

Chapter 2

Mechanosensitive K2P channels, TREKking through the autonomic nervous system

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2.1 Introduction

The TOK1/YORK channel from the yeast *Saccharomyces cerevisiae* was the first channel containing two-pore loop-forming domains to be cloned and sequenced (Ketchum et al. 1995; Lesage et al. 1996b). Although the TOK1/YORK channel comprises eight transmembrane segments (8TMS-2P), its discovery anticipated the flourishing of a new family of 4TMS mammalian potassium channels now known as the “two-pore domain potassium channels” (K2P, KCNK). The sequence of a family of 4TM channels containing two-pore domains (the CeK family) was obtained from the genome of the nematode *Caenorhabditis elegans* and it was also deposited in DNA databases in 1995 (Salkoff and Jegla 1995). One year later, the first member of the mammalian 4TMS-K2P family was described in human tissue and named TWIK-1 for “Tandem of pore domains in a Weak Inward rectifying K^+ channel” (Lesage et al. 1996a). Within less than 10 years 15 such channels had been categorized into six subfamilies: TWIK, TREK, TASK, TALK, THIK and TRESK (for extensive reviews on K2P channels see: Lesage and Lazdunski 2000; Lotshaw 2007; Enyedi and Czirják 2010).

The second member of the mammalian K2P family, TREK-1 (TWIK-1 Related K^+ channel), was first isolated from mouse brain in 1996 (Fink et al. 1996). This channel was the first member of the TREK subfamily discovered, soon followed by the mouse TRAAK (TWIK-Related Arachidonic Acid-stimulated K^+ channel: Fink et al. 1998) and finally by the rat (Bang et al. 2000) and human (Lesage et al. 2000b) TREK-2.

The members of the TREK subfamily are non-inactivating background potassium channels that can open at any membrane potential, thus playing an important role in the control of the resting membrane potential and in neuronal excitability. Moreover, their open rectification and slight voltage-dependency suggests a role in action potential repolarization, and consequently in repetitive firing (Goldstein et al. 2001).

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TREK channels are widely expressed throughout the nervous system, as well as in several non-neuronal tissues (Talley et al. 2001, 2003), indicating a broad and complex range of physiological and pathological roles, depending on the function of the cells, tissues and organs in which they are expressed.

All three members of the TREK subfamily share the distinctive characteristic of being activated by a mechanical membrane stretch (Patel et al. 1998; Maingret et al. 1999a; Bang et al. 2000). In addition, TREK channels are modulated via G protein-coupled receptors and by a extremely high number of physical and chemical physiological stimuli, including membrane deformation, temperature, pH, polyunsaturated fatty acids, lysophospholipids and neurotransmitters, (Lesage 2003; Kim 2003; Franks and Honore 2004; Mathie 2007; Mathie and Veale 2007; Lotshaw 2007; Huang and Yu 2008; Sabbadini and Yost 2009; Dedman et al. 2009; Noël et al. 2011). Non-physiological stimuli such as neuroprotective agents and volatile general anesthetics also potently regulate these channels. Indeed, their strong reactivity confers TREK channels with an additional complex set of putative cellular functions, apparently derived from the interaction of these factors with the C-terminus region of the protein.

2.2 Electrophysiological Properties

We usually think of leak channels as pores that produce non-rectifying ohmic currents, thereby producing a linear relationship between current and voltage (linear I - V) similar to that of an ohmic resistor in an electrical circuit. However, in theory, the current-voltage relationship (I - V) for a potassium-selective leak channel is expected to rectify in the outward direction, due to the difference in potassium concentration between the extracellular and intracellular fluids in physiological conditions. This behavior was already predicted by the constant field theory (GHK-current equation) and it is often referred to as “open channel rectification”, meaning that K^+ ions travel more easily through an open channel when moving from a compartment with high K^+ concentration to one with low K^+ concentration, producing higher conductance in this direction (Goldman 1943; Hodgkin and Katz 1949; Lesage and Lazdunski 2000; Goldstein et al. 2001; Plant et al. 2005; Honore 2007). However, current passing through a single-channel is most commonly studied at equimolar (symmetrical) potassium concentrations (often 150 mM, both inside and outside of the membrane). In these ideal conditions the concentration gradient vanishes and the I - V becomes linear, at least for a perfect leak channel.

