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Mechanisms underlying excitatory effects of group I metabotropic glutamate receptors via inhibition of 2P domain K^+ channels

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Group I metabotropic glutamate receptors (mGluRs) are implicated in diverse processes such as learning, memory, epilepsy, pain and neuronal death. By inhibiting background K^+ channels, group I mGluRs mediate slow and long-lasting excitation. The main neuronal representatives of this K^+ channel family (K_{2P} or KCNK) are TASK and TREK. Here, we show that in cerebellar granule cells and in heterologous expression systems, activation of group I mGluRs inhibits TASK and TREK channels. D-*myo*-inositol-1,4,5-triphosphate and phosphatidyl-4,5-inositol-biphosphate depletion are involved in TASK channel inhibition, whereas diacylglycerols and phosphatidic acids directly inhibit TREK channels. Mechanisms described here with group I mGluRs will also probably stand for many other receptors of hormones and neurotransmitters.

Keywords: diacylglycerol/glutamate/IP3/phosphatidic acid/PIP2

Introduction

Glutamate is a major excitatory neurotransmitter in the central nervous system. To mediate fast excitatory transmission, glutamate activates postsynaptic ionotropic receptors. Glutamate also activates metabotropic receptors (mGluRs) (Sladeczek *et al*., 1985; Sugiyama *et al*., 1987), which modulate neuronal excitability and synaptic transmission, resulting in a slow depolarization and increase in cell firing (Pin and Duvoisin, 1995). There are currently eight subtypes of mGluRs (mGluR1–8) and multiple splice variants (Conn and Pin, 1997). Group II and III receptors are coupled to Gi, leading to a decrease of cAMP. Group II and III mGluRs (except mGluR6) predominate in presynaptic elements, where they regulate the release of glutamate and other neurotransmitters. Conversely, group I mGluRs (mGluR1 and mGluR5) are coupled to Gq and stimulate the phospholipase C (PLC) pathway, leading to increased phosphoinositide hydrolysis (Conn and Pin, 1997). They are located essentially in postsynaptic areas, where they contribute to the enhancement of cellular excitability via interactions with other postsynaptic processes including background potassium (K^+) current inhibition (Charpak *et al*., 1990; Glaum and Miller, 1992; McCormick and von Krosigk, 1992; Guerineau *et al*., 1994; Pin and Duvoisin, 1995).

Background or 'leak' K^+ currents are central to electrical excitability by controlling the resting membrane potential and cell membrane resistance. Inhibition of these background K^+ currents by several neurotransmitters induces membrane depolarization and subsequent action potential firing, as well as an increase in cell membrane resistance amplifying responses of synaptic inputs (Charpak *et al*., 1990; Nicoll *et al*., 1990; Glaum and Miller, 1992; McCormick and von Krosigk, 1992; Guerineau *et al*., 1994; Pin and Duvoisin, 1995; Millar *et al*., 2000; Talley *et al*., 2000). In contrast, their opening by volatile anesthetics contributes to neuronal hyperpolarization leading to analgesia and immobilization (Patel *et al*., 1999). Background K^+ channels have two pore domains (K_{2P}) and four transmembrane segments (Lesage and Lazdunski, 2000; Patel and Honore, 2001a). In the nervous system, important representatives of this family are the TASK and TREK channels (Fink *et al*., 1996; Duprat *et al*., 1997; Lauritzen *et al*., 2000; Lesage and Lazdunski, 2000; Talley *et al*., 2001). They are relatively insensitive to the broad-spectrum K^+ channel blockers (tetraethylammonium, 4-aminopyridine, Cs^+ and Ba^{2+}) but their activities are increased by volatile anesthetics (Patel *et al*., 1999; Patel and Honore, 2001b). TREK channels (TREK1 and TREK2) are the targets of many physicochemical stimuli, including internal pH, temperature, osmolarity and membrane stretch (Patel *et al*., 1998; Lesage *et al*., 2000; Patel and Honore, 2001a). TREK1 and TREK2 channels are potently activated by polyunsaturated fatty acids and lysophospholipids, and inhibited by neurotransmitters that increase cAMP, via a protein kinase A-dependent phosphorylation process (Patel *et al*., 1998; Lesage *et al*., 2000) and by Gq-coupled receptors via an unknown mechanism (Lesage *et al*., 2000). TASK channels (TASK1 and TASK3) are highly sensitive to small pH variations near physiological pH and to hypoxia (Duprat *et al*., 1997; Buckler *et al*., 2000; Kim *et al*., 2000; Patel and Honore, 2001a). These channels are also modulated by various neurotransmitters and hormones but the precise transduction mechanism through which these molecules exert their control is still unknown (Millar *et al*., 2000; Talley *et al*., 2000).

In this study, we show that by acting on group I Gq-coupled metabotropic receptors, glutamate strongly inhibits both TASK and TREK channels using two distinct pathways. TASK1 and TASK3 currents are inhibited by phosphatidyl-4,5-inositol-biphosphate (PIP_2) depletion and D-*myo*-inositol-1,4,5-triphosphate (IP_3) liberation following PLC activation, while TREK1 and TREK2 currents are directly blocked by diacylglycerols (DAG) and phosphatidic acids (PA).

