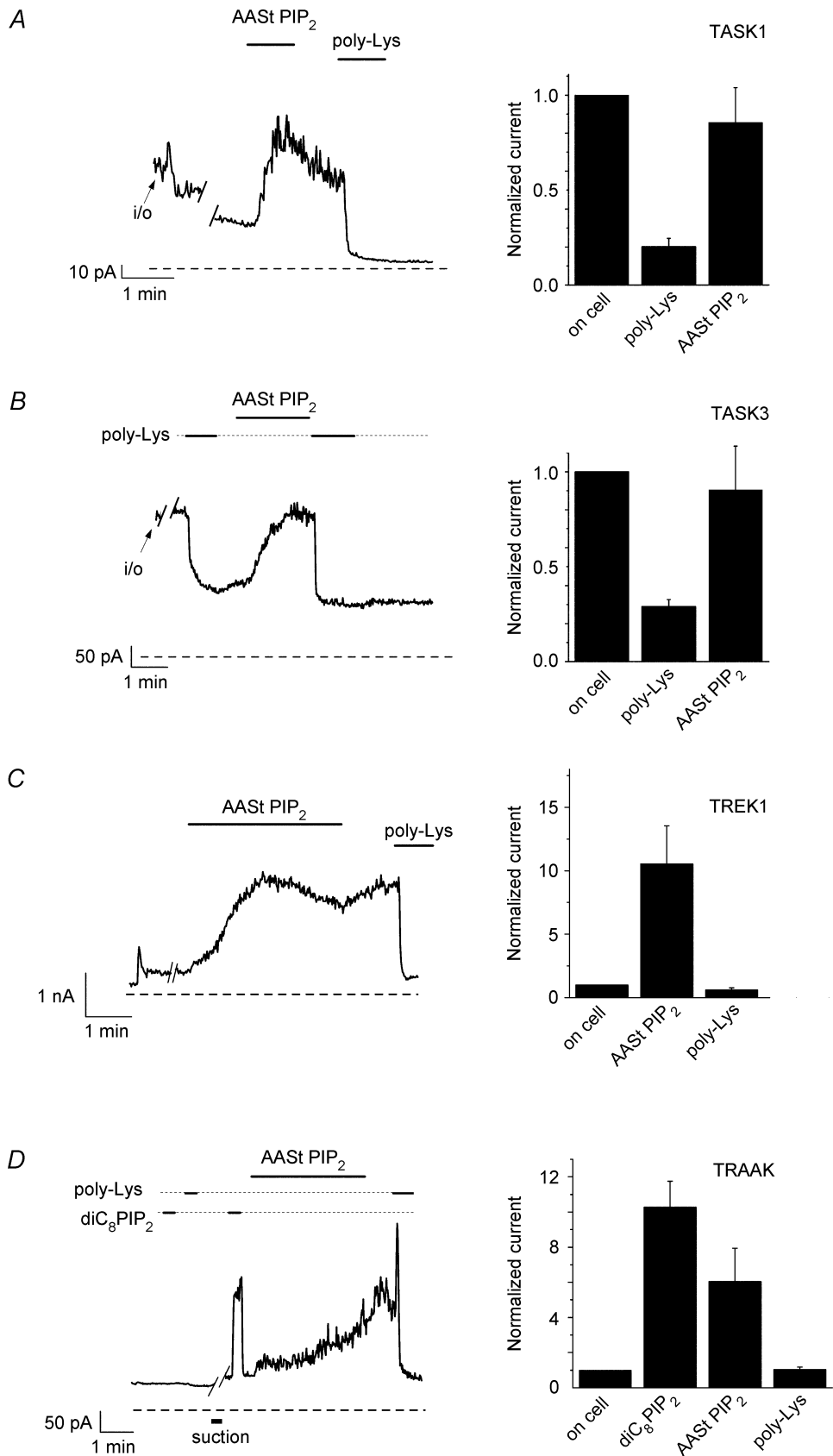
TREK1 and TRAAK are mechanosensitive members of the 2-P domain channel family. We tested whether these channels were also activated by PIP<sub>2</sub>. We expressed TREK1 and TRAAK channels in *Xenopus* oocytes and measured channel activity in inside-out macropatches. As the representative traces in Fig. 1C and D show, PIP<sub>2</sub> application activated both channels. For TREK1 in most cases (*n* = 7 out of 10) activation of the channel

occurred without application of a mechanical stimulus (suction) after seal formation (Fig. 1C). In the remaining three patches, PIP<sub>2</sub> activation could be observed only after the application of a mechanical stimulus to the patch following the seal formation (data not shown). For these experiments, a -16 mmHg negative pressure was applied to the patch pipette (tip diameter 4–5 μm) for 40–70 s. For TRAAK, currents were elicited by PIP<sub>2</sub> in most cases only after a mechanical stimulus had been applied (Fig. 1D, *n* = 4 out of 5). In one patch, activation of TRAAK currents occurred without application of a mechanical stimulus (data not shown). To test PIP<sub>2</sub> sensitivity before and after mechanical stimulation, we used short chain (diC<sub>8</sub>), water soluble PIP<sub>2</sub>, which activates ion channels in a reversible fashion (Rohacs *et al.* 2002, 2003; Zhang *et al.* 2003). DiC<sub>8</sub> PIP<sub>2</sub> activated these channels to a comparable extent to AASt PIP<sub>2</sub>, but with faster kinetics. Interestingly, the effect of suction on PIP<sub>2</sub> sensitivity persisted even after relieving application of the negative pressure. It should be noted, however, that mild negative pressure (2–5 mmHg) was necessary for seal formation in all experiments, and therefore it cannot be excluded that this promoted PIP<sub>2</sub> activation in cases where further suction was not required in the inside-out patch configuration. Suction applied to the patch pipette also increased channel activity in the cell-attached configuration with intact PIP<sub>2</sub> levels (data not shown). In non-injected oocytes 100 μM diC<sub>8</sub> PIP<sub>2</sub> did not activate any current before or after application of negative pressure (*n* = 6).

### TASK1, TASK3 and TREK1, but not TRAAK are sensitive to agonist-induced PIP<sub>2</sub> hydrolysis

Several G-protein coupled receptors are known to couple to PLC through G<sub>q</sub>/G<sub>11</sub>. Upon receptor stimulation PLC hydrolyses PIP<sub>2</sub>. Hydrolysis of PIP<sub>2</sub> inhibited the activity of several PIP<sub>2</sub>-dependent channels (Baukowitz *et al.* 1998; Xie *et al.* 1999; Kobrinisky *et al.* 2000; Zhang *et al.* 2003). The extent of current inhibition observed is dependent on the strength of channel-PIP<sub>2</sub> interactions (Kobrinisky *et al.* 2000; Zhang *et al.* 2003; Lopes *et al.* 2002). As we have seen, TASK1, TASK3 and TREK1 channels are activated by PIP<sub>2</sub>, whereas activation of TRAAK channels by PIP<sub>2</sub> generally happened only after mechanical stimulation. To test whether hydrolysis of PIP<sub>2</sub> inhibits these channels, we coexpressed all four channels with different G<sub>q</sub> coupled receptors and measured the degree of inhibition upon agonist stimulation.

