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REVIEW

The mechano-gated K_{2P} channel TREK-1

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Abstract The versatility of neuronal electrical activity is largely conditioned by the expression of different structural and functional classes of K^+ channels. More than 80 genes encoding the main K^+ channel alpha subunits have been identified in the human genome. Alternative splicing, heteromultimeric assembly, post-translational modification and interaction with auxiliary regulatory subunits further increase the molecular and functional diversity of K^+ channels. Mammalian two-pore domain K^+ channels (K_{2P}) make up one class of K^+ channels along with the inward rectifiers and the voltage- and/or calcium-dependent K^+ channels. Each K_{2P} channel subunit is made up of four transmembrane segments and two pore-forming (P) domains, which are arranged in tandem and function as either homo- or heterodimeric channels. This novel structural arrangement is associated with unusual gating properties including "background" or "leak" K^+ channel activity, in which the channels show constitutive activity at rest. In this review article, we will focus on the lipid-sensitive mechano-gated K_{2P} channel TREK-1 and will emphasize on the polymodal function of this "unconventional" K^+ channel.

Keywords Ion channels · Stretch · Mechanotransduction · Potassium · Pharmacology · Physiology

K^+ channels are responsible for cell repolarization, thus controlling both the resting and the dynamic electrical activity of cells. Mammalian K^+ channel subunits can contain two, four or six/seven transmembrane segments. Members of the two and six/seven transmembrane segment classes are characterized by the presence of a single P domain, whereas the more recently discovered class of four transmembrane segments subunits contain two P domains in tandem (Fig. 1a) (Goldstein et al. 2001; Kim 2003; Lesage and Lazdunski 2000; Patel and Honoré 2001b; Talley et al. 2003). In K_{1P} channels, four matching P loops are assembled in a homo- or hetero-tetramer (all subunits have a similar P domain sequence GYG), whereas, in the dimeric K_{2P} channels, the P1 and P2 domains are different (Doyle et al. 1998; O'Connell et al. 2002).

The inward rectifiers are made of two transmembrane segments and one pore-forming K^+ channel subunit. Some of these channels (IRK) are constitutively active at rest (i.e., they leak K^+), while the activity of others is influenced by modulators such as G proteins (G-protein-coupled inwardly rectifying K^+ (GIRK) channels or nucleotides (K_{ATP}). Conductance increases on hyperpolarization and consequently, the inward K^+ currents recorded at potentials below the equilibrium potential EK^+ (approximately ~90 mV in a physiological K^+ gradient) are much larger than the outward K^+ currents recorded at depolarized potentials (inward rectification). Although the amplitude of the outward currents flowing through the inward rectifiers is limited, they profoundly influence the resting membrane potential.

The outward rectifiers are encoded by the 6/7 transmembrane segment and one pore-forming subunit opening upon depolarization (Kv channels) and/or after intracellular Ca^{2+} increase (BK and SK channels). Depolarization is sensed by the positively charged fourth transmembrane segment of Kv channels which is coupled to the activation gates. Opening of the voltage-gated K^+ channels is time-dependent (delayed rectifiers) and contributes to termination of the action potential.

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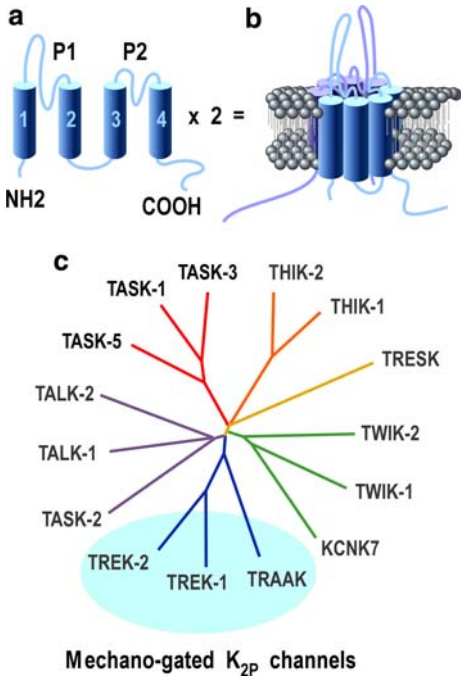

Fig. 1 Membrane topology of K_{2P} channel subunits and molecular diversity. **a** Membrane topology of a four transmembrane segment (TMS) 2 P domain K^+ channel subunit. Both the amino and carboxy terminal domains face the cytosol. **b** A functional channel is a dimer of subunits. **c** Phylogenetic tree of human 2P domain K^+ channels. So far 15 subunits have been cloned forming 6 structural and functional subfamilies. TREK-1, TREK-2 and TRAAK are stretch-activated K^+ channels

The *Saccharomyces cerevisiae* TOK-1 channel (composed of eight transmembrane segments) was the first K^+ channel discovered to contain two P domains in tandem (Goldstein et al. 1996; Ketchum et al. 1995; Lesage et al. 1996b; Reid et al. 1996; Zhou et al. 1995). Subsequently, subunits comprising four transmembrane segments and two P domains in tandem have been cloned in mammals, *Drosophila melanogaster*, *Caenorhabditis elegans* and plants (Czempinski et al. 1997; Goldstein et al. 1996; Lesage et al. 1996a; Wei et al. 1996). A functional K_{2P} channel consists of a dimer of subunits which may also heteromultimerize (for reviews see (Goldstein et al. 2001; Honoré 2007; Lesage and Lazdunski 2000; O'Connell et al. 2002; Patel and Honoré 2001b) (Fig. 1b). Many K_{2P} channels have a phenylalanine in the GXG motif (where X represents any amino acid) of the selectivity filter in the second pore domain, instead of a tyrosine as in K_{1P} channels (Goldstein et al. 2001; Lesage and Lazdunski 2000; O'Connell et al. 2002). This means that in K_{2P} channels the pore is predicted to have a two-fold symmetry, rather than the classical four-fold arrangement of other K^+ channels. Although the selectivity of K_{2P} channels for K^+ over Na^+ is high [permeability ratio (P_{Na}/P_K) <0.03], these structural differences suggest more varied permeation and gating properties compared to K_{1P} channels (O'Connell et al. 2002; Patel and Honoré 2001b).