As classically hypothesized, a leak current should also be voltage-independent, meaning that the open probability of the channel should be the same at any membrane voltage and that channels should not undergo inactivation. This also implies that rapid changes in the voltage of the membrane result in instantaneous alterations in the macroscopic current (whole-cell current) with only negligible time course constants, simply depending on the driving force for potassium ($V_m - E_K$). In summary, in ideal conditions (*e.g.*, equimolar potassium concentrations) the current generated by leak

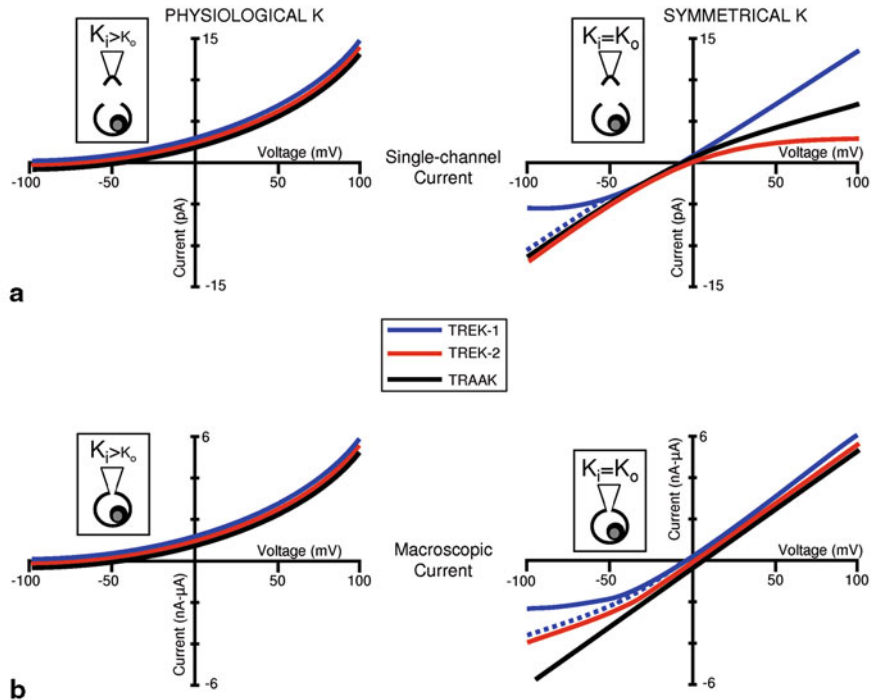


Fig. 2.1 Current-voltage relationships for TREK channels. At near physiological potassium concentrations, both single-channel (**a**, *left*) and macroscopic whole-cell currents (**b**, *left*) rectify strongly in the outward direction. This rule is applicable to all three members of the TREK subfamily. At high symmetrical potassium concentrations, TREK-1 single-channel currents still rectify in the outward direction (**a**, *right*; *blue*). This rectification essentially disappears when cations are removed (**a**, *right*; *dotted-blue*). TREK-2 and TRAAK single-channels exhibit inward rectification at symmetrical potassium concentrations (**a**, *right*; *red* and *black*). In the same conditions, TREK-1 and TREK-2 macroscopic whole-cell currents exhibit outward rectification (**b**, *right*; *blue* and *red*), although the stronger rectification of TREK-1 can be reduced by removing extracellular cations (**b**, *right*; *dotted blue*). TRAAK macroscopic I-Vs at high potassium concentrations are essentially linear (**b**, *right*; *black*). The data shown in this figure are not real but rather, they serve to summarize the cumulative findings of many authors (see text). “nA-μA” roughly refers to values obtained from experiments performed following heterologous expression in small cells or in oocytes, respectively

channels (macroscopic or single-channel) should follow Ohm’s law, maintaining a linear relationship with the voltage (linear I–V), while at physiological potassium concentrations the I–V should exhibit a slight outward rectification, as predicted by the GHK equation (outwardly rectifying I–V). TREK channels are universally considered leak (or baseline, background or resting) channels, and although they generally conform to the rules outlined above, some important deviations from this ideal behavior occur (as discussed below and in Fig. 2.1).