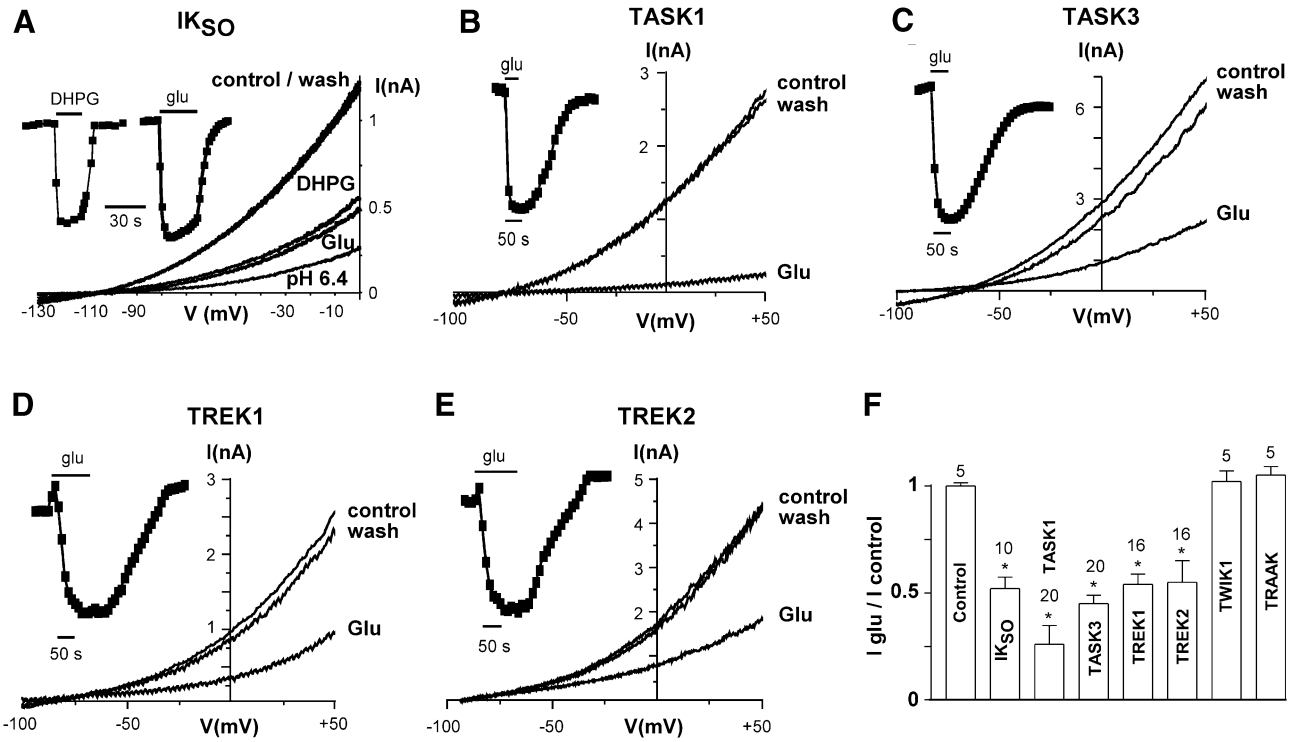

Fig. 1. Glutamate inhibits background potassium currents in native neurons and in heterologous expression systems. (**A**) Sustained outward potassium currents (IK_{SO}) recorded in cerebellar granule neurons before and after 100 μM glutamate, 100 μM DHPG, pH 6.4, application. The time-course of glutamate and DHPG inhibition (measured at 0 mV) is indicated as an inset. (**B**–**E**) Glutamate (50 μM) inhibits recombinant TASK1 (B), TASK3 (C), TREK1 (D) and TREK2 (E) channels as expressed in COS-7 cells containing mGLUR1a receptors. Currents were elicited by voltage ramp (1 mV/7 ms) ranging between indicated potentials and applied every 10 s. (**F**) Percent of current inhibition induced by glutamate (measured at 0 mV) on various K_{2P} channels expressed in brain. $^*P < 0.01$.

Results

Group I metabotropic glutamate receptors inhibit TASK and TREK background potassium channels

Cerebellar granule neurons express both background K^+ currents (named IK_{SO}, for standing outward; Millar *et al*., 2000; Brickley *et al*., 2001; Han *et al*., 2002) and group I mGluRs (Ango *et al*., 2001). Using the perforated patch technique, we recorded robust K^+ currents with a mean amplitude of 1.16 ± 0.16 nA (n = 14) at 0 mV in control condition (Figure 1A). Application of glutamate reversibly inhibited IK_{SO} at all tested potentials, showing no voltage dependence (Figure 1A and F). The inhibition was reproduced by 100 μM (RS)-3,5-dihydroxyphenylglycine (DHPG; 49.7 ± 5% inhibition, n = 14; Figure 1A) indicating the involvement of group I mGluRs (mainly mGluR1a; Ango *et al*., 2001). IK_{SO} is very sensitive to extracellular acidification (72 ± 3% inhibition, n = 14, pH 6.4; Figure 1A) indicating the involvement of TASK channels (Duprat *et al*., 1997; Kim *et al*., 2000). Furthermore, IK_{SO} is blocked by 5 μM ruthenium red (76 ± 2% inhibition, n = 4), but is insensitive to 3 μM methanandamide (14 ± 16% inhibition, n = 4), demonstrating a major contribution of TASK3 currents (Maingret *et al*., 2001; Czirjak and Enyedi, 2002; Lauritzen *et al*., 2003). However, IK_{SO} is also generated by other K_{2P} channels, mainly TASK1 and TREK2 (Millar *et al*., 2000; Han *et al*., 2002). Thus, these channels were co-expressed in COS-7 cells with mGluR1a and tested for their glutamate sensitivity. As observed with IK_{SO}, glutamate reversibly inhibited TASK1, TASK3, TREK1 and TREK2 currents (Figure 1B–F), whereas no effect was observed on TASK2 (n = 5; data not shown), TWIK1 and TRAAK currents (Figure 1F), or in COS-7 cells lacking mGluR1a (n = 5; data not shown) and COS-7 cells expressing only mGluR1a (control, Figure 1F). This lack of effect on TWIK-1 and TRAAK channels is interesting since they are both highly expressed in the brain (Lesage *et al*., 1996; Fink *et al*., 1998; Lauritzen *et al*., 2000; Talley *et al*., 2001). Similar glutamate effects on TREK and TASK channels were obtained using COS-7 cells containing mGluR5 (n = 12; data not shown).

Glutamate inhibition displayed faster kinetics for TASK compared with TREK currents ($P < 0.01$; Figure 1B–E), suggesting distinct mechanisms of inhibition. Moreover, TREK inhibition was often preceded by small increases of the currents after glutamate application (+7 ± 2%, n = 16 for TREK1 currents and +8 ± 2%, n = 16 for TREK2 currents; Figure 1D and E).