The class of mammalian K_{2P} channel subunits now includes 15 members (Fig. 1c). Although these subunits display the same structural arrangement, with four transmembrane segments, two P domains, and an extended M1P1 extracellular loop with intracellular N- and C-termini, they share rather low sequence identity outside the P regions (Fig. 1c) (Patel and Honoré 2001b; Patel et al. 2001). The K_{2P} channel subunits are subdivided into six main structural and functional classes (Fig. 1c): tandem of P domains in a weak inwardly rectifying K^+ (TWIK)-1, TWIK-2 and KCNK7 channels (functional expression of KCNK7 has not yet been reported); mechano-gated and arachidonic acid-activated TWIK-related K^+ (TREK)-1, TREK-2 and TRAAK channels; TWIK-related acid-sensitive K^+ (TASK)-1, TASK-3 and TASK-5 channels (functional expression of TASK-5 has not yet been reported); tandem pore domain halothane-inhibited K^+ (THIK)-1 and THIK-2 channels (functional expression of THIK-2 has not yet been reported); TWIK-related alkaline-pH-activated K^+ (TALK-1), TALK-2 and TASK-2 channels; and the TWIK-related spinal cord K^+ (TRESK) channel, which is regulated by intracellular calcium.

In this article, we will review the functional properties of the K_{2P} channels, with particular focus on TREK-1 (K2P2.1 or KCNK2), the most extensively studied K_{2P} channel. The complex gating properties of TREK-1 and its modulation by cellular lipids, membrane-receptor-coupled second messengers and pharmacological agents will be described. We will then discuss the possible role of TREK-1 in various disease states.

Functional properties of K_{2P} channels

Mammalian K_{2P} channels (Goldstein et al. 2001; Kim 2003; Lesage and Lazdunski 2000; Patel and Honoré 2001b; Talley et al. 2003) show either a weak inward rectification [as is the case for tandem of P domains in a weak inwardly rectifying K^+ (TWIK)-1 channel] (Lesage et al. 1996b), an open rectification [as for TWIK-related acid-sensitive K^+ (TASK)-1 channel] (Duprat et al. 1997), or an outward rectification (as for TREK-1) (Maingret et al. 2002; Patel et al. 1998). Some channels, such as TASK-1 and TASK-3, are constitutively open at rest (Bayliss et al. 2001; Duprat et al. 1997; Kim et al. 1998; Talley and Bayliss 2002; Talley et al. 2000), whereas other channels, including TREK-1, require physical or chemical stimulation to open (Chemin et al. 2005a; Maingret et al. 1999, 2000a, b; Patel et al. 1998). The key feature of the K_{2P} channels is that they open over the whole voltage range, and therefore qualify as leak or background K^+ channels

(Goldstein et al. 2001; Kim 2003; Lesage 2003; Patel and Honoré 2001b). In TWIK-1, the first mammalian K_{2P} channel to be identified, a cysteine residue located at position 69 in the M1P1 extracellular loop is implicated in the formation of a disulfide bond between subunits (Lesage et al. 1996c). This covalent association is necessary for the functional expression of TWIK-1. However, a disulfide bridge is not essential for correct functional expression of other K_{2P} channels including TWIK-2 and TASK-2, so this requirement might be restricted to TWIK-1 (Niemeyer et al. 2003; Patel et al. 2000).

K_{2P} channels protein partners

Various proteins that interact and modulate the function and localization of K_{2P} channels have been recently identified. Enzymes in the plasma membrane that conjugate small ubiquitin-like modifier (SUMO) assemble in tandem with P domains in a weak inwardly rectifying K^+ (TWIK-1) (Rajan et al. 2005). Covalent modification at lysine 274 with SUMO silences TWIK-1. Removal of this peptide by SUMO protease has revealed that TWIK-1 is a K^+-selective, pH-sensitive, openly rectifying channel regulated by reversible peptide linkage (Rajan et al. 2005). However, these results have recently been disputed and the role of sumoylation in the regulation of TWIK-1 activity is obscure (Feliciangeli et al. 2007).

EFA6, an exchange factor for the small G protein ADP-ribosylation factor 6 (ARF6), is another protein partner of TWIK-1 (Decressac et al. 2004). EFA6 interacts with TWIK1 only when it is bound to ARF6. Because ARF6 modulates endocytosis at the apical surface of epithelial cells, the ARF6–EFA6–TWIK-1 association is probably important for TWIK-1 internalization and recycling (Decressac et al. 2004).

The EF hand superfamily protein p11 (*S100A10*, annexin 2 light chain) directly interacts with the C-terminal domain of TASK-1 (Girard et al. 2002). Conflicting results concerning the exact site of interaction and the functional effect of P11 on TASK-1 have been reported: P11 either promotes the expression of TASK-1 at the plasma membrane, or it acts as a "retention factor" that causes localization of TASK-1 to the endoplasmic reticulum (Girard et al. 2002; Renigunta et al. 2006).