2.2.1 *TREK Single-Channel Properties*

Heterologously expressed members of the TREK subfamily, TREK-1 (Fink et al. 1996), TREK-2 (Lesage et al. 2000b) and TRAAK (Fink et al. 1998; Maingret et al. 1999a; see also Lesage et al. 2000a), exhibit flickering-bursting behavior, and a strongly outwardly rectifying single-channel I-V when recorded at asymmetrical (close to physiological) potassium concentrations (Fig. 2.1a, left). In atrial myocytes (Terrenoire et al. 2001), recordings from native TREK-1-like single-channels also revealed outward rectification at asymmetrical potassium concentrations. Thus, it seems likely that TREK channels do not permit the passage of significant inward potassium current in physiological conditions.

By contrast, there are clear differences among TREK channels when single-channels are studied at symmetrical potassium concentrations (approximately 150 mM on each side; Fig. 2.1a, right). In these conditions TREK-1 single-channel currents still exhibit clear outward rectification and a conductance of about 100 pS at positive potentials (Patel et al. 1998; Kim et al. 2001a; Maingret et al. 2002; Li et al. 2006). This single-channel outward rectification disappears in the absence of extracellular divalent cations (Ca^{2+} and Mg^{2+}) and the single-channel I-V becomes linear (Fig. 2.1a, right; dotted blue line) or very slightly inwardly rectifying (Bockenhauer et al. 2001; Maingret et al. 2002; Han et al. 2003; Li et al. 2006). Native TREK-1-like channels showing high conductance (84–120 pS) at positive potentials have also been described in rat atrial (Terrenoire et al. 2001) and ventricular (Tan et al. 2002; Kang et al. 2005; Li et al. 2006) myocytes, as well as rat hippocampal (Bockenhauer et al. 2001), Dorsal Root Ganglia (DRG; Kang et al. 2005) and supraoptic (Han et al. 2003) neurons.

TREK-2 single-channels exhibit clear inward rectification at symmetrical potassium concentrations (Fig. 2.1a, right; red line), with much lower conductance at positive (approx. 65 pS at +40 mV) than at negative (approx. 110 pS at -40 mV) voltages (Bang et al. 2000; Kim et al. 2001b; Han et al. 2002, 2003; Kang et al. 2004). Interestingly, human TREK-2 channels display much weaker rectification (Lesage et al. 2000b). Similar disparities in conductance at different membrane potentials have been reported for native TREK-2-like single-channel currents in cerebellar (Han et al. 2002; Kang et al. 2005), supraoptic (Han et al. 2003), pancreatic (Kang et al. 2004), DRG (Kang et al. 2005) and ganglionic sympathetic (Cadaveira-Mosquera et al. 2011) cells.

Heterologously expressed single TRAAK channels exhibit inward rectification at symmetrical potassium concentrations, although to a lesser degree than the TREK-2 channel (Fig. 2.1a, right; black line), showing a conductance of about 75 pS and > 100 pS at positive and negative membrane potentials respectively (Kim et al. 2001a; Han et al. 2003). Similar results were obtained for native TRAAK-like channels from supraoptic (Han et al. 2003) and DRG (Kang et al. 2005; Kang and Kim 2006) neurons.

In summary, the outward rectification induced by external divalent cations when single TREK channels are recorded at equimolar potassium concentrations appears to

be exclusive to TREK-1 (Enyedi and Czirják 2010). The single-channel rectification observed in divalent cation-free and symmetrical solutions is probably an intrinsic property of the channel produced by the structural characteristics of the channel pore. Interestingly, at least two different isoforms of TREK-1 (one of them permeable to sodium) and TREK-2, with clearly different conductances, can be expressed in heterologous systems by alternative translation initiation mechanisms (Thomas et al. 2008; Simkin et al. 2008). Several sub-conductance levels have also been reported (Li et al. 2006; Kang et al. 2007).