We coexpressed TASK1 or TASK3 channels with either muscarinic (M<sub>1</sub>) or angiotensin II (AT1a) receptors in *Xenopus* oocytes. Channel inhibition upon activation of either of these heterologously expressed receptors or the endogenous lysophosphatidic acid (LPA) receptor was seen for both channels (Fig. 2A and B). The extent of



agonist-induced inhibition in all cases was lower for TASK3 channels than for TASK1 channels. TREK1 or TRAAK channels were coexpressed with M<sub>1</sub> receptors in *Xenopus* oocytes. TREK1 was potently inhibited by ACh application. TRAAK channels on the other hand were only minimally inhibited (10%) by the agonist-induced PIP<sub>2</sub> hydrolysis (Fig. 2C). The mechanism of this small inhibition is unclear; it is unlikely to be mediated by PIP<sub>2</sub> depletion. Endogenous Ca<sup>2+</sup>-activated Cl<sup>-</sup> currents were used to monitor M<sub>1</sub> receptor expression. The Cl<sup>-</sup> current develops quickly after ACh application, but inactivates rapidly, not contributing significantly to the currents measured in the later phases of the experiment. ACh inhibition of TREK1 channels is not affected by coexpression of M<sub>1</sub> receptors with IP<sub>3</sub> phosphatase, which abolishes IP<sub>3</sub>-induced Ca<sup>2+</sup> release and consequently the Cl<sup>-</sup> currents (data not shown). We attempted to stimulate whole-cell TRAAK currents in *Xenopus* oocytes by low osmolarity, shear-stress and direct mechanical stimulation (gentle prodding of the oocyte with thin forceps). None of these interventions resulted in increased current, due probably to the convoluted nature of the oocyte membrane or the presence of the vitelline layer. Therefore we were not able to test in the whole-cell configuration whether mechanical stimulation confers sensitivity to agonist-induced PIP<sub>2</sub> hydrolysis.

### Strength of channel-PIP<sub>2</sub> interactions with TASK and TREK-1 channels correlate with kinetics of recovery from inhibition

Poly Lys or PIP<sub>2</sub>Ab (an antibody against PI(4,5)P<sub>2</sub>) competes with channels for PIP<sub>2</sub>, thus scavenging PIP<sub>2</sub> away from channels and reducing current levels (Huang *et al.* 1998; Lopes *et al.* 2002; Rohacs *et al.* 2002; Zhang *et al.* 2003). Slower inhibition by PIP<sub>2</sub> scavengers is likely to reflect higher binding affinity of the channel to PIP<sub>2</sub> or alternatively, stronger allosteric coupling between PIP<sub>2</sub> molecules and the channel. Regardless of

the precise mechanism, kinetics of current inhibition by PIP<sub>2</sub> scavengers reflects a different apparent affinity of the channels for PIP<sub>2</sub>. We often refer to this apparent affinity as 'strength of channel-PIP<sub>2</sub> interactions' (Zhang *et al.* 1999, 2003; Kobrinsky *et al.* 2000; Lopes *et al.* 2002; Rohacs *et al.* 2002). To assess the apparent channel affinity for PIP<sub>2</sub> we measured the rate of current inhibition by poly Lys (Fig. 3A–C) and quantified the kinetics of the effect by measuring the percentage inhibition of the current after a 60 s poly Lys application (Fig. 3C). All three channels were fully inhibited by poly Lys suggesting that PIP<sub>2</sub> is required for channel activity and that PIP<sub>2</sub> electrostatically interacts with both channels. Spontaneous hydrolysis of PIP<sub>2</sub> by lipid phosphatases in excised patches causes run-down of PIP<sub>2</sub>-sensitive channels (Hilgemann & Ball, 1996; Huang *et al.* 1998; Zhang *et al.* 1999). To avoid run-down, this set of experiments were performed in FVPP solution (see Methods) to inhibit lipid phosphatases and prevent breakdown of endogenous phosphoinositides throughout the experiment (Huang *et al.* 1998; Lopes *et al.* 2002). TASK3 channels often exhibited some run-down after excision even in the presence of FVPP solutions. Experiments were performed after currents were stable. We found that the kinetics of poly Lys inhibition were the fastest for TREK1, followed by TASK1, then TASK3 channels. These findings suggest that the interactions with PIP<sub>2</sub> are the weakest for TREK1 channels, followed by TASK1 and TASK3 channels (Fig. 3C). We also tested the effect of PIP<sub>2</sub>Ab on TASK3 channels. PIP<sub>2</sub>Ab inhibited the channel with very slow kinetics, where the time to inhibit 50% of channel activity (*T*<sub>50</sub>) was 290 ± 50 s (*n* = 5) (Fig. 3D). In addition, PIP<sub>2</sub>Ab prevented poly Lys from inhibiting the channel (Fig. 3D, *n* = 6). Our results suggest that poly Lys inhibits the channel by specifically competing for PIP<sub>2</sub> binding and not by scavenging other negatively charged lipids. These data also suggest that PIP<sub>2</sub>Ab does not inhibit completely channel activity.

Wortmannin at low nanomolar concentrations specifically blocks phosphoinositide 3-kinases (PI 3-kinases) but at micromolar concentrations it also blocks the activity of several PI 4-kinases (Nakanishi *et al.* 1995).

### Figure 1. Activation of 2-P domain channels by PIP<sub>2</sub>

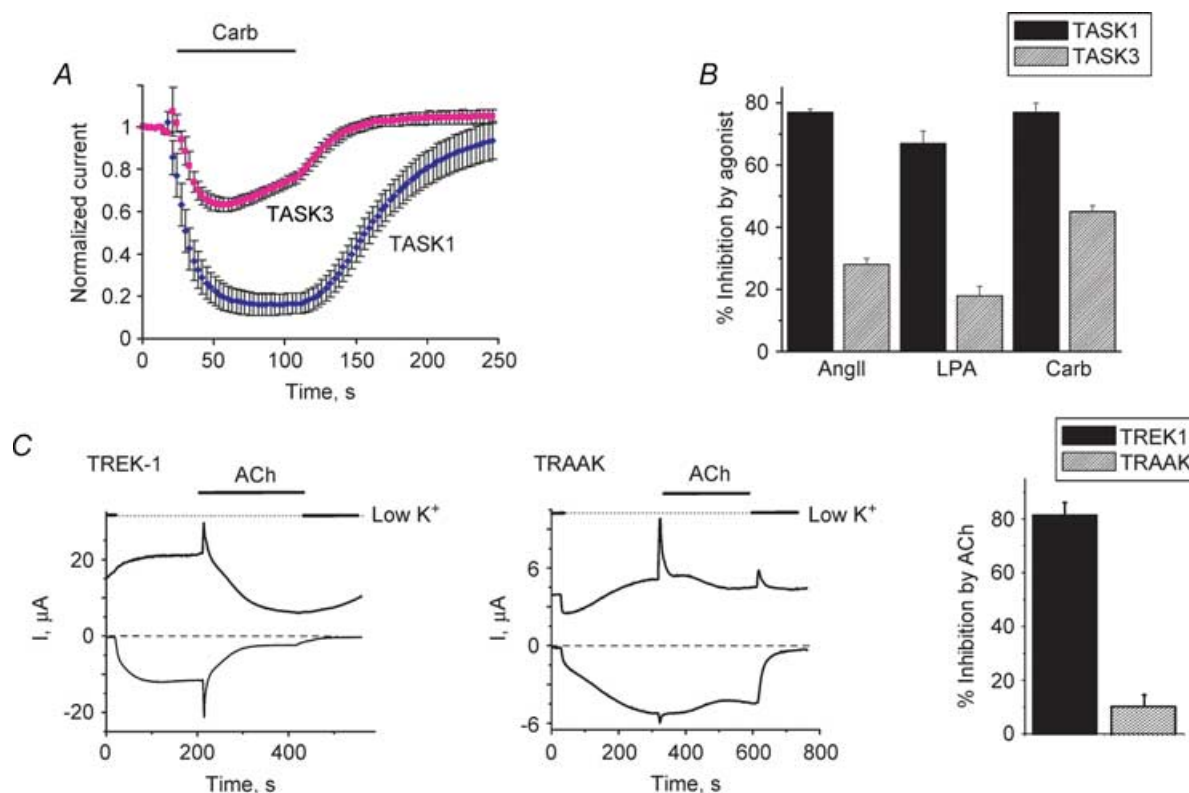
A, left, representative trace of TASK1 currents in an inside-out macropatch, measured at +100 mV. Applications of 25 μM AAST PIP<sub>2</sub> and 3 μg ml<sup>-1</sup> poly Lys are indicated by the horizontal lines. Right, statistical summary of the data. The average current level in the cell-attached configuration was 65 ± 21 pA (*n* = 3). B, left, a representative trace for TASK3 currents is shown, measured at +40 mV. Applications of 5 μM AAST PIP<sub>2</sub> and 3 μg ml<sup>-1</sup> poly Lys are indicated by the horizontal lines. Right, statistical summary of the data, normalized to the cell-attached current level. The average current in the cell-attached level was 580 ± 160 pA (*n* = 8). C, left, representative trace of TREK1 currents measured in macropatches at +100 mV. Applications of 2.5 μM AAST PIP<sub>2</sub> and 3 μg ml<sup>-1</sup> poly Lys are indicated by the horizontal lines. Right, statistical summary of the data (*n* = 6–10), normalized to the cell-attached level. The average current in the cell-attached level was 143 ± 35 pA (*n* = 10). D, left, representative trace for TRAAK currents is shown, measured in macropatches at +100 mV. Applications of 100 μM diC<sub>8</sub> PIP<sub>2</sub>, 5 μM AAST PIP<sub>2</sub> and 3 μg ml<sup>-1</sup> poly Lys are indicated by the horizontal lines. Right, statistical summary of the data (*n* = 4), normalized to the cell-attached current level. The average current in the cell-attached level was 80 ± 9 pA (*n* = 8).