Gq/11 and PLC are involved in TASK and TREK inhibition independently of calcium release and protein kinase C activation

Glutamatergic mGluR1 receptors usually couple via the G proteins Gq/11 and PLC (Sladeczek *et al*., 1985; Sugiyama *et al*., 1987). Therefore, we tested whether TASK and TREK currents are modulated via this pathway. In the presence of 1 mM GTPγS in the patch-pipette, glutamate induced an irreversible inhibition of TASK3 and TREK2 currents (<5% of wash, n = 5; $P < 0.01$; Figure 2A). To

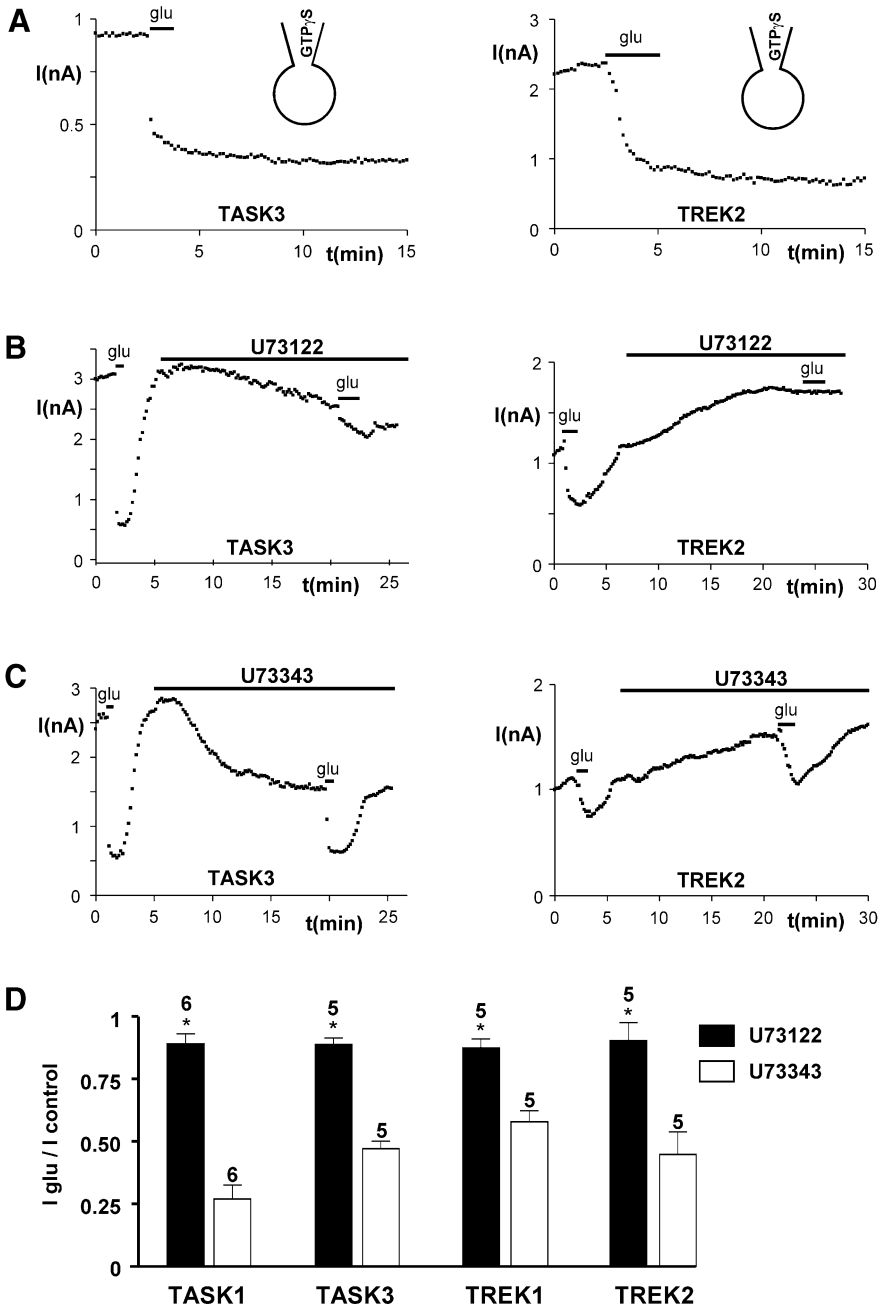

Fig. 2. Glutamate inhibition of background potassium currents involved G proteins and PLC. (**A**) Recovery of TASK3 and TREK2 currents in the presence of 1 mM GTPγS in the patch–clamp pipette. (**B** and **C**) Glutamate inhibition of TASK3 and TREK2 currents before and after 15 min treatment with 5 μM PLC inhibitor U73122 (B) or its inactive analog U73343 (C). (**D**) Summary of the effects produced by U73122 (black bars) and U73343 (white bars). *$P < 0.01$.

simplify, and because IK_{SO} comprises mainly TASK3 and TREK2 (Han *et al*., 2002), all further presented data relate to these channels. The same results were obtained with TASK1 and TREK1 ($n = 5$, $P < 0.01$; data not shown). Neither TASK nor TREK inhibition was affected after 16–24 h of treatment with 200 ng/ml pertussis toxin ($n = 20$; data not shown) or 500 ng/ml cholera toxin ($n = 10$; data not shown). Furthermore, the PLC inhibitor U73122 (5 μM) perfused for 15 min, i.e. in conditions similar to those which have been used to establish the PLC-mediated inhibition of I_M (KCNQ) currents (Suh and Hille, 2002), suppressed TASK and TREK inhibitions by glutamate. U73122 also induced a down-regulation of TASK currents but an up-regulation of TREK currents (Figure 2B). These effects are probably unrelated to the action of U73122 on PLC, since they are reproduced by a U73122 structural analog, U73343, which is inactive on PLC. Importantly, unlike U73122, U73343 had no effect on TASK and TREK current inhibitions by glutamate (Figure 2B–D). Activation of PLC leads to the cleavage of PIP_2 into two second messengers, the membrane-bound DAG that activates protein kinase C (PKC) and the soluble IP_3 that mobilizes Ca^{2+} from intracellular stores via fixation to IP_3 receptors. Buffering intracellular Ca^{2+} concentration to