Interaction of the scaffolding protein 14-3-3 with the C-terminal domain of TASK-1 overcomes retention of the channel in the endoplasmic reticulum that is mediated by dibasic signals in TASK-1 binding to β-COP (O'Kelly et al. 2002). Therefore, 14-3-3 promotes forward transport of TASK-1 to the surface of the membrane (O'Kelly et al. 2002).

Finally, AKAP150 interacts with TREK-1 and changes the regulatory properties of the channel, as outlined in the main text (Sandoz et al. 2006).

K_{2P} channels permeation

Mammalian K_{2P} channels, unlike the yeast two-pore-domain channel TOK-1 (Ketchum et al. 1995; Lesage et al. 1996a; Reid et al. 1996; Zhou et al. 1995), can pass large inward K^+ currents in elevated concentrations of extracellular K^+ at negative membrane potentials (Duprat et al. 1997; Fink et al. 1998; Goldstein et al. 1996; Lesage et al. 1996b, 2000; Reyes et al. 1998). The rectification of a constitutively open K^+-selective pore (leak channel) is a direct function of the difference in concentration of K^+ across the membrane, as expected from the Goldman–Hodgkin–Katz (GHK) constant field theory (Hille 1992). The GHK equation predicts an increase in conductance when ions flow from the more concentrated side (Hille 1992). The equation also assumes that permeant ions are not interacting. However, K_{2P} channels, as previously demonstrated for the classical K_{1P} channels, fail to respect this rule of ionic independence, which indicates that the pore can simultaneously accommodate multiple ions, with their movement influenced by each other (Ilan and Goldstein 2001). The GHK equation also anticipates that leak channels lack voltage and time dependency—that is, K^+ flow should remain stable over time (Hille 1992). However, several mammalian K_{2P} channels show both striking voltage- and time-dependent gating (Bockenhauer et al. 2001; Honoré et al. 2006; Lopes et al. 2000; Maingret et al. 2002). We will now concentrate on the functional properties of the mechano-gated lipid-sensitive TREK-1 K_{2P} channel (Fig. 1c), the most thoroughly studied K_{2P} channel at the biophysical, pharmacological and physiological levels.

Pattern of TREK-1 expression

Human TREK-1 is highly expressed in the brain, where it is particularly abundant in γ-aminobutyric acid (GABA) ergic interneurons of the caudate nucleus and putamen (Hervieu et al. 2001). TREK-1 is also expressed in the prefrontal cortex, in the hippocampus, in the hypothalamus, in the midbrain serotoninergic neurons of the dorsal raphé nucleus and in sensory neurons of dorsal root ganglia (Heurteaux et al. 2006; Medhurst et al. 2001).

Moreover, TREK-1 is also found in peripheral tissues such as the gastrointestinal tract (Fink et al. 1996; Medhurst et al. 2001; Talley et al. 2001). Immunohistochemical labeling on mesenteric artery sections have shown that TREK-1 expression is distributed throughout the

mesenteric wall in both myocytes and endothelial cell layers (Garry et al. 2007) (Fig. 3a). TREK-1 is also expressed in mouse cutaneous arteries (Garry et al. 2007). A particularly strong staining, confirmed by electron microscopy analysis, is observed in the endothelium of the blood vessel network surrounding and interconnecting hair follicles (Garry et al. 2007). Additionally, RT PCR experiments have detected TREK-1 mRNA expression in rat and mouse basilar arteries, but this subunit is absent from both carotid and femoral arteries (Blondeau et al. 2007). Expression of TREK-1 in the myocytes and endothelial cells of the basilar artery was confirmed by in situ hybridization, Western blot analysis and immunostaining (Blondeau et al. 2007).

TREK-1 voltage-dependency

Deletional and chimeric analyses have shown that the C-terminal domain of TREK-1 conditions the voltage-dependent gating of the channel (Maingret et al. 2002). Phosphorylation by protein kinase A of serine 333 in the distal C-terminal domain has been proposed to be responsible for the interconversion between the voltage-dependent and the leak phenotype of rat TREK-1 (Bockenhauer et al. 2001; Maylie and Adelman 2001) (see below). However, there must be additional mechanisms controlling TREK-1 voltage-dependent gating because, in a significant number of recordings, the TREK-1 S333A mutant reveals an intermediate phenotype between leak and voltage dependency (Bockenhauer et al. 2001). Furthermore, the activation kinetics and the voltage dependency of the mouse TREK-1 S333A mutant do not differ from those of the wild-type channel (Maingret et al. 2002).

TREK-1 therefore combines a leak channel (an instantaneous component) with a voltage-dependent outward rectifier (a delayed component) (Bockenhauer et al. 2001; Chemin et al. 2005a, b; Maingret et al. 2002). As K_{2P} channels diverge from the constant-field GHK current formulation and are characterized by complex and sophisticated permeation and gating mechanisms, they should not be considered as genuine leak K^+ channels (Bockenhauer et al. 2001; Patel and Honoré 2001a).

Mechano-activation of TREK-1

Membrane stretch reversibly induces TREK-1 channel opening in both cell-attached and excised inside–out configurations (Maingret et al. 1999; Patel et al. 1998) (Figs. 2a, b, 4). Mechano-activation of TREK-1 occurs independently of intracellular Ca^{2+} and ATP levels (Patel et al. 1998). In the inside–out patch configuration (i.e. the intracellular side of the membrane is facing the bath), positive pressure is significantly less effective than negative pressure at opening channels, which implies that a specific membrane deformation (convex curving) probably preferentially opens these channels (Patel et al. 1998). The relationship between channel activity and pressure is sigmoidal with half-maximal activation at about −40 mm Hg (Honoré et al. 2006) (Fig. 2b). At the whole cell level, when the osmolarity of the external solution is increased, the basal TREK-1 current amplitude is strongly reduced, which suggests that cellular volume (presumably by influencing tension on the cell membrane) also regulates channel activity (Maingret et al. 2000a; Patel et al. 1998).