While leak channels are expected to be voltage-independent, the open probability of TREK-1 channels increases at positive membrane potentials, such that their voltage-dependency is not negligible (Maingret et al. 2002; Li et al. 2006). Interestingly, phosphorylation by Protein Kinase A (PKA) of TREK-1 and TREK-1-like channels expressed in hippocampal cells induces voltage-dependency in the otherwise leak-like channel (Bockenhauer et al. 2001; but see Maingret et al. 2002). The open probability of both human (Lesage et al. 2000b) and rat (Bang et al. 2000; Kang et al. 2007) TREK-2 channels also increases at positive potentials. This observation may explain the unexpected outward rectification of TREK-2 macroscopic currents at symmetrical K^+ concentrations, even when the single-channel I-V is clearly inwardly rectifying in the same conditions. Also TRAAK channels have been described as slightly voltage-dependent, with significant increases in P_o observed at positive membrane potentials (Maingret et al. 1999a; Kim et al. 2001a).

2.2.2 *TREK Macroscopic-Current Properties*

Unlike single-channel currents, macroscopic (whole-cell) currents are usually studied at physiological (asymmetrical) potassium concentrations. In these conditions the I-Vs for heterologously expressed TREK-1 (Fink et al. 1996; Meadows et al. 2000; Bockenhauer et al. 2001; Koh et al. 2001; Enyeart et al. 2002; Maingret et al. 2002; Kennard et al. 2005), TREK-2 (Bang et al. 2000; Lesage et al. 2000b) and TRAAK (Fink et al. 1998; Maingret et al. 1999a; Duprat et al. 2000; Lesage et al. 2000a; Meadows et al. 2001) currents are all strongly outwardly rectifying, with almost no inward current (Fig. 2.1b, left). This behavior appears to be independent of the subunit source (mouse, rat or human) and the heterologous system employed (oocyte (OO), COS or HEK), and rectification often exceeds that predicted by the constant field theory. Native TREK-1-like macroscopic currents from adrenocortical cells (Enyeart et al. 2002; Danthi et al. 2003) and TREK-2-like currents from cortical astrocytes and sympathetic neurons (Ferroni et al. 2003; Kucheryavykh et al. 2009; Cadaveira-Mosquera et al. 2011) are also predominantly outward, crossing the voltage axis near the Nernst equilibrium potential for potassium.

Although uncommon, analysis of TREK macroscopic currents in symmetrical conditions reveals that I-Vs can exhibit clear inward currents at negative potentials (Fig. 2.1b, right), indicating that the strong outward rectification observed at physiological concentrations (Fig. 2.1b, left) is not (or at least not completely) due

to voltage dependency. This phenomenon has been described in heterologously expressed TREK-1 (Patel et al. 1998; Koh et al. 2001; Maingret et al. 2002; Lopes et al. 2005; Sandoz et al. 2006; Cohen et al. 2008), TREK-2 (Lesage et al. 2000b) and TRAAK (Fink et al. 1998; Lesage et al. 2000a; Meadows et al. 2001) channels. Interestingly, TREK-1 and TREK-2 currents do not completely linearize at equimolar potassium concentrations and a clear outward rectification is still evident (Fig. 2.1b; right, blue and red lines). The macroscopic rectification of TREK-1 can be attenuated by removing divalent Mg^{2+} and Ca^{2+} cations and the residual outward rectification in these conditions has been ascribed to the intrinsic voltage-dependency of TREK-1 channels (Maingret et al. 2002; Sandoz et al. 2006; Li et al. 2006). However, this remaining rectification was recently attributed to voltage-dependent inhibition by protons as in the absence of Mg^{2+} , mutant TREK-1 channels that are insensitive to H^+ exhibit an essentially linear I-V at symmetrical K^+ concentrations (Cohen et al. 2008). Linear, or very slightly outwardly-rectifying I-Vs have been described for TRAAK, indicating a lack of intrinsic voltage dependency for this channel or possibly a compensation for the slight voltage dependency by inward single-channel rectification (Fink et al. 1998; Lesage et al. 2000a; Ozaita and Vega-Saenz de Miera 2002). At symmetrical K^+ concentrations, linear or weak outwardly-rectifying I-Vs have also been described for native TREK-1-like (Danthi et al. 2003) and TREK-2-like (Ferroni et al. 2003; Cadaveira-Mosquera et al. 2011) macroscopic currents.