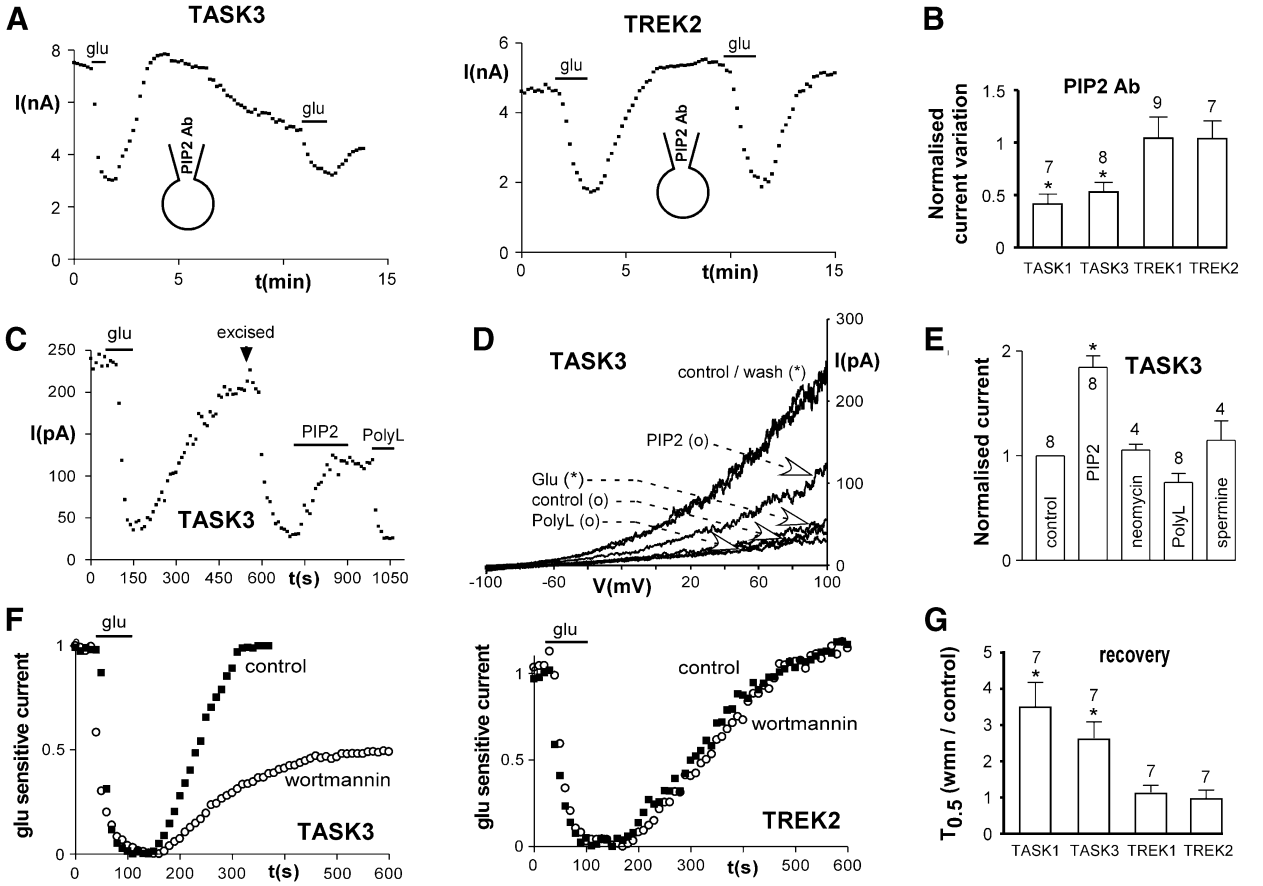

Fig. 3. PIP_2 depletion is involved in TASK current inhibition. (**A**) Addition of PIP_2 antibody (1:50) in the patch–clamp pipette inhibits TASK3 currents without effect on TREK2 currents. (**B**) Summary of the effects of PIP_2 antibody application (10 min). (**C**) Time-course of inhibition of TASK3 currents by glutamate (measured at +100 mV) in the cell-attached configuration. After excision to the inside-out configuration (arrow), PIP_2 (25 μM) was applied after current run-down. Poly-lysine (30 μg/ml) inhibits PIP_2-stimulated currents. (**D**) TASK3 currents obtained with voltage-ramps from the same cell as in (C). (*), Cell-attached mode; (o), inside-out mode. (**E**) Summary of the effects of 25 μM PIP_2 and of 100 μM neomycin, 30 μg/ml poly-lysine or 100 μM spermine applied after 25 μM PIP_2 as in (C). (**F**) Inhibition of PIP_2 synthesis by 10 μM wortmannin (perfused during 5 min) slows down the recovery of TASK currents without effect on TREK currents (black squares, first glutamate application in control conditions; white circles, second glutamate application during wortmannin perfusion). (**G**) Effects of wortmannin on current recovery, quantified as the time for 50% recovery after glutamate application ($T_{0.5}$). *$P < 0.01$.

10 nM or 5 μM with EGTA did not significantly affect TASK and TREK currents or their inhibition by glutamate (variation/control = 10 ± 12%, $n = 10$ for TASK currents; 15 ± 14%, $n = 10$ for TREK currents). The same results were obtained with the more potent calcium chelator BAPTA (used at 5 mM) on TASK and TREK currents ($n = 8$; not shown, but see Figure 4C). To explore the potential role of PKC under our conditions, we pre-treated cells expressing TASK or TREK currents with 100 nM staurosporine, a broad-spectrum kinase inhibitor. Staurosporine did not prevent TASK and TREK current inhibitions by glutamate (variation/control = 14 ± 16%, $n = 6$ for TASK currents; 16 ± 13%, $n = 6$ for TREK currents). The same results were obtained in the presence of 50 μM PKC peptide inhibitor (pseudosubstrate fragment 19–36) in the patch pipette ($n = 12$; data not shown). Similarly, the PKC activator phorbol 12,13-dibutyrate (PdBU; 5 μM perfused 15 min) did not significantly affect TASK and TREK currents or their inhibition by glutamate (variation/control = 8 ± 17%, $n = 11$ for TASK currents; 14 ± 16%, $n = 13$ for TREK currents). The same results were obtained using 100 nM phorbol 12-myristate 13-acetate (PMA; $n = 9$; data not shown). The absence of effect of the generic mediators of Gq/11-coupled receptors lead us to turn our investigation to the possibility of direct roles of PIP_2 and its downstream products IP_3 and DAG.

Depletion of PIP_2 is involved in glutamate-mediated TASK inhibition

Monoclonal antibodies against PIP_2 have been used recently by other authors to analyze the regulatory function of PIP_2 on ion channels or transporters (Chuang *et al.*, 2001; Runnels *et al.*, 2002; Wu *et al.*, 2002). The addition of anti-PIP_2 (1:50) in the patch–clamp pipette induced a strong run-down of both TASK1 and TASK3 currents (Figure 3A and B) without effect on TREK currents. In contrast, an unrelated IgM antibody (1:50) was ineffective ($n = 6$; data not shown). We then tested whether PIP_2 could directly modulate TASK currents in inside-out patches. In the cell-attached mode, glutamate potently inhibited TASK3 currents (56 ± 9.7% inhibition, $n = 4$; Figure 3C and D). After excision to the inside-out mode, TASK3 currents strongly decreased (run-down of current: 57 ± 6.6%, $n = 8$; Figure 3C and D). Bath application of the naturally occurring PIP_2 (1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho-1-D-myo-inositol-4,5-bisphosphate,