Since PI 4-kinase is required for the synthesis of PI(4,5)P<sub>2</sub>, its inhibition interferes with replenishment of PI(4,5)P<sub>2</sub>, thus decreasing PI(4,5)P<sub>2</sub> levels (Zhang *et al.* 2003). Micromolar but not low nanomolar concentrations of wortmannin have been shown to affect TASK1 channel activity (Czirjak *et al.* 2001). Incubation of oocytes expressing either TREK1, TASK1 or TASK3 with wortmannin (10 μM) for 60–90 min suppressed channel activity. Wortmannin showed the smallest effect on TASK3 channels followed by TASK1 and TREK1 channels (Fig. 3E). These data together with the difference in the kinetics of poly Lys block, indicate that the order of the strength of channel–PIP<sub>2</sub> interactions is TASK3, TASK1 and TREK1 channels.

There is a clear correlation between the slower poly Lys inhibition kinetics (Fig. 3C), the weaker wortmannin inhibition (Fig. 3E), and the smaller agonist-induced inhibition observed for TASK3 channels when compared

to TASK1 channels (Fig. 2A and B). These results indicate that the level of agonist-induced inhibition of these channels correlates with the strength of interaction of the channel with PIP<sub>2</sub>, and suggest therefore that the inhibition of TASK channels is mediated by PIP<sub>2</sub> hydrolysis.

We also measured the recovery from inhibition by ACh. The recovery of TASK1 channels from inhibition was shown to be affected by wortmannin at micromolar concentrations implicating PIP<sub>2</sub> hydrolysis as the mechanism that underlies this inhibition (Czirjak *et al.* 2001). Similarly, recovery from PIP<sub>2</sub> hydrolysis mediated agonist-induced inhibition of KCNQ (Suh & Hille, 2002; Zhang *et al.* 2003) and Kir (Xie *et al.* 1999) channels was inhibited by micromolar concentrations of wortmannin. These results suggest that for PIP<sub>2</sub>-sensitive channels, recovery from agonist-induced inhibition is dependent on PIP<sub>2</sub> resynthesis. We found that the recovery from inhibition was the slowest for TREK1 channels, followed by



**Figure 2. Inhibition of 2-P domain channels by agonist-induced PIP<sub>2</sub> hydrolysis**

TASK1 and TASK3 currents were assessed by two-electrode voltage clamp. *A*, normalized effect of 1 μM carbachol application on whole-cell currents measured at –100 mV in 80 mM K<sup>+</sup> extracellular solution, average of *n* = 5–6 cells. *B*, summary data for current inhibition by various agonists: angiotensin II (10 nM); lysophosphatidic acid (0.5 μM) and carbachol (1 μM) (for experiments done as in *A*). *C*, left, representative trace of the effect of 10 μM ACh on whole-cell TREK1 currents measured at +100 and –100 mV using a ramp protocol. Centre, representative trace of the effect of ACh on whole-cell TRAAK currents measured at +100 and –100 mV using a ramp protocol. Right, summary of the ACh-induced inhibition measured at –100 mV (*n* = 6 and *n* = 8 for TREK1 and TRAAK, respectively). Current level was estimated using the difference between current measured in high K<sup>+</sup> (mm: 96 KCl, 1 NaCl, 1 MgCl<sub>2</sub>, 5 Hepes, pH 7.4) and current measured in low K<sup>+</sup> (mm: 2 KCl, 95 NaCl, 1 MgCl<sub>2</sub>, 5 Hepes, pH 7.4) solutions at –100 mV. Note activation and fast inactivation of native Cl<sup>–</sup> current by ACh indicating expression of M<sub>1</sub> receptors despite the lack of TRAAK inhibition.

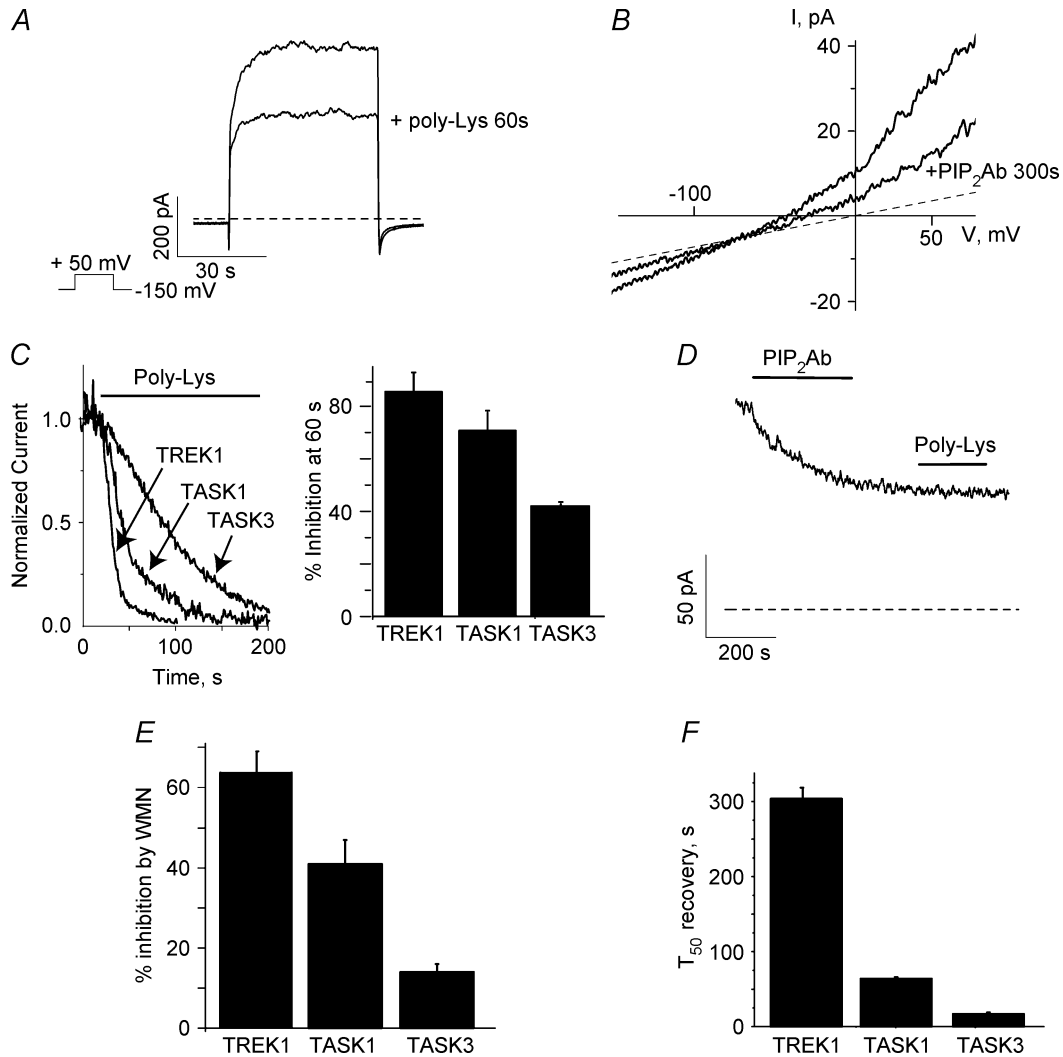
TASK1 and TASK3 channels (Fig. 3F), further supporting the idea that the agonist-induced inhibition is mediated by PIP<sub>2</sub> hydrolysis.