While initial analyses of macroscopic currents evoked by voltage steps indicated no time dependency accompanying TREK-1 outward rectification (Meadows et al. 2000; Maingret et al. 2000a), clear time-dependent activation was reported subsequently (Bockenhauer et al. 2001; Maingret et al. 2002; Kennard et al. 2005). Thus, it has been proposed that two distinct populations of TREK-1 channels may coexist in physiological conditions, one phosphorylated and hence voltage-dependent, and the other dephosphorylated and voltage-independent. If true, step-activated macroscopic current should exhibit both instantaneous and time-dependent components, which indeed has been frequently reported (Enyeart et al. 2002; Kennard et al. 2005). Similarly, voltage changes result in rapid activation of human TREK-2 currents, exhibiting instantaneous and delayed components (Lesage et al. 2000b; Kim et al. 2005). By contrast, TRAAK channels appear to produce instantaneous non-inactivating currents when the membrane is voltage-stepped (Fink et al. 1998; Duprat et al. 2000; Lesage et al. 2000a; Ozaita and Vega-Saenz de Miera 2002).

A second and distinct form of voltage-dependence has been described for TREK-1 macroscopic currents expressed in oocytes. A slow, progressive increase in TREK-1 currents in response to membrane hyperpolarization has been reported, which declined when the membrane was depolarized (Segal-Hayoun et al. 2010). As a result, it was proposed that the resting membrane potential (or holding voltage) modulates the activity of Gq-coupled receptors in the absence of agonist, such that persistent membrane depolarization activates the Gq cascade, depleting PIP2 levels and thereby inhibiting TREK-1 activity.

Regulation of TREK-1 activity by extracellular sodium and potassium concentrations has been reported in heterologous systems (oocytes). A reduction in the $[Na]_o$ clearly inhibited macroscopic outward currents, whereas the outward current slope

conductance was augmented when the $[K]_o$ increased from 2–98 mM, indicating a potentiating effect of both extracellular Na^+ and K^+ (Fink et al. 1996; Meadows et al. 2000; Ma et al. 2011). TREK-2 outward currents are also larger than expected from the driving force at high external K^+ concentrations, and they are reduced when the Na^+ concentration drops (Lesage et al. 2000b), suggesting a stimulating effect of extracellular K^+ and Na^+ similar to that described for TREK-1 channels. The modulation of TREK channels by external K^+ may be important in pathologies involving large variations in extracellular potassium concentrations, such as epilepsy and brain or heart ischemia.

In summary, the strong outward rectification observed in TREK currents at physiological K^+ concentrations appears to result from the interplay of “open rectification” (for all three channel types), weak voltage-dependence (mainly TREK-1 and probably TREK-2) and blockage (exclusively TREK-1) by divalent cations (Enyedi and Czirják 2010). While TREK channels deviate from the classical definition of leak channels, they can still be considered resting channels, as they remain open across the physiological voltage range and do not inactivate, despite of their voltage-dependency. In addition, TREK channels lack the classical positively charged S4 transmembrane domain and their apparent “activation threshold” follows the potassium equilibrium potential rather than depending on the membrane voltage (Fink et al. 1996).