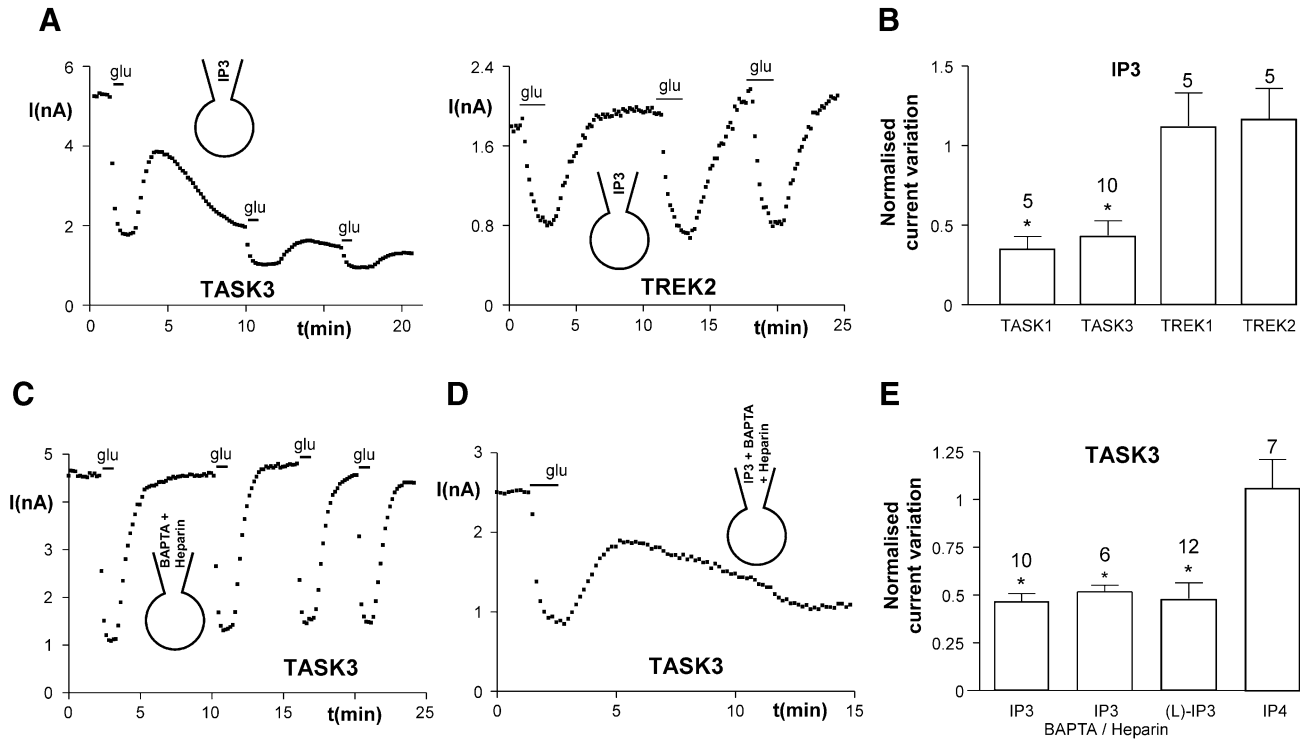

Fig. 4. IP_3 inhibits TASK currents independently of IP_3 receptors. (**A**) Inhibition of TASK3 currents by 50 μM IP_3 added in the patch–clamp pipette. (**B**) Summary of the effects of IP_3 application (10 min). (**C**) Inhibition of TASK3 currents by glutamate in the presence of 5 mM BAPTA and 5 mg/ml heparin added in the patch–clamp pipette. (**D**) Inhibition of TASK3 currents by 50 μM IP_3 in the presence of 5 mM BAPTA and 5 mg/ml heparin. (**E**) The pharmacologically inactive and non-metabolizable analog L-*myo*-Ins(1,4,5)P_3 (50 μM) inhibits TASK3 currents, while Ins(1,3,4,5)P_4 (50 μM) has no effect. *$P < 0.01$.

25 μM) in inside-out patches increased TASK3 currents by 84% (Figure 3C–E). Application of poly-cationic molecules, such as poly-lysine, neomycin and spermine, which act as PIP_2 scavengers (Huang *et al*., 1998), resulted in a fast and complete block of the current, following reactivation by PIP_2 (Figure 3C–E). Despite the persistence of the current block upon poly-lysine wash-out, a second application of PIP_2 fully reactivated TASK3 currents ($n = 4$; data not shown). Therefore, PIP_2 appears to be necessary to maintain TASK channels active, which suggests strongly that depletion of PIP_2 could be involved in glutamate-mediated inhibition of TASK currents. The replenishment of PIP_2 requires lipid kinases, especially the phosphatidylinositol-4-OH kinase [PI(4)K]. Application of wortmannin at concentrations 100-fold higher than those used to inhibit PI(3)K (10–100 nM) decreases replenishment of PIP_2 after receptor-mediated depletion through inhibition of PI(4)K (Nakanishi *et al*., 1995). In the whole-cell configuration, perfusion of 10 μM wortmannin for 5 min decreased TASK currents (29 ± 6% inhibition, $n = 11$, and 27 ± 5% inhibition, $n = 11$, for TASK1 and TASK3 currents, respectively; $P < 0.01$) and slowed their recovery following glutamate inhibition (Figure 3C and D) without effect on TREK currents. These results were not due to inhibition of PI(3)K, since 100 nM wortmannin was ineffective ($n = 5$; data not shown). Furthermore, we excluded a direct block of TASK currents by wortmannin, since 10 μM wortmannin had no effect in inside-out patches expressing TASK3 currents, even after PIP_2 application ($n = 2$; data not shown). Also, wortmannin did not produce run-down of TASK3 currents in the whole-cell mode after their inhibition by PIP_2 antibodies ($n = 3$; data not shown). Interestingly, recovery of TASK currents after glutamate application was not completely blocked by 50 μM wortmannin (percent recovery: 31 ± 13%, $n = 7$, and 38 ± 18%, $n = 6$, for TASK1 and TASK3 currents, respectively), suggesting that although PIP_2 depletion is involved in inhibition of TASK currents, another mechanism also contributes to glutamate mediated inhibition.