We attempted to identify molecular determinants of PIP<sub>2</sub> interactions with 2-P channels. First we truncated the whole C-terminus of TASK3 channels, and this channel was still activated by PIP<sub>2</sub> (data not shown). In addition we mutated several conserved positive residues in TASK3 (K137A, K144D–K145A and R150A) in the coupling region between the 2nd and 3rd TM domains. None of these mutations increased the level

of agonist-induced inhibition suggesting they are not PIP<sub>2</sub>-interacting residues (data not shown). An increase in the agonist-induced inhibition would be expected if the residue was interacting with PIP<sub>2</sub> and the mutant channel presented weaker channel–PIP<sub>2</sub> interactions.

**Agonist-induced inhibition of TREK1 currents is not mediated by protein kinase C or diacylglycerol**

Agonist induced inhibition of TASK1 channels has been shown not to be mediated by downstream effectors of



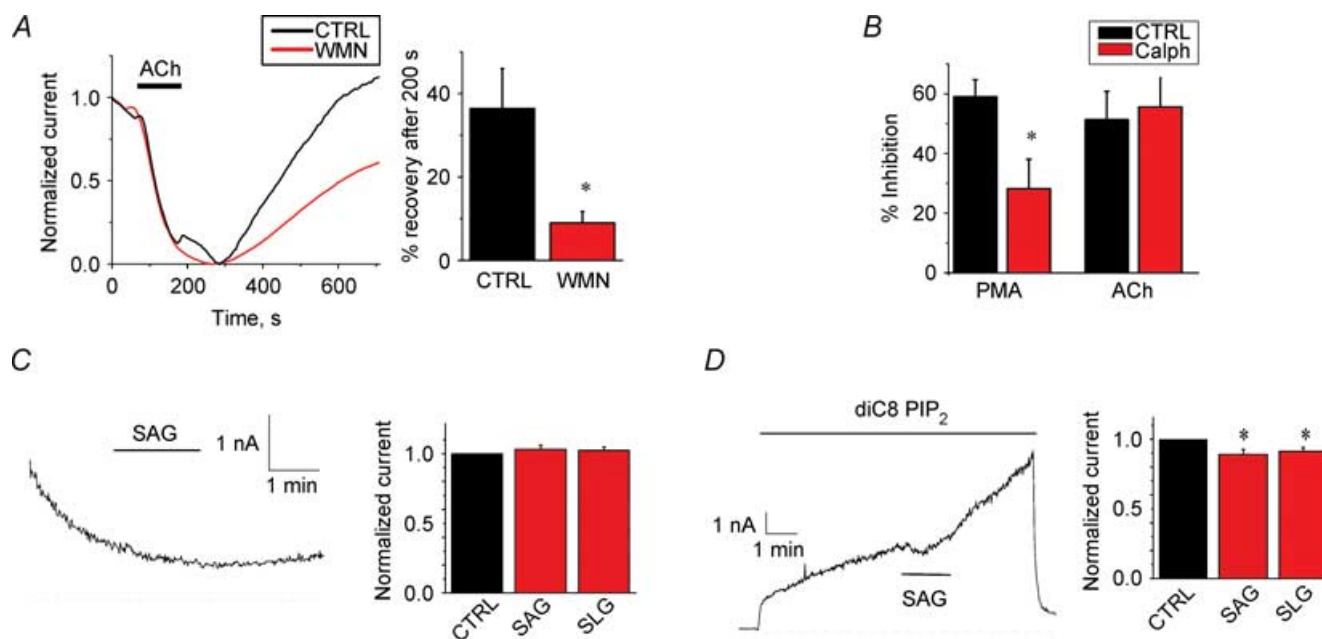
**Figure 3. Kinetics of inhibition of TASK1 and TASK3 channels by the PIP<sub>2</sub> scavenger poly Lys correlates with wortmannin inhibition and recovery from agonist-induced inhibition**

A, typical response of TASK3 channels measured in oocyte macropatches to a depolarizing voltage step to +50 mV from a –150 mV holding voltage before and after 60 s application of poly Lys. B, typical response of TASK3 channels measured in oocyte macropatches to a voltage ramp (0.25 mV ms<sup>-1</sup>) before and after 300 s of PIP<sub>2</sub>Ab application. C, TREK1, TASK1 and TASK3 currents were measured in oocyte macropatches. Left, typical inhibition of either TREK1, TASK1 or TASK3 currents by poly Lys; right, summary data of kinetics of inhibition by poly Lys (30 μg ml<sup>-1</sup>, see Methods) for TREK1 (n = 5) TASK1 (n = 7) and TASK3 (n = 5) channels. D, typical effect of poly Lys application after PIP<sub>2</sub>Ab application. After PIP<sub>2</sub>Ab is bound to PIP<sub>2</sub>, poly Lys application had no effect on TASK3 channels (n = 6). E, summary data for inhibition of the whole-cell current by 60–90 min treatment with 10 μM wortmannin (n = 10–18). F, average whole-cell current recovery from carbachol inhibition for TREK1, TASK1 and TASK3 channels.

PLC activation (Czirjak *et al.* 2001). Our data indicate that this inhibition is mediated by PIP<sub>2</sub> depletion. It has recently been suggested that the mechanism underlying agonist-induced inhibition was different for TASK and TREK channels (Chemin *et al.* 2003). Our data showing that TREK1 channels are activated by PIP<sub>2</sub> and inhibited by PIP<sub>2</sub> scavengers (Fig. 1D) suggest otherwise. Furthermore, incubation of oocytes for 2–3 h with 10 μM wortmannin, a concentration that inhibits PI 4-kinases (Nakanishi *et al.* 1995; Zhang *et al.* 2003), decreased TREK1 currents, consistent with PIP<sub>2</sub> being necessary for maintaining channel activity (Fig. 3E). In addition, shorter incubations with 10 μM wortmannin (10–15 min) slowed recovery from ACh-induced inhibition (Fig. 4A), suggesting that PIP<sub>2</sub> hydrolysis mediates the inhibition for these channels. Generally, these shorter incubations seem to affect replenishment of PIP<sub>2</sub> without a significant effect on basal PIP<sub>2</sub> levels.

TREK1 channels were also reported to be inhibited by protein kinase C (PKC) (Fink *et al.* 1996). To test whether PKC contributes to the agonist-induced inhibition we measured ACh inhibition in the presence

of the specific PKC inhibitor calphostin-C (10 μM). Phorbol 12-myristate 13-acetate (PMA)-induced, but not ACh-induced, inhibition of TREK1 currents was diminished by calphostin-C treatment (Fig. 4B). Our data suggest that inhibition by PKC phosphorylation does not underlie agonist-induced inhibition. It was recently suggested that agonist-induced inhibition of TREK1 channels by metabotropic glutamate receptors is mediated by diacylglycerol (DAG) (Chemin *et al.* 2003). In this study, the DAG analogues SAG (10 μM) and SLG (10 μM) directly inhibited TREK2 and TREK1 currents previously activated by arachidonic acid (AA). To test whether DAG inhibits TREK1 currents in the absence of exogenously applied AA, we examined the effect of SAG (10 μM) and SLG (10 μM) on TREK1 current after patch excision or after activation by diC<sub>8</sub> PIP<sub>2</sub>. Figure 4C left panel shows a representative TREK1 current trace in an inside-out patch from *Xenopus* oocytes shortly after seal formation. TREK1 currents usually showed an immediate increase after excision (not shown), followed by run-down (Fig. 4C). SAG and SLG showed no inhibition in these patches (*n* = 4 for each DAG analogue). Figure 4D shows