The number of active TREK-1 channels is enhanced after treatment of cell-attached patches with agents that disrupt the actin cytoskeleton or after patch excision (Honoré et al. 2006; Lauritzen et al. 2005; Patel et al. 1998). Mechanical force is likely to be transmitted to the channel through the bilayer, with the cytoskeleton acting as a tonic repressor, limiting channel activation by membrane tension (Lauritzen et al. 2005; Patel et al. 1998, 2001) (Fig. 4). Conversely, the expression of TREK-1 markedly alters the

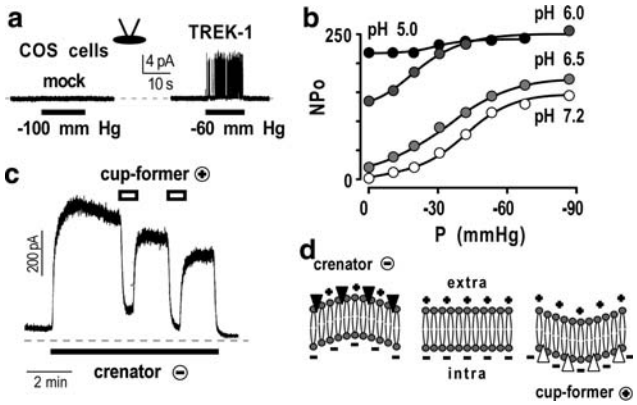

Fig. 2 Polymodal activation of TREK-1 by physical and chemical stimuli. **a** TREK-1 is activated by membrane stretch. COS cells were transiently transfected with the cDNA encoding TREK-1 and channel activity was monitored at +50 mV in the cell-attached patch configuration. Mechano-gated K^+ channels are absent in mock transfected COS cells. Mechano-sensitivity of TREK-1 persists upon patch excision in the inside–out patch configuration. **b** The dose-effect curve between channel activity and negative pressure is described by a sigmoidal relationship. At intracellular pH 7.2, 50% of TREK-1 channels are opened at a patch pressure value of −36 mm Hg. Intracellular acidification gradually shifts the dose-effect curve towards more positive values and ultimately at pH 5.0 all channels are opened in the absence of stretch. Intracellular acidosis converts mechano-gated TREK-1 channels into constitutively active K^+ channels. **c** Anionic amphipaths, including polyunsaturated fatty acids such as arachidonic acid, are openers of TREK-1 (recorded in the whole cell configuration at 0 mV), while cationic amphipaths reverse channel activation. **d** Anionic amphipaths preferentially insert in the external leaflet of the bilayer, while cationic amphipaths insert in the inner leaflet thus differentially altering the membrane curvature. The bilayer is naturally asymmetric with an excess of negatively charged phosphatidylserines in the inner leaflet. Adapted with permission from (Maingret et al. 1999; Patel et al. 1998)