IP_3 inhibits TASK currents independently of IP_3 receptors

We then investigated the role of IP_3 in glutamate-mediated current inhibition. The addition of IP_3 in the patch–clamp pipette (50 μM) strongly decreased TASK currents (Figure 4A and B) with no effect on TREK. However, glutamate could still inhibit residual TASK currents despite the presence of IP_3, which is consistent with a PIP_2 depletion-mediated inhibition. Glutamate effects (54 ± 4% inhibition, $n = 5$; Figure 4C) persisted in the presence of heparin (5 mg/ml), which inhibit IP_3 receptors, and BAPTA (5 mM), which prevents increase in intracellular calcium. IP_3 receptors are not involved in the IP_3-mediated inhibition of the TASK currents since IP_3 could still inhibit TASK3 in the presence of BAPTA and heparin (Figure 4D and E). Application of 50 μM IP_3 to inside-out patches expressing TASK3 currents had no effect, even after PIP_2 application and in the presence or absence of 5 μM Ca^{2+} ($n = 8$; data not shown). The effects of IP_3 are

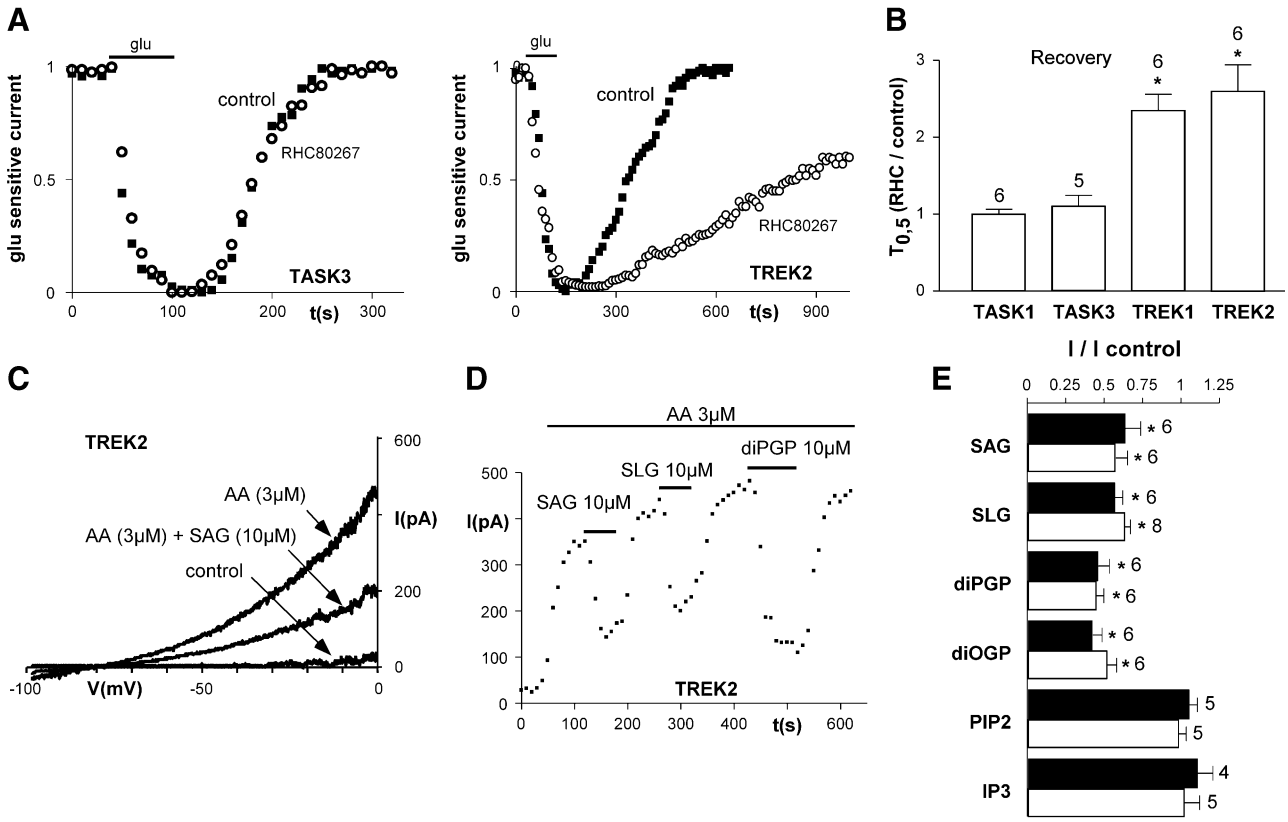

Fig. 5. DAGs and PAs inhibit TREK currents. (**A** and **B**) Fifteen minute perfusion of 50 μM RHC80267 (rhc), a DAG lipase inhibitor, slows down the recovery of TREK currents without effect on TASK currents (black squares, first glutamate application in control conditions; white circles, second glutamate application during RHC80267 application). (**C**) Direct inhibition of TREK2 currents by 10 μM SAG in inside-out patch configuration. TREK2 currents are activated by 3 μM AA. (**D**) Time-course of TREK2 current inhibition by 10 μM SAG, SLG and diPGP. (**E**) Percent of inhibition of various DAGs and PAs and phosphoinositides in the presence of 3 mM internal Mg^{2+}, on TREK1 (black bars) and TREK2 (white bars). $*P < 0.01$.

independent of its metabolism, since its non-metabolizable and IP_3 receptor inactive analog L-*myo*-Ins(1,4,5)P_3 (Willcocks *et al*., 1987; Hirata *et al*., 1990) also potently inhibits TASK3 currents in the whole-cell configuration (Figure 4E). IP_3-mediated TASK current inhibition is not due to unspecific interaction with charged phosphate groups, since Ins(1,3,4,5)P_4 had no effect (Figure 4E). Taken together, these results indicate that TASK currents are inhibited directly by depletion of PIP_2 and indirectly by IP_3, whereas PLC-induced inhibition of TREK currents, under the conditions used in this work, does not involve intracellular Ca^{2+}, PKC activation, PIP_2 or IP_3. Therefore, we then explored whether DAG or its metabolites could be involved in the inhibition of TREK currents, as previously observed in the activation of TRPC3 and TRPC6 by histamine (Hofmann *et al*., 1999).