**Figure 4. Modulation of TREK1 channels by PIP<sub>2</sub>**

**A**, effect of wortmannin on whole-cell current recovery from carbachol inhibition for TREK1 channels. Left, a typical normalized recovery from inhibition for TREK1 channels. Right, summary data for recovery of inhibition after 200 s of removal of ACh in the presence or absence of wortmannin (*n* = 16). Oocytes were treated with 10 μM wortmannin during the recovery and for at least 10 min prior to ACh application. **B**, effect of the PKC inhibitor calphostin-C (10 μM) on ACh-induced inhibition. Summary data of whole-cell current inhibition by either 10 nM PMA or 10 μM ACh in either presence or absence of calphostin-C (*n* = 5–7). **C**, effect of 10 μM SAG and 10 μM SLG on TREK1 currents on inside-out macropatches. Left, a typical inside-out macropatch current at +90 mV; SAG was applied as indicated. Establishment of the inside-out configuration is indicated by the arrow. Right, the summary data of effects of SAG and SLG on basal current. **D**, effects of 10 μM SAG and 10 μM SLG on PIP<sub>2</sub>-activated TREK1 currents on inside-out macropatches. Left, a typical inside-out macropatch current at +90 mV, 25 μM diC<sub>8</sub> PIP<sub>2</sub> and SAG were applied as indicated. Right, summary data of effects of SAG and SLG on inside-out macropatch currents activated by diC<sub>8</sub> PI(4,5)P<sub>2</sub>.



a representative measurement in which TREK1 currents were re-activated by diC<sub>8</sub> PIP<sub>2</sub>. Both SAG and SLG showed a mild but statistically significant inhibition, averaging around 10% (for measurement of inhibition see Methods). This small inhibition is unlikely, however, to account for the marked inhibition by M<sub>1</sub> receptor activation.

Our data suggest that depletion of PIP<sub>2</sub> by PLC accounts for the agonist-induced inhibition of TREK1 as well as TASK1 and TASK3 channels.

### Agonist-induced PIP<sub>2</sub> hydrolysis and PIP<sub>2</sub> applications shift voltage sensitivity of TREK1 channels

2-P domain K<sup>+</sup> channels are generally open at all membrane potentials, but their activity has also been shown to be regulated by voltage. Most 2-P domain channels, including TASK and TREK, present an instantaneous current component and a second fast activation component in response to depolarizing steps (Lopes *et al.* 2000; Bockenhauer *et al.* 2001; Maingret *et al.* 2003) (see also Fig. 3A). The voltage sensitivity of TREK1 channels has been shown to be regulated by PKA phosphorylation and partially by external Mg<sup>2+</sup> ions (Bockenhauer *et al.* 2001; Maingret *et al.* 2003). We studied the ability of agonist-induced PIP<sub>2</sub> hydrolysis to regulate the voltage dependence of 2-P domain channels. The measurements were performed in constant external Mg<sup>2+</sup> concentration. Figure 5A left panel shows the time course of M<sub>1</sub> receptor mediated inhibition of TREK1 currents in *Xenopus* oocytes at +80 and −150 mV. The right panel shows individual representative traces in response to a slow voltage ramp (0.1 mV ms<sup>−1</sup>), which allowed measurements of steady-state current at a range of voltages before and after ACh application. Endogenous K<sup>+</sup> currents were negligible compared to the expressed TREK1 currents measured in this voltage range. We assessed the voltage dependence by measuring the chord conductance of the current *versus* voltage plot. We measured currents at high external [K<sup>+</sup>]. This chord conductance reflects the ability of the channel to open at different voltages. Figure 5B left panel shows a plot of the chord conductance measured using the current–voltage relationships in Fig. 5A. The voltage dependence of TREK1 currents was affected by ACh suggesting that PIP<sub>2</sub> hydrolysis modulates the voltage dependence of this channel. The right panel shows calculated V<sub>1/2</sub> values as the voltage where conductance was half-way between the conductance at +100 mV and −100 mV (*n* = 12). Because of the limited voltage range where currents could be measured, it was not possible to fit all data with a Boltzmann equation. An equivalent and significant shift in V<sub>1/2</sub> values during ACh stimulation was obtained for current–voltage traces (*n* = 6–9) that could be well fitted with a Boltzmann curve (*R*<sup>2</sup> > 0.9) (V<sub>1/2</sub> (in mV): before

ACh: −74 ± 11; 1 min after ACh: 7 ± 22; steady state inhibition: 124 ± 40).

To further test whether PIP<sub>2</sub> directly modulates voltage dependence of this channel, we measured the effect on the TREK1 voltage dependence in excised inside-out patches before and after PIP<sub>2</sub> applications. The outwardly rectifying current present after the washout of 100 μM diC<sub>8</sub> PIP<sub>2</sub> is reflecting the activity of TREK-1 due to endogenous PIP<sub>2</sub> present in the patch and residual effect of the diC<sub>8</sub> PIP<sub>2</sub> applied. We observed a left shift in the voltage dependence of TREK1 currents in response to diC<sub>8</sub> PIP<sub>2</sub>. Figure 5C shows the time course of the activation of TREK1 currents by the water-soluble diC<sub>8</sub> PIP<sub>2</sub> analogue (100 μM). Figure 5C (left panel) shows averaged ramp currents from the periods. Figure 5D (left panel) shows the chord conductance of the ramp traces. The right panel shows summary of V<sub>1/2</sub> values (*n* = 6), calculated as the voltage where conductance was half-way between the conductance at +100 mV and −150 mV. Endogenous currents in non-injected oocytes were negligible compared to TREK1 currents.

Our data indicate that the presence of PIP<sub>2</sub> is required for the voltage-dependent activation of the channel at more hyperpolarized voltages. For channels where PIP<sub>2</sub> is removed either by agonist-induced PIP<sub>2</sub> hydrolysis or wash out of PIP<sub>2</sub>, a stronger depolarization is necessary to activate the channel.