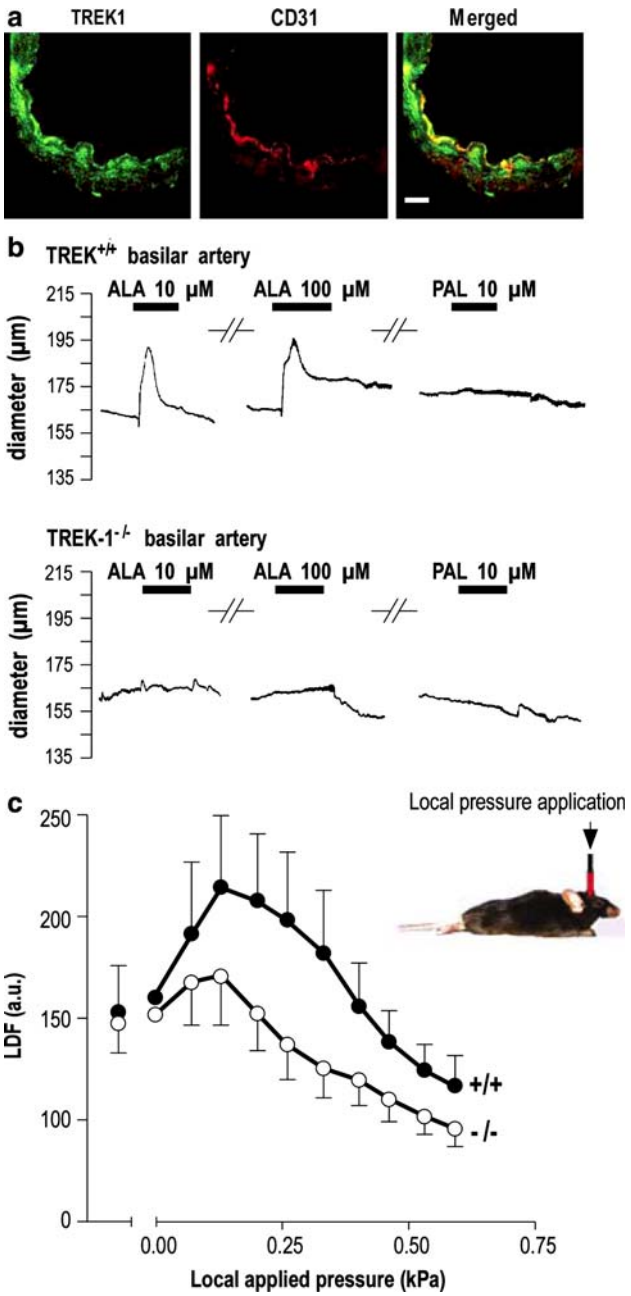

Fig. 3 Role of TREK-1 in vascular physiology. **a** TREK-1 localization in mouse mesenteric arteries. TREK-1 immunoreactivity is shown in green, the endothelial cell marker CD31 in red or both in yellow merged. The double staining indicates endothelial localization of TREK-1. **b** Alpha-linolenic acid (ALA)-induced vasodilation is abolished in the basilar artery of TREK-$1^{-/-}$ mice. The saturated fatty acid palmitic acid (PAL) had no effect. In TREK-$1^{-/-}$ mice, ALA-induced vasodilation is lost. **c** In vivo changes in cutaneous blood flow in response to a progressive local pressure applied at 2.2 Pa/s in TREK-$1^{-/-}$ and WT mice. The inset shows the mechanical device used to apply progressive pressure on the skin and simultaneously measure cutaneous blood flow with a laser Doppler probe at the same site. Adapted with permission from (Blondeau et al. 2007; Garry et al. 2007)

cytoskeletal network and induces the formation of membrane protrusions that are rich in actin and ezrin (a protein that links the cytoskeleton to the membrane) (Lauritzen et al. 2005). Thus, there is a dynamic interaction between TREK-1 and the actin cytoskeleton that will influence both neuronal electrogenesis and synaptogenesis (Lauritzen et al. 2005).

Fig. 4 Polymodal activation of TREK-1 by physical and chemical stimuli. TREK-1 is up-modulated by stretch, heat, intracellular acidosis, depolarization, lipids, volatile general anaesthetics and down-modulated by PKA and PKC phosphorylation pathways. TREK-1 is tonically inhibited by the actin cytoskeleton. When the C-terminal domain is partially coupled to the membrane, TREK-1 channel activity can be increased by stretch, intracellular acidosis and depolarization. Protonation of E306 within the C terminus of TREK-1 increases the affinity of this domain for inner leaflet phospholipids and dramatically increases channel activity. When the negative charge of E306 is titrated at acidic pH or when it is mutated by amino acid substitution (E306A), TREK-1 becomes constitutively active (leak K^+-selective channel). Stimulation of Gq-coupled receptors, including the metabotropic mGluR1 and mGluR5 receptors, inhibits TREK-1 activity. Several mechanisms occur: hydrolysis of PtdIns(3,4)P_2 and uncoupling of the C-terminal domain of TREK-1 from the membrane; a direct inhibition of TREK-1 by diacylglycerol and phosphatidic acid and phosphorylation of S300 and S333 by protein kinase C. Stimulation of Gs-coupled receptors, including the 5HT4 receptor, inhibits TREK-1 activity via the cAMP/PKA/S333 phosphorylation pathway

TREK-1 shows pronounced desensitization within 100 ms of membrane stretch (Honoré et al. 2006). This phenomenon is independent of the cytoskeleton and remains after patch excision. Mechanosensitive currents can be assigned to a four-state cyclic kinetic model, without the need to introduce adaptation of the stimulus, which therefore implies the presence of an inactivation mechanism that is intrinsic to the channel (Honoré et al. 2006). Progressive deletion of the C-terminal domain of TREK-1 gradually renders the channels more resistant to stretch, with a faster inactivation demonstrating that this domain is central to channel mechano-gating (Honoré et al. 2006; Maingret et al. 1999; Patel et al. 1998).

Modulation of TREK-1 by internal pH

Lowering the internal pH shifts the pressure-activation relationship of TREK-1 toward positive pressure values (the

opposite occurs following C- terminal deletion) and ultimately leads to channel opening at atmospheric pressure (Honoré et al. 2002; Maingret et al. 1999) (Figs. 2b, 4). Moreover, TREK-1 inactivation is gradually inhibited by intracellular acidosis (Honoré et al. 2006). Acidosis essentially converts a TREK-1 mechanogated channel into a constitutively active, leak K^+ channel (Honoré et al. 2002; Maingret et al. 1999) (Fig. 2b). Residue E306 in the proximal C-terminal domain acts as a proton sensor and is therefore important in the internal pH modulation of TREK-1 (Honoré et al. 2002) (Fig. 4). Removing this negative charge, by protonation or amino acid substitution, is central to the regulation of TREK-1 gating (Honoré et al. 2002).

Temperature sensitivity of TREK-1

A progressive rise in temperature induces a gradual and reversible stimulation of TREK-1 activity (Maingret et al. 2000a) (Fig. 4). The maximal temperature sensitivity of TREK-1 is observed between 32 and 37°C, with a 0.9-fold increase in current amplitude per degree centigrade (Maingret et al. 2000a). Patch excision results in the loss of the response of TREK-1 to heat, whereas stretch still maximally opens channels (Maingret et al. 2000a). Therefore, thermal activation of TREK-1 critically requires cell integrity, and implies that an indirect mechanism is acting on the channel.

Modulation of TREK-1 by membrane phospholipids

The cationic molecules poly-lysine or spermine have a high affinity for anionic phospholipids and inhibit TREK-1 currents when administered intracellularly on excised inside-out patches (Chemin et al. 2007; Lopes et al. 2005). However, channel activity can be restored and even stimulated by applying phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P_2) on the intracellular side of the channel (Chemin et al. 2007; Lopes et al. 2005). Other inner leaflet phospholipids such as phosphatidylinositol, phosphatidylethanolamine or phosphatidylserine also increase the activity of TREK-1 (Chemin et al. 2007). The presence of a large polar head is not an absolute requirement, as phosphatidic acid also stimulates TREK-1 channel activity (Chemin et al. 2007), but diacylglycerol cannot increase this activity, which indicates that the negative phosphate group at position 3 of the glycerol is critical (Chemin et al. 2007; Lopes et al. 2005).