DAG and PA inhibit TREK currents

To assess the role of DAG, we first measured TREK currents during application of the membrane-permeable DAG analog 1,2-dioctanoyl-*sn*-glycerol (DOG). DOG (50 μM) rapidly decreased TREK currents (percent inhibition of TREK2 currents after 3 min of perfusion: 54 ± 17%, $n = 10$, $P < 0.01$). In mammalian cells DAG is mainly metabolized by DAG lipase. If DAG is implicated in glutamate-mediated TREK inhibition, then the block of DAG lipase should slow the recovery of TREK currents after glutamate application. Indeed, the DAG lipase inhibitor 1,6-bis (cyclohexyloximinocarbonyl-amino) hexane (RHC80267) slowed the recovery of TREK currents (Figure 5A and B) without effect on TASK currents. This result prompted us to investigate whether DAG or its phosphorylated metabolite PAs, which are mainly generated from DAG via DAG kinase or from phospholipids via phospholipase D, could directly inhibit TREK currents. Various physiological DAGs and PAs were applied to inside-out patches expressing TREK currents. In inside-out patches, TREK currents only display a small activity, but this activity can be potently stimulated by acidic pH, negative pressures or in the presence of polyunsaturated fatty acids such as arachidonic acid (AA) (Patel *et al*., 1998; Lesage *et al*., 2000; Patel and Honore, 2001a). Strong TREK currents were obtained in the presence of AA (Figure 5C and D). Under these conditions, application of the endogenous impermeable DAG analogs 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG, 10 μM) or 1-stearoyl-2-linoleoyl-*sn*-glycerol (SLG, 10 μM) to the internal side of the membrane patch reversibly inhibited TREK1 and TREK2 currents (Figure 5D–F). Interestingly, as observed with DAG, PA analogs, 1,2-dipalmitoyl-*sn*-glycerol-3-phosphate (diPGP 10 μM) and 1,2-dioleoyl-*sn*-glycerol-3-phosphate (diOGP 10 μM), strongly decreased TREK currents (Figure 5D and E). The inhibitory effects of DAG and PA analogs were not observed in inside-out patches expressing TASK currents, even after reactivation with PIP_2 ($n = 5$; data not shown). In

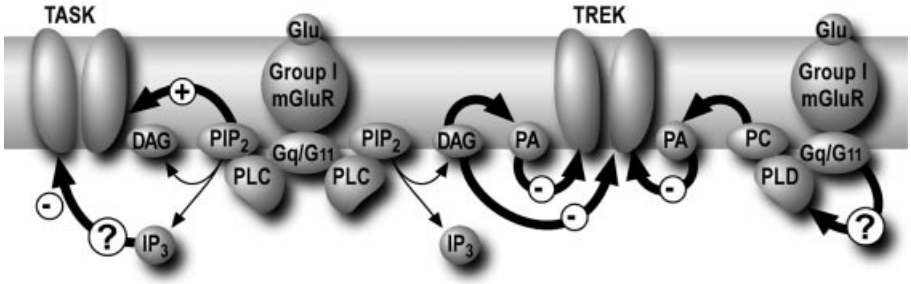

Fig. 6. A model of TASK and TREK modulation by group I mGluRs. Activation of PLC by group I Gq-coupled metabotropic receptors inhibit both TASK (TASK1 and TASK3) and TREK (TREK1 and TREK2) channels using two distinct pathways. TASK channels are inhibited directly by PIP_2 depletion and by IP_3 via an as yet unknown (?) mechanism. In contrast, TREK channels are directly blocked by DAGs independently of PKC activation. In addition, PA, which is mainly generated from DAG via DAG kinase or from phospholipids [mainly phosphatidylcholine (PC)] via phospholipase D (PLD), is a direct blocker of TREK channels.

agreement with the results of the whole-cell experiments, we observed that neither PIP_2 nor IP_3 (in the presence or absence of 5 μM Ca^{2+}) altered AA-activated TREK2 currents in inside-out patch configurations (Figure 5E).

Discussion

Various neurotransmitters induce slow excitation in diverse regions of the CNS by inhibiting background K^+ currents (Charpak *et al*., 1990; Nicoll *et al*., 1990; Glaum and Miller, 1992; McCormick and von Krosigk, 1992; Guerineau *et al*., 1994; Pin and Duvoisin, 1995; Millar *et al*., 2000; Talley *et al*., 2000). K_{2P} channels are now known to be major contributors of background K^+ current (Lesage and Lazdunski, 2000). Among these K_{2P} channels, TASK and TREK channels have been recently shown to mediate neurotransmitter-sensitive background K^+ currents in cerebellar granule cells, hypoglossal motoneurons or in adrenal cortex (Millar *et al*., 2000; Talley *et al*., 2000; Czirjak *et al*., 2001; Enyeart *et al*., 2002). This study shows that mGluR1 glutamate receptors inhibit both K^+ background currents in cerebellar granule cells and heterologously expressed TASK and TREK channels. Activation of mGluR1 receptors has no effect on other K_{2P} channels that are abundantly expressed in the brain such as TWIK-1 and TRAAK (Lesage *et al*., 1996; Fink *et al*., 1998; Lauritzen *et al*., 2000; Talley *et al*., 2001).

It was known that Gq-coupled receptors inhibit background K^+ currents (Lesage *et al*., 2000; Millar *et al*., 2000; Talley *et al*., 2000; Czirjak *et al*., 2001); however, the signaling elements involved in the transduction cascades were unknown. We have now shown that inhibition of TASK1, TASK3, TREK1 and TREK2 by glutamate via mGluR1 receptors requires PTX-insensitive G proteins coupled to PLC. If inhibition of both TASK and TREK channels requires PLC activation, the downstream molecular events leading to TASK and TREK inhibition are clearly different (Figure 6). TASK1 and TASK3 currents are inhibited by PIP_2 depletion following PLC activation. This effect is mimicked by PIP_2 antibodies, while PIP_2 application to inside-out patches reactivates TASK currents after run-down. Inhibition of PIP_2 replenishment induced run-down of TASK currents and slowed their recovery after glutamate inhibition. Thus, TASK channels belong to the growing family of proteins, including several transporters (Hilgemann *et al*., 2001) and ion channels such as the inward rectifier potassium channels (K_{ATP}, IRK, GIRK and ROMK), M-type channels (KCNQ2/KCNQ3), epithelial sodium channels (ENaC), transient receptor potential (TRPL, TRPM7, VR1) and calcium channels (α_{1A}, α_{1B}), that are regulated by variations of the PIP_2 level (Huang *et al*., 1998; Chuang *et al*., 2001; Hilgemann *et al*., 2001; Runnels *et al*., 2002; Suh and Hille, 2002; Wu *et al*., 2002). However, we have also shown that IP_3 drastically decreases TASK currents. The effect is independent of IP_3 metabolism but is indirect, since IP_3 had no effect on inside-out patches expressing TASK channels. We have no definite mechanism for the IP_3 inhibition of TASK currents but we suggest that IP_3 might modulate PIP_2 metabolism by acting as a feed-back molecule on phospholipases or kinases. Therefore, the IP_3 regulation of TASK currents would be via PIP_2 levels. Regulation of TASK channels involves first the depletion of membrane-bound PIP_2 and second the liberation of its soluble metabolite IP_3.