### Agonist-induced PIP<sub>2</sub> hydrolysis inhibits current and shifts voltage dependence of KCNQ1/KCNE1 channels

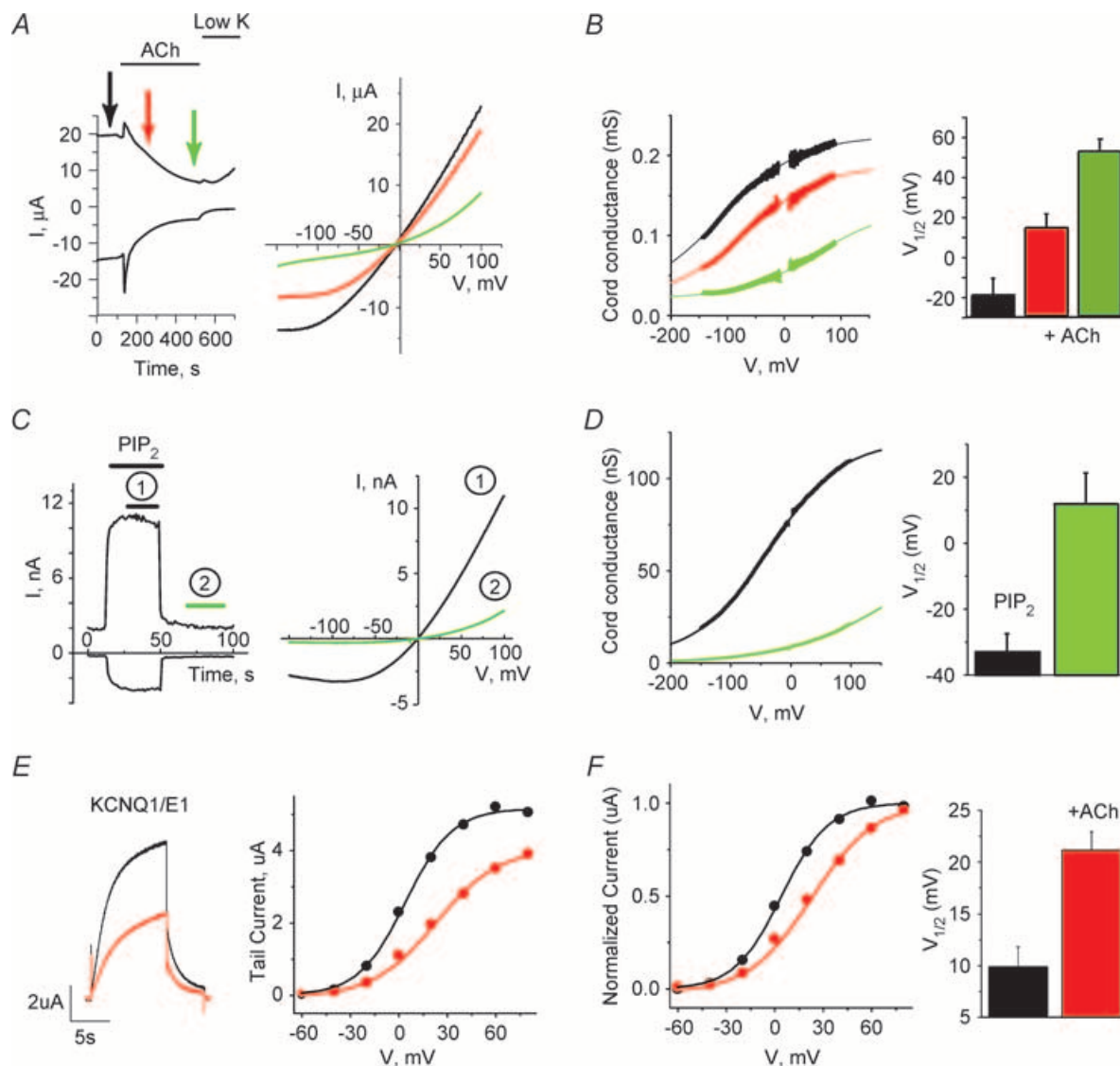
We compared the effects of ACh on TREK1 with the effect of PIP<sub>2</sub> hydrolysis in the voltage-gated KCNQ1/KCNE1 channel. PIP<sub>2</sub> has been shown to activate KCNQ channels and its hydrolysis to underlie inhibition by agonist activation of PLC (Zhang *et al.* 2003). As for the TREK1 channels, ACh-induced PIP<sub>2</sub> hydrolysis inhibited maximal KCNQ1/KCNE1 channel conductance and shifted the voltage dependence of activation towards more depolarized voltages (Fig. 5E and F). Our data are consistent with a recent report showing that application of PIP<sub>2</sub> to excised patches expressing KCNQ1/KCNE1 channels shifted their voltage dependence to the left (Loussouarn *et al.* 2003). These channels were coexpressed with IP<sub>3</sub> phosphatase to minimize Ca<sup>2+</sup>-dependent Cl<sup>−</sup> currents and Ca<sup>2+</sup> modulation. These results suggest a conserved role of PIP<sub>2</sub> as a gating molecule for structurally unrelated K<sup>+</sup> channel families.

## Discussion

The role of PIP<sub>2</sub> as a second messenger that directly regulates transmembrane effector proteins is being increasingly appreciated (Hilgemann *et al.* 2001). In the

present study, we have characterized the effects of PIP<sub>2</sub> on members of the 2-P domain K<sup>+</sup> channel family. We have shown that all four members of this family tested are activated by PIP<sub>2</sub>. Our data indicate that hydrolysis

of PIP<sub>2</sub> mediates inhibition of channel activity by hormones and neurotransmitters. In addition, we show that PIP<sub>2</sub> hydrolysis modulates voltage dependence of 2-P channels. We also demonstrate that agonist-induced PIP<sub>2</sub>



**Figure 5. Agonist-induced PIP<sub>2</sub> hydrolysis and PIP<sub>2</sub> applications shift the voltage dependence of TREK1 and KCNQ1/KCNE1 channels**

**A**, left, time course of whole-cell currents for oocytes expressing TREK1 channels at +80 and –100 mV during ACh application in high K<sup>+</sup> (96 mM) solution. Right, whole-cell currents in response to a slow voltage ramp (0.1 mV ms<sup>-1</sup>) before and after 1 min (red) and steady-state (green) ACh inhibition. **B**, left, plot of the chord conductance ( $I/V$ ) measured using the current–voltage relationship shown in **A**. Continuous lines represent a Boltzmann fit of the data. Right, summary data of the effects of ACh on  $V_{1/2}$ .  $V_{1/2}$  was estimated by the conductance half-way between the conductance at –100 and +100 mV ( $n = 12$ ). **C**, left, activation of TREK1 currents in inside-out macropatches by 100  $\mu$ M DiC<sub>8</sub> PIP<sub>2</sub> at +90 and –90 mV in symmetrical high K<sup>+</sup> (96 mM) solutions. Right, currents in response to a slow voltage ramp (0.16 mV ms<sup>-1</sup>) during (black) and after (green) application of PIP<sub>2</sub>. **D**, left, plot of the chord conductance ( $I/V$ ) measured using the current–voltage relationship shown in **C**. Continuous lines represent a Boltzmann fit of the data. Right, summary data of effects of the application of DiC<sub>8</sub> PIP<sub>2</sub> on  $V_{1/2}$  estimated by the voltage where the conductance was half-way between of the conductance measured at +100 and –150 mV ( $n = 6$ ). **E**, left, representative current traces of KCNQ1/KCNE1 channels, activated by a voltage step from the holding potential of –80 mV to +60 mV and then –40 mV before and after the application of ACh. Right, current–voltage relationship measured from the tail currents at –40 mV after steps to different voltages. **F**, left, normalized current–voltage relationship before and after the application of ACh. Right, summary data ( $n = 15$ ). All conductance measurements were fit by a Boltzmann equation (continuous line).

hydrolysis modulates voltage dependence of both 2-P and voltage-gated KCNQ channels. Our results suggest that PIP<sub>2</sub> is a common gating molecule for K<sup>+</sup> channels and that its function is conserved despite the lack of homology between the putative PIP<sub>2</sub>-binding cytoplasmic domains of the different K<sup>+</sup> channel families.

For TASK1 channels, PLC activation was shown to be required for the agonist-induced inhibition and downstream signals did not mediate the inhibition. In addition, wortmannin, at concentrations inhibiting PIP<sub>2</sub> replenishment in the cell membrane, was shown to retard recovery from carbachol-induced inhibition (Czirjak *et al.* 2001). Although these results are consistent with PIP<sub>2</sub> hydrolysis underlying the inhibition, they did not provide direct evidence for this mechanism. Recently, direct activation of TASK3 channels by PIP<sub>2</sub> has been demonstrated (Chemin *et al.* 2003). It was suggested that PIP<sub>2</sub> depletion underlies PLC-mediated inhibition of TASK, but not TREK, channels. Here we provide several lines of evidence that agonist-induced inhibition of TASK1 and TASK3 as well as TREK1 channels is mediated by PIP<sub>2</sub> depletion. First TASK1, TASK3 and TREK1 channels are all activated by PIP<sub>2</sub> (Fig. 1). Second, activity of all three channels is blocked by PIP<sub>2</sub> scavengers (Fig. 1). Third all three channels are inhibited by wortmannin at concentrations that inhibit PI 4-kinases, depleting PIP<sub>2</sub> (Fig. 3E). Fourth, wortmannin retards recovery of TREK1 channels from ACh-induced inhibition (Fig. 4A), similarly to TASK1 (Czirjak *et al.* 2001) and TASK3 (Chemin *et al.* 2003) channels. Finally, we show that the downstream products of PIP<sub>2</sub> hydrolysis, DAG and PKC, do not underlie agonist-induced inhibition of TREK1 channels (Fig. 4B–D). Our results also indicate that IP<sub>3</sub> does not mediate agonist-induced inhibition, as coexpression of IP<sub>3</sub> phosphatase, eliminating the Ca<sup>2+</sup>-activated Cl<sup>-</sup> current, did not affect the M<sub>1</sub>-induced inhibition on TREK1 currents (data not shown). In conclusion, our results indicate that agonist-induced inhibition of TASK and TREK channels is mediated by depletion of PIP<sub>2</sub>.