A cluster of five positive charges in the proximal C-terminal domain of TREK-1, which encompass the proton sensor E306, is central to the effect of phospholipids (Chemin et al. 2007) (Fig. 4). This cationic region is required for the interaction between the C-terminal domain of TREK-1 and the inner leaflet of the plasma membrane (Chemin et al. 2007). The positively charged nature of this region is increased at acidic pH_i (or by the E306A mutation), which probably favours a stronger electrostatic interaction with the inner leaflet phospholipids (Chemin et al. 2007) (Fig. 4). This region is also involved in the interaction with AKAP and comprises a serine residue that can be phosphorylated by protein kinase C (see below) (Murbartian et al. 2005; Sandoz et al. 2006).

Agonist-induced PtdIns(4,5)P_2 hydrolysis inhibits TREK-1 activity by shifting its voltage-dependency of activation towards more depolarized voltages (Lopes et al. 2005). Conversely, stimulation of channel activity as a result of a left shift in the voltage dependency of TREK-1 currents occurs in response to addition of PtdIns(4,5)P_2 (Chemin et al. 2007; Lopes et al. 2005). The fine regulation of TREK-1 voltage dependency by PtdIns(4,5)P_2 might be involved in the down-regulation of TREK-1 by G_q-coupled membrane receptors (see below) (Lopes et al. 2005).

Polyunsaturated fatty acids modulation of TREK-1

The TREK-1 channel is reversibly opened by anionic amphipaths including polyunsaturated fatty acids, such as arachidonic acid (Patel et al. 1998) (Figs. 2c, 4). The activation is observed in excised patch configurations and in the presence of cyclooxygenase and lipoxygenase inhibitors, which indicates that the effect is direct (Patel et al. 2001). Chain length (docosahexaenoic acid C22:6 being the most potent polyunsaturated acid tested), the extent of unsaturation (at least one double bond) and a negative charge are critically required for channel stimulation (Patel et al. 1998).

The bilayer–couple hypothesis assumes that the effects of amphipaths derive entirely from interactions within the bilayer (Martinac et al. 1990; Sheetz and Singer 1974) (Fig. 2d). Anionic amphipaths, including arachidonic acid, preferentially insert into the outer leaflet, presumably because of the natural asymmetric distribution of negatively charged phosphatidylserines in the inner leaflet (Martinac et al. 1990; Sheetz and Singer 1974) (Fig. 2d). This differential insertion produces a convex, positive curvature of the membrane. By contrast, positively charged amphipaths are expected to preferentially insert into the inner leaflet of the bilayer and thereby generate a concave negative curvature (Martinac et al. 1990; Sheetz and Singer 1974). Assuming that TREK-1 is preferentially opened by negative mechanical pressure (which causes convex or negative curvature of the membrane) rather than positive mechanical pressure, it is indeed expected from the bilayer–couple hypothesis that anionic amphipaths, such as

trinitrophenol, open the channel, whereas cationic amphipaths, including chlorpromazine, close it (Patel et al. 1998, 2001) (Fig. 2d). Moreover, as anticipated by the bilayer couple hypothesis, the stimulation of TREK-1 by arachidonic acid is voltage-dependent, decreasing e-fold for 41 mV depolarization (Meadows et al. 2000).

Deletional analysis demonstrates that the C terminus of TREK-1 is critical for the response to arachidonic acid, as previously demonstrated for membrane stretch (Patel et al. 1998). These results strongly suggest that activation of TREK-1 by polyunsaturated fatty acids and stretch might be related. However, the possible existence of a specific binding site for polyunsaturated fatty acids on the channel protein itself cannot be entirely ruled out (Kim 2003; Patel et al. 1998, 2001).

Lysophospholipid modulation of TREK-1

Extracellular lysophospholipids (including lysophosphatidylcholine) but not phospholipids open TREK-1 (Maingret et al. 2000b) (Fig. 4). At low doses, arachidonic acid and lysophosphatidylcholine induce additive activation. The effect of lysophospholipids is critically dependent on the length of the carbonyl chain and the presence of a large polar head, but is independent of the global charge of the molecule (Maingret et al. 2000b). The conical shape of lysophospholipids is the key parameter conditioning TREK-1 stimulation. By contrast, intracellular lysophospholipids inhibit TREK-1 activation (Patel et al. 2001).

TREK-1 is also strongly activated by lysophosphatidic acid (LPA), but only when applied on the intracellular side of the channel following patch excision in the inside-out patch configuration (Chemin et al. 2005a). LPA reversibly converts the voltage-, internal pH- and stretch-sensitive K^+ channel TREK-1 into a leak conductance channel (Chemin et al. 2005a).

Membrane receptors and second messengers modulation of TREK-1

When co-expressed with the G_s-coupled $5HT_4$ receptor, serotonin inhibits TREK-1 (Fink et al. 1996; Patel et al. 1998). This effect is mimicked by a membrane-permeant derivative of cyclic AMP and is mediated by protein kinase A-mediated phosphorylation of S333 in the C-terminal domain of TREK-1 (Patel et al. 1998) (Fig. 4). Binding of A-kinase-anchoring protein 150 (AKAP150) to a key regulatory charged domain in the proximal C-terminal domain of TREK-1 (the same region that is involved in modulation by PtdIns(4,5)P_2 and internal pH) dramatically stimulates TREK-1 activity (Chemin et al. 2005b; Honoré et al. 2002; Sandoz et al. 2006). Furthermore, in the presence of AKAP150, inhibition of TREK-1 by G_s-coupled receptors is enhanced, which suggests that AKAP150 might cluster TREK-1 with protein kinase A to facilitate protein kinase A-mediated phosphorylation of TREK-1 (Sandoz et al. 2006).