In contrast, PLC activation inhibits TREK1 and TREK2 currents via DAGs independently of PKC activation. The DAG inhibition is most likely direct since it has been observed in excised patches expressing both TREK1 and TREK2 channels. Therefore, the recovery of glutamate inhibition via the mGluR1 receptor is delayed when DAG metabolism is blocked in the presence of a DAG lipase inhibitor. Interestingly, PAs are also direct inhibitors of both TREK1 and TREK2 channels, suggesting that PLD activation also modulates TREK channels activities. Many molecular stimuli activate PLD, including activation of Gq-coupled receptors, which comprise mGluR1 (Klein *et al*., 1997). Therefore, inhibition of TREK currents depends on both hydrolysis of phosphatidylinositol by PLC and other phospholipids (mainly phosphatidylcholine) by PLD (Figure 6). To our knowledge, TREK1 and TREK2 represent the first class of K^+ channels directly modulated by both DAGs and PAs. Our work also shows for the first time a role of PAs in the regulation of ionic channels.

Group I mGluRs are postsynaptic and enhance cellular excitability through different mechanisms, including background K^+ current inhibition (Charpak *et al*., 1990; Glaum and Miller, 1992; McCormick and von Krosigk, 1992; Guerineau *et al*., 1994; Pin and Duvoisin, 1995).

Group I mGluR1 and mGluR5 are both present in several CNS structures such as hippocampus, cortex, thalamus and cerebellum, where they are involved in many brain functions (Bordi and Ugolini, 1999). In the cerebellum, mGluR1 plays a key role in motor learning and motor coordination (Aiba *et al*., 1994; Conquet *et al*., 1994), whereas mGluR5 in the hippocampus contributes to the induction of long-term potentiation and associative learning (Anwyl, 1999). Group I mGluRs are also implicated in a variety of disorders including epilepsy, ischemia, nociception and neurodegenerative diseases (Bordi and Ugolini, 1999). In pathological states, such as ischemia, overstimulation of glutamate receptors is excitotoxic and results in neuronal death (Choi, 1988). Group I mGluRs agonists amplify the excitotoxic neuronal degeneration induced by NMDA in cultured murine cortical cells, whereas antagonists are neuroprotectors (Nicoletti *et al*., 1996). On the other hand, TREK channels are activated by a neuroprotective agent such as riluzole, and also by polyunsaturated fatty acids and lysophospholipids (Patel *et al*., 1998; Duprat *et al*., 2000; Lauritzen *et al*., 2000; Lesage *et al*., 2000; Patel and Honore, 2001a), which are potent protectors against global ischemia and epilepsy (Hibbeln, 1998; Lauritzen *et al*., 2000). They are also neuroprotective in *in vitro* models of excitotoxicity using primary cultures of cerebellar granule cells (Blondeau *et al*., 2002). The inhibition of TASK and TREK currents by glutamate via group I metabotropic receptors provides a molecular mechanism by which glutamate induces cellular excitability, but also probably overexcitability, leading to neuronal death.

Both TASK and TREK channels are the targets of many regulatory processes that are commanded via intracellular (TREK) or extracellular (TASK) pH variations, hypoxia (TASK), osmolarity and membrane stretch (TREK). On the other hand, these K_{2P} channels now appear as central targets of transduction pathways including PIP_2, IP_3, DAG and PA. This sophistication of regulation is uncommon in the K^+ channel family and is reminiscent of that observed in the TRP family (Kiselyov and Muallem, 1999).

Materials and methods

Whole-cell recordings in rat cerebellar granule neurons

Primary cultures of cerebellar granule neurons were prepared from 6- to 8-day-old Wistar rats (Iffacredo, France). Cells were plated at a density of 2×10^6 cells per 35 mm culture dish. Eight to 12 days after plating, perforated patch measurements were obtained using amphotericin B added to the pipette solution to a final concentration of 240 μg/ml. The pipette solution contained (in mM): 125 KCl, 5 $MgCl_2$, 0.1 BAPTA and 5 HEPES at pH 7.4 with KOH; and the bath solution contained (in mM): 135 NaCl, 2.5 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, and 10 HEPES at pH 7.4 with NaOH. Whole-cell recordings were performed at room temperature using a RK400 patch–clamp amplifier (Bio-Logic, Grenoble, France). Data were analysed using pClamp software. All results are expressed as mean ± SEM, with *n* indicating the number of cells tested.

Electrophysiology in transfected COS cells

COS cells were seeded at a density of 20 000 cells per 35 mm culture dish, 24 h before transfection. Cells were transfected by the classical DEAE-dextran method with 1 μg of pIRES-CD8 containing hTASK1, hTASK3, hTREK1 or hTREK2, with or without 1 μg of pRK/mGluR1a or pRK/mGluR5a expression vectors (a generous gift of Drs J.P.Pin and A.Dumuis). Transfected cells were visualized 48 h after transfection using anti-CD8 antibody-coated beads. For whole-cell experiments, the pipette solution (INT) contained (in mM):155 KCl, 3 $MgCl_2$, 5 EGTA and 10 HEPES, adjusted to pH 7.3 with KOH; the external solution (EXT) contained (in mM): 150 NaCl, 5 KCl, 3 $MgCl_2$, 1 $CaCl_2$ and 10 HEPES, adjusted to pH 7.4 with NaOH. In inside-out experiments the pipette solution contained the EXT solution and the external solution was INT. In experiments with PIP_2 and TASK channels, Mg was omitted in the INT solution (Huang *et al*., 1998). Recordings were performed at room temperature using a RK400 patch–clamp amplifier (Bio-Logic). Data were analyzed using pClamp software. All currents were measured at 0 mV. All results are expressed as mean ± SEM, with *n* indicating the number of cells tested. Student's *t*-tests and one-way ANOVA combined with a Student–Newman–Keuls *post hoc* tests were used to compare the different values, and were considered significant at $P < 0.05$.

Reagents

All reagents were obtained from Sigma except (RS)-3,5-dihydroxyphenylglycine (Tocris), pertussis toxin (Calbiochem), PIP_2 antibodies (Molecular Probes), PKC peptide inhibitor (fragment 19–36), SAG, SLG, DOG, RHC80267, diPGP and diOGP (Biomol). All reagents were dissolved in appropriate solvents (H_2O, ethanol or DMSO), with DMSO and ethanol never exceeding 1% of final solutions. Aqueous solution of SAG, SLG, DOG, diPCP, diOGP, PIP_2 and RHC80267 were sonicated on ice for 30 min before use.

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