Our data also show that the kinetics of poly Lys block of TREK1 and TASK channels (Fig. 3C) correlate with the level of agonist-induced inhibition (Fig. 2) and the kinetics of recovery from inhibition (Fig. 3F). In addition, effects of wortmannin on TREK1 and TASK channel activity (Fig. 3E) also correlate with agonist-induced inhibition. These results indicate that TASK3 channels have the strongest interaction with PIP<sub>2</sub> followed by TASK1 and TREK1 and that the extent of agonist-induced inhibition by depletion of PIP<sub>2</sub> is determined by the strength of channel–PIP<sub>2</sub> interactions.

Inwardly rectifying K<sup>+</sup> channels are best characterized in their interactions with PIP<sub>2</sub> (Huang *et al.* 1998; Rohacs *et al.* 2003; Lopes *et al.* 2002). The highly conserved C and N terminal cytoplasmic portions of these channels presumably form a well-defined binding site that is

formed by positively charged residues (Lopes *et al.* 2002). Mutations in the PIP<sub>2</sub>-interacting positive residues of these channels confer lower PIP<sub>2</sub> affinity, resulting in either non-functional channels, or channels with lower activity and higher sensitivity to inhibition by agonist-induced PIP<sub>2</sub> hydrolysis (Zhang *et al.* 1999; Kobrinsky *et al.* 2000; Lopes *et al.* 2002). The cytoplasmic domains of 2-P domain channels show a much lower level of conservation than those of Kir channels. As described earlier, our initial attempt to test potential PIP<sub>2</sub> interacting sites comparable to those found in other channels did not prove successful. A five residue region (VLRFM<sub>T</sub>) in the proximal C-terminus has been implicated in agonist- and halothane-induced inhibition. Mutation of the positively charged Arg residue reduced agonist-induced inhibition by TRH and did not seem to inhibit basal current level (Talley & Bayliss, 2002). This suggests increased apparent PIP<sub>2</sub> affinity, similarly to the I229L mutant in Kir3.4 channels (Zhang *et al.* 1999; Kobrinsky *et al.* 2000). Therefore this residue is unlikely to be a direct PIP<sub>2</sub>-interacting residue. Although unlikely, it is possible that PIP<sub>2</sub> activates these channels through an intermediate PIP<sub>2</sub> binding protein. This putative protein has to be membrane bound, show different apparent PIP<sub>2</sub> affinities when bound to different channels and not be affected by the deletion of the whole C-terminus of the channel.

Mechanosensitive channels are sensitive to non-specific membrane perturbations. It could therefore be possible that incorporation of excess PIP<sub>2</sub> into the membrane changes the physical properties of the membrane and affects channel activity. Our data, however, suggest that the effects of PIP<sub>2</sub> are not mediated by such non-specific membrane perturbations. First, such perturbations should not be caused by the scavenging of PIP<sub>2</sub> by poly Lys. Second, PIP<sub>2</sub> does not activate stretch-activated channels, known to be present in the oocyte membrane in non-injected oocytes. Finally, since PIP<sub>2</sub> is a minor (~1%) component of the plasma membrane, decreasing its levels by wortmannin and ACh is highly unlikely to change the mechanical properties of the membrane significantly. Therefore, the correlation between whole-cell (Figs 2C, 3E, 4A and 5A and B) and excised-patch measurements (Figs 1C and 5C and D) on TREK1 channels strongly suggests that PIP<sub>2</sub> activation does not proceed through non-specific membrane perturbation when PIP<sub>2</sub> is directly applied to the patches.

We have also shown that PIP<sub>2</sub> plays an important role in the regulation of 2-P channels by mechanical stimulation. TRAAK channels were generally only activated by PIP<sub>2</sub> after applying negative pressure to the membrane patch. This raises the possibility that pressure regulates these channels by allowing the otherwise silent PIP<sub>2</sub> interactions to exert their stimulatory effect.

Potassium channels inhibit cell excitability by holding the membrane potential below the firing threshold for

action potential. Leak potassium channels contribute to the resting membrane potential and generally maintain it close to the potassium equilibrium potential. A decrease in leak potassium conductance is thought to increase cell excitability. The mechanism underlying this increase in excitability is believed to be a simple decrease in channel conductance and increased contribution of other ion channels, which will tend to depolarize the cell. Here we show that hormones and neurotransmitters not only decrease channel conductance but also modulate their voltage dependence. This change in the channel voltage dependence is also expected to contribute to changes in cell excitability.

We show that PIP<sub>2</sub> hydrolysis inhibited channel activity and shifted the voltage dependence of both the 2-P domain TREK1 channel and the voltage-gated KCNQ1/KCNE1 channel. Our data are consistent with a recent report showing that addition of excess PIP<sub>2</sub> to inside-out patches shifts the voltage sensitivity of KCNQ1/KCNE1 channels (Loussouarn *et al.* 2003). The nature of the voltage sensor of the 2-P domain K<sup>+</sup> channels is not known, but positive charges outside the transmembrane domains, may serve this function, provided they are embedded in the membrane. For both channel families we observed a decrease in channel activity and shift on the voltage dependence towards more depolarized potentials by PIP<sub>2</sub> hydrolysis. This conserved mechanism suggests a common role for PIP<sub>2</sub> in setting the voltage sensitivity of different K<sup>+</sup> channel families. Furthermore, PIP<sub>2</sub> constitutes a common regulatory molecule for the activity of all three major K<sup>+</sup> channel families, underlining a general role for PIP<sub>2</sub> in the gating of K<sup>+</sup> channels.