Stimulation of G_q-coupled receptors, including metabotropic glutamate receptors 1 or 5, inhibits TREK-1 (Chemin et al. 2003; Lopes et al. 2005; Murbartian et al. 2005). Several pathways are involved: first, phospholipase C hydrolyses PtdIns(4,5)P_2 (Lopes et al. 2005); second, TREK-1 may be directly inhibited by diacylglycerol and/or phosphatidic acid (Chemin et al. 2003); and, finally, protein kinase C sequentially phosphorylates S333 and S300 (which is also located in the AKAP150 binding site) (Murbartian et al. 2005). As AKAP150 reverses the downregulation of TREK-1 by G_q-coupled receptor stimulation (or stimulation with the phorbol ester phorbol-12-myristate-13-acetate), this suggests that it might prevent the access of protein kinase C to the S300 phosphorylation site (Sandoz et al. 2006).

Finally, nitric oxide donors, as well as 8-bromo-cGMP, increase TREK-1 currents by protein kinase G-mediated phosphorylation of S351 (Koh et al. 2001). This effect is apparently very labile as it can only be detected using the perforated whole cell patch clamp configuration (Koh et al. 2001).

Pharmacology of TREK-1

TREK-1 is unusual in terms of its pharmacology, as it is resistant to all the classical blockers of K_{1P} channels, including tetraethylammonium and 4-aminopyridine (Fink et al. 1996; Patel et al. 1998). However, it is inhibited by a variety of other pharmacological agents, including the antidepressant selective serotonin reuptake inhibitors such as fluoxetine (Heurteaux et al. 2006; Kennard et al. 2005). This is apparently a direct effect, as channels are inhibited in the outside–out patch configuration (Kennard et al. 2005).

TREK-1 is opened by another important class of pharmacological agents, the volatile general anaesthetics (Franks and Honoré 2004; Franks and Lieb 1999; Patel and Honoré 2001a; Patel et al. 1998, 1999) (Fig. 4). Clinical doses of chloroform, diethyl ether, halothane and isoflurane open TREK-1 (Patel et al. 1999). Nitrous oxide, xenon and cyclopropane, which are gaseous anaesthetics known for their potent analgesic action in addition to their euphoric and neuroprotective effects, similarly stimulate TREK-1 at clinical doses (Gruss et al. 2004), as does chloral hydrate (Harinath and Sikdar 2004). Stimulation of TREK-1 activity by these anaesthetics occurs in excised patches, which

again suggests a direct effect. The C-terminal domain of TREK-1 also has a major role in its modulation by anaesthetics (Gruss et al. 2004; Harinath and Sikdar 2004; Patel et al. 1999).

TREK-1 and general anaesthesia

TREK-1$^{-/-}$mice show a marked decrease in sensitivity to chloroform, halothane, sevoflurane and desflurane (Franks and Honoré 2004; Heurteaux et al. 2004; Patel and Honoré 2001a; Patel et al. 1999). A concentration of halothane that is sufficient to anaesthetize 100% of a population of wild-type mice has no effect in the knockout mice (Heurteaux et al. 2004). No difference between wild-type and TREK-1-knockout mice was observed following administration of the barbiturate pentobarbital, which fails to affect TREK-1 (Heurteaux et al. 2004; Patel et al. 1999). Furthermore, knockout of the anaesthetic-resistant K_{2P} channel TRAAK fails to affect anaesthetic sensitivity, thus demonstrating the specificity of this phenotypeb (Heurteaux et al. 2004; Patel et al. 1999). Although the neuronal pathways that are responsible remain to be determined, these data demonstrate that the opening of TREK-1 contributes, along with the modulation of other targets including the γ-aminobutyric acid A receptors, to the cellular mechanisms of general anaesthesia (Franks and Honoré 2004; Heurteaux et al. 2004; Patel and Honoré 2001a; Patel et al. 1999).

TREK-1, lipids and neuroprotection

TREK-1$^{-/-}$ mice, unlike TRAAK$^{-/-}$ mice, are more sensitive to both ischaemia and epilepsy compared with wild-type mice (Franks and Honoré 2004; Heurteaux et al. 2004). The polyunsaturated fatty acid linolenate (C18:3) or lysophospholipids administered either intracerebroventricularly or intravenously are, unlike the saturated fatty acid palmitate, neuroprotective against global ischaemia and kainate-induced seizures (Blondeau et al. 2002a, b; Lang-Lazdunski et al. 2003; Lauritzen et al. 2000). Remarkably, such neuroprotection is absent in the TREK-1$^{-/-}$ mice (Franks and Honoré 2004; Heurteaux et al. 2004).

During brain ischaemia, arachidonic acid is released, the intracellular pH falls and neurons swell. These pathological alterations might contribute to the opening of TREK-1 and hyperpolarize both presynaptic neurons (which would limit the opening of voltage-dependent Ca^{2+} channels) and postsynaptic neurons (which would increase NMDA (*N*-methyl-D-aspartate) receptor Mg^{2+} block) and thus protect against glutamate excitotoxicity (Franks and Honoré 2004). It is interesting to note that various neuroprotective agents, including riluzole, nitrous oxide and xenon, are also potent openers of TREK-1 (Duprat et al. 2000; Gruss et al. 2004).