## References

- Baukowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, Ruppersberg JP & Fakler B (1998). PIP<sub>2</sub> and PIP as determinants for ATP inhibition of K<sub>ATP</sub> channels. *Science* **282**, 1141–1144.
- Bockenbauer D, Zilberberg N & Goldstein SA (2001). KCNK2: reversible conversion of a hippocampal potassium leak into a voltage-dependent channel. *Nat Neurosci* **4**, 486–491.
- Brickley SG, Revilla V, Cull-Candy SG, Wisden W & Farrant M (2001). Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* **409**, 88–92.
- Chemin J, Girard C, Duprat F, Lesage F, Romey G & Lazdunski M (2003). Mechanism underlying excitatory effects of group I metabotropic glutamate receptors via inhibition of 2P domain K<sup>+</sup> channels. *EMBO J* **22**, 5403–5411.
- Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV & Julius D (2001). Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)<sub>2</sub> mediated inhibition. *Nature* **411**, 957–962.
- Czirjak G & Enyedi P (2002). TASK-3 dominates the background potassium conductance in rat adrenal glomerulosa cells. *Mol Endocrinol* **16**, 621–629.
- Czirjak G, Fischer T, Spat A, Lesage F & Enyedi P (2000). TASK (TWIK-related acid-sensitive K<sup>+</sup> channel) is expressed in glomerulosa cells of rat adrenal cortex and inhibited by angiotensin II. *Mol Endocrinol* **14**, 863–874.
- Czirjak G, Petheo GL, Spat A & Enyedi P (2001). Inhibition of TASK-1 potassium channel by phospholipase C. *Am J Physiol Cell Physiol* **281**, C700–C708.
- Duprat F, Lesage F, Fink M, Reyes R, Heurteaux C & Lazdunski M (1997). TASK, a human background K<sup>+</sup> channel to sense external pH variations near physiological pH. *EMBO J* **16**, 5464–5471.
- Fink M, Duprat F, Lesage F, Reyes R, Romey G, Heurteaux C & Lazdunski M (1996). Cloning, functional expression and brain localization of a novel unconventional outward rectifier K<sup>+</sup> channel. *EMBO J* **15**, 6854–6862.
- Goldstein SA, Bockenbauer D, O'Kelly I & Zilberberg N (2001). Potassium leak channels and the KCNK family of two-P-domain subunits. *Nat Rev Neurosci* **2**, 175–184.
- Hilgemann DW & Ball R (1996). Regulation of cardiac Na<sup>+</sup>/Ca<sup>2+</sup> exchange and K<sub>ATP</sub> potassium channels by PIP<sub>2</sub>. *Science* **273**, 956–959.
- Hilgemann DW, Feng S & Nasuhoglu C (2001). The complex and intriguing lives of PIP<sub>2</sub> with ion channels and transporters. *Sci. STKE* 2001, RE19.
- Huang CL, Feng S & Hilgemann DW (1998). Direct activation of inward rectifier potassium channels by PIP<sub>2</sub> and its stabilization by G<sub>βγ</sub>. *Nature* **391**, 803–806.
- Kim Y, Bang H, Gnatenco C & Kim D (2001). Synergistic interaction and the role of C-terminus in the activation of TRAAK K<sup>+</sup> channels by pressure, free fatty acids and alkali. *Pflugers Arch* **442**, 64–72.
- Kobrinisky E, Mirshahi T, Zhang H, Jin T & Logothetis DE (2000). Receptor-mediated hydrolysis of plasma membrane messenger PIP<sub>2</sub> leads to K<sup>+</sup> current desensitization. *Nature Cell Biol* **2**, 507–514.
- Lesage F & Lazdunski M (2000). Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol Renal Physiol* **279**, F793–F801.
- Liman ER, Tytgat J & Hess P (1992). Subunit stoichiometry of a mammalian K<sup>+</sup> channel determined by construction of multimeric cDNAs. *Neuron* **9**, 861–871.
- Lopes CM, Gallagher PG, Buck ME, Butler MH & Goldstein SA (2000). Proton block and voltage gating are potassium-dependent in the cardiac leak channel Kcnk3. *J Biol Chem* **275**, 16969–16978.
- Lopes CMB, Zhang H, Rohacs T, Jin T & Logothetis DE (2002). Alterations in conserved Kir channel–PIP<sub>2</sub> interactions underlie channelopathies. *Neuron* **34**, 933–944.
- Loussouarn G, Park KH, Bellocq C, Baro I, Charpentier F & Escande D (2003). Phosphatidylinositol-4,5-bisphosphate, PIP<sub>2</sub>, controls KCNQ1/KCNE1 voltage-gated potassium channels: a functional homology between voltage-gated and inward rectifier K<sup>+</sup> channels. *EMBO J* **22**, 5412–5421.
- Maingret F, Honore E, Lazdunski M & Patel AJ (2003). Molecular basis of the voltage-dependent gating of TREK-1, a mechano-sensitive K<sup>+</sup> channel. *Biochem Biophys Res Commun* **292**, 339–346.
- Millar JA, Barratt L, Southan AP, Page KM, Fyffe RE, Robertson B & Mathie A (2000). A functional role for the two-pore domain potassium channel TASK-1 in cerebellar granule neurons. *Proc Natl Acad Sci U S A* **97**, 3614–3618.

- Nakanishi S, Catt KJ & Balla T (1995). A wortmannin-sensitive phosphatidylinositol 4-kinase that regulates hormone-sensitive pools of inositolphospholipids. *Proc Natl Acad Sci U S A* **92**, 5317–5321.
- Patel AJ & Honore E (2001). Properties and modulation of mammalian 2P domain K<sup>+</sup> channels. *Trends Neurosci* **24**, 339–346.
- Patel AJ, Honore E, Lesage F, Fink M, Romey G & Lazdunski M (1999). Inhalational anesthetics activate two-pore-domain background K<sup>+</sup> channels. *Nat Neurosci* **2**, 426.
- Rohacs T, Lopes CM, Jin T, Ramdya PP, Molnar Z & Logothetis DE (2003). Specificity of activation by phosphoinositides determines lipid regulation of Kir channels. *Proc Natl Acad Sci U S A* **100**, 745–750.
- Rohacs T, Lopes C, Mirshahi T, Jin T, Zhang H & Logothetis DE (2002). Assaying phosphatidylinositol bisphosphate regulation of potassium channels. *Meth Enzymol* **345**, 71–92.
- Runnels LW, Yue L & Clapham DE (2002). The TRPM7 channel is inactivated by PIP<sub>2</sub> hydrolysis. *Nat Cell Biol* **4**, 329–336.
- Shyng SL & Nichols CG (1998). Membrane phospholipid control of nucleotide sensitivity of K<sub>ATP</sub> channels. *Science* **282**, 1138–1141.
- Sirois JE, Lei Q, Talley EM, Lynch C III & Bayliss DA (2000). The TASK-1 two-pore domain K<sup>+</sup> channel is a molecular substrate for neuronal effects of inhalation anesthetics. *J Neurosci* **20**, 6347–6354.
- Stauffer TP, Ahn S & Meyer T (1998). Receptor-induced transient reduction in plasma membrane PtdIns(4,5)<sub>2</sub>P<sub>2</sub> concentration monitored in living cells. *Curr Biol* **8**, 343–346.
- Suh BC & Hille B (2002). Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. *Neuron* **35**, 507–520.
- Sui JL, Petit Jacques J & Logothetis DE (1998). Activation of the atrial K<sub>ACh</sub> channel by the βγ subunits of G proteins or intracellular Na<sup>+</sup> ions depends on the presence of phosphatidylinositol phosphates. *Proc Natl Acad Sci U S A* **95**, 1307–1312.
- Talley EM & Bayliss DA (2002). Modulation of TASK-1 (Kcnk3) and TASK-3 (Kcnk9) potassium channels: volatile anesthetics and neurotransmitters share a molecular site of action. *J Biol Chem* **277**, 17733–17742.
- Talley EM, Lei Q, Sirois JE & Bayliss DA (2000). TASK-1, a two-pore domain K<sup>+</sup> channel, is modulated by multiple neurotransmitters in motoneurons. *Neuron* **25**, 399–410.
- Varnai P & Balla T (1998). Visualization of phosphoinositides that bind pleckstrin homology domains: calcium- and agonist-induced dynamic changes and relationship to myo-[<sup>3</sup>H]inositol-labeled phosphoinositide pools. *J Cell Biol* **143**, 501–510.
- van der Wal J, Habets R, Varnai P, Balla T & Jalink K (2001). Monitoring agonist-induced phospholipase C activation in live cells by fluorescence resonance energy transfer. *J Biol Chem* **276**, 15337–15344.
- Wu L, Bauer CS, Zhen XG, Xie C & Yang J (2002). Dual regulation of voltage-gated calcium channels by PtdIns(4,5)<sub>2</sub>P<sub>2</sub>. *Nature* **419**, 947–952.
- Xie LH, Horie M & Takano M (1999). Phospholipase C-linked receptors regulate the ATP-sensitive potassium channel by means of phosphatidylinositol 4,5-bisphosphate metabolism. *Proc Natl Acad Sci U S A* **96**, 15292–15297.
- Zhang H, Craciun LC, Mirshahi T, Rohacs T, Lopes CMB, Jin T & Logothetis DE (2003). PIP<sub>2</sub> activates KCNQ channels and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron* **37**, 963–975.
- Zhang H, He C, Yan X, Mirshahi T & Logothetis DE (1999). Activation of inwardly rectifying K<sup>+</sup> channels by distinct PtdIns(4,5)<sub>2</sub>P<sub>2</sub> interactions. *Nature Cell Biol* **1**, 183–188.

### Acknowledgements

We thank Dr Bensheng Liu, Ms Elizabeth Findeis and Ms Irén Veres for preparing oocytes; Dr M. Lazdunski and Dr E. Honoré for providing cDNA clones for TASK1, TREK1 and TRAAK; Dr Christophe Erneux for providing the type-1 IP<sub>3</sub> 5-phosphatase clone; and Dr J. Yang for critically reading the manuscript. This work was supported by NIH grant HL59949 to D.E.L., grants OTKA (T046954) and ETT-085/2003 to P.E. D.E.L. is an Established Investigator of the American Heart Association (AHA). T.R. was supported by a postdoctoral fellowship from the Revson Foundation and a Scientist Development Grant (0330224 N) from AHA. C.B.L. was supported by a postdoctoral fellowship and a Scientist Development Grant from AHA.