TREK-1 and pain perception

TREK-1 is expressed in both small and medium-sized sensory neurons of mouse dorsal root ganglion cells where it is co-localized with the capsaicin-activated nonselective ion channel TRPV1 (Alloui et al. 2006; Maingret et al. 2000a; Talley et al. 2001). TREK-1$^{-/-}$ mice are more sensitive than wild-type mice to painful heat sensations near the threshold between non-painful and painful heat (Alloui et al. 2006). Moreover, experiments using single polymodal C-fibres demonstrate that TREK-1$^{-/-}$ mice are more sensitive to heat stimulation (30–45°C) (Alloui et al. 2006). However, no difference is observed in the sensitivity to cold induced by a drop of acetone to the dorsal surface of the paw (Alloui et al. 2006).

Touching the skin with von Frey hairs of increasing stiffness revealed that TREK-1$^{-/-}$ mice are more sensitive to mechanical stimuli than are wild-type mice (Alloui et al. 2006). Thus, TREK-1 deletion results in allodynia, suggesting that this K_{2P} channel is probably important for tuning the mechanosensitivity of nociceptors. Chronic pain syndromes involve the sensitization of nociceptors and thermal receptors. Mediators of pain that bind to receptors coupled to G_q or G_s proteins, such as prostaglandin E2 receptors, result in TREK-1 inhibition (Maingret et al. 2000a). Interestingly, inflammation-induced mechanical and thermal hyperalgesia is lower in TREK-1$^{-/-}$ mice than in wild-type mice, suggesting that TREK-1 is, at least in part, involved in the peripheral sensitization of nociceptors during inflammation (Alloui et al. 2006).

These results suggest that TREK-1 controls the excitability of nociceptors, probably by opposing the depolarization that results from the opening of non-selective transient receptor potential channels, including TRPV1, that are also stimulated by pressure, heat and acidosis. Therefore, TREK-1 is an attractive pharmacological target for the development of novel types of analgesic drug.

TREK-1 and depression

Neither reflex nor cognitive functions are significantly altered in TREK-1$^{-/-}$ mice (Heurteaux et al. 2004). However, TREK-1 is reported to have an important functional role in mood regulation (Heurteaux et al. 2006). In the Porsolt forced swim test (FST) (Gordon and Hen 2006), TREK-1$^{-/-}$ mice swam for much longer than wild-type mice before feeling despair and giving up (Heurteaux et al. 2006). Administration of the antidepressants fluoxetine or

paroxetine in the FST reduce the immobility time of wild-type mice (i.e., they have an antidepressant action), but fail to affect TREK-1$^{-/-}$ mice (Heurteaux et al. 2006). Various other behavioural tests used to assay for the antidepressant effects of drugs confirmed the "antidepressant" state of the TREK-1$^{-/-}$ mice (Heurteaux et al. 2006).

TREK-1 and TRAAK are both expressed in the 5-hydroxytryptamine neurons of the midbrain dorsal raphé nucleus (Heurteaux et al. 2006). Importantly, combined treatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine methyl ester and fluoxetine, which together depletes serotonin from the nerve terminals, completely eliminates the antidepressant phenotype of the TREK-1$^{-/-}$ (Heurteaux et al. 2006). Moreover, the firing rate of neurons from the dorsal raphé nucleus is more than two-fold higher in the TREK-1$^{-/-}$ mice (although it is not altered in TRAAK$^{-/-}$ mice), as compared to wild-type mice (Heurteaux et al. 2006). These results indicate that TREK-1, unlike TRAAK, has a major role in mood regulation (Gordon and Hen 2006).

TREK-1 and vascular physiology

Alpha-linolenic acid injections increase cerebral blood flow and induce vasodilation of the basilar artery, but not the carotid artery where TREK-1 is absent (Blondeau et al. 2007) (Fig. 3b). The saturated fatty acid palmitic acid, which does not open TREK-1, fails to produce vasodilation (Fig. 3b). Vasodilation induced by PUFAs in basilar artery and the resultant increase in blood flow are abolished in TREK-1$^{-/-}$ mice (Blondeau et al. 2007) (Fig. 3b). Furthermore, deletion of TREK-1 leads to an important alteration in vasodilation of mesenteric arteries induced by acetylcholine and bradykyinin (Blondeau et al. 2007). Similarly, TREK-1 plays a major role in cutaneous endothelium dependent vasodilation (Garry et al. 2007). Additionally, the vasodilator response to local pressure application is also markedly decreased in TREK-1$^{-/-}$ mice, mimicking the decreased response to pressure observed in diabetes (Garry et al. 2007) (Fig. 3c). Therefore, deletion of TREK-1 is associated with a marked alteration in the efficacy of the G-protein-coupled receptor-associated cascade producing NO that leads to major endothelial dysfunction and impairment of vasodilation (Blondeau et al. 2007; Garry et al. 2007).

Conclusions

TREK-1-knockout mice provide very strong evidence for the important functional role of this polymodal K^+ channel in both neuronal and vascular cells (Alloui et al. 2006; Blondeau et al. 2007; Garry et al. 2007; Heurteaux et al. 2004, 2006). This "unconventional" K^+ channel, with its complex gating and regulation by membrane receptors and second messengers, is central to ischaemic and epileptic neuroprotection, pain sensing and depression. Furthermore, TREK-1 is an important pharmacological target playing a significant role in the cellular mechanism of general anesthesia by volatile and gaseous agents (Franks and Honoré 2004; Franks and Lieb 1999; Gordon and Hen 2006; Patel and Honoré 2001a).

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