2.2.3 *Resting Membrane Potential and Excitability*

The heterologous expression of TREK channels in oocytes, COS or HEK cells clearly modifies the resting membrane potential by shifting its value (20–50 mV) towards the equilibrium potential for potassium, as described for TREK-1 (Fink et al. 1996; Gruss et al. 2004a; Thomas et al. 2008), TREK-2 (Kim et al. 2005) and TRAAK (Fink et al. 1998; Meadows et al. 2001; Ozaita and Vega-Saenz de Miera 2002). The influence of TREK channels on the resting membrane potential of real neurons is more difficult to determine, nevertheless, several authors have reported rather soft effects following pharmacological modulation or genetic deletion/overexpression of TREK channels (Ferroni et al. 2003; Heurteaux et al. 2004; Alloui et al. 2006; Yang and Jan 2008; Deng et al. 2009).

Modulation of TREK-2-like native currents mildly influences the resting membrane potential and excitability of mouse sympathetic neurons (Cadaveira-Mosquera et al. 2011). A depolarization of 10 mV is observed only when TREK channels are blocked strongly by fluoxetine (100 μ M), while relatively high concentrations of riluzole (300 μ M) induce a comparable hyperpolarization (Cadaveira-Mosquera et al. 2011). Fluoxetine also decreases the latency to the first action potential evoked by current-injections, but it fails to attenuate the adaptation of sympathetic neurons as expected (Cadaveira-Mosquera et al. 2011). This may seem a disappointing result for the channel touted as the long-awaited “leak channel”. However, we should bear in mind that the sympathetic neurons of the superior cervical ganglion (SCG) are

endowed with a number of other voltage-dependent (potassium M-type, cationic H-type, sodium persistent) and voltage-independent (unidentified chloride and sodium) channels that will influence the resting membrane potential (Lamas 1998; Lamas et al. 2002; Romero et al. 2004; Lamas 2005; Lamas et al. 2009). Whether TREK channels are additional contributors or the main regulators of the resting membrane potential remains to be determined.

2.3 Pharmacology

The pharmacology of K2P channels is extremely complex (Lesage 2003). Traditionally, researchers have sought to identify the most selective channel blocker in order to study a given ion current. However, K2P channels in general, and the TREK subfamily in particular, have demonstrated the importance of channel enhancers as useful tools to study the channels themselves but also as putative useful tools in clinical pharmacology.

2.3.1 *Channel Blockers*

A distinctive characteristic of K2P channels is that they are not sensitive to most classic potassium channel blockers. It is generally agreed that all members of the TREK subfamily, TREK-1 (Fink et al. 1996; Patel et al. 1998; Meadows et al. 2000; Koh et al. 2001; Han et al. 2003; Moha ou Maati et al. 2011), TREK-2 (Bang et al. 2000; Lesage et al. 2000b; Han et al. 2002, 2003; Kang et al. 2007; Deng et al. 2009; Kucheryavykh et al. 2009; Xiao et al. 2009; Cadaveira-Mosquera et al. 2011) and TRAAK (Fink et al. 1998; Lesage et al. 2000a; Ozaita and Vega-Saenz de Miera 2002; Han et al. 2003) are insensitive to millimolar concentrations of TEA, 4-AP and Cs⁺. TREK channels are also insensitive to blockers of the calcium-dependent potassium channels such as apamin, charybdotoxin, iberiotoxin and paxilline (Kang et al. 2007; Mazella et al. 2010; Cadaveira-Mosquera et al. 2011; Moha ou Maati et al. 2011), ATP-sensitive potassium channels like glibenclamide and tolbutamide (Kucheryavykh et al. 2009; Moha ou Maati et al. 2011), inward rectifier potassium channels like tertiapin (Deng et al. 2009), voltage-dependent sodium channel such as TTX and valproate (Cadaveira-Mosquera et al. 2011) and calcium channels such as Cd (Cadaveira-Mosquera et al. 2011). However, other calcium channel blockers such as mibefradil, penfluridol and pimozide do effectively inhibit heterologously expressed and native TREK-1 channels (Enyeart et al. 2002; Chemin et al. 2005a).

The effect of Ba²⁺ on TREK channels has originated some discrepancies (see Patel and Honore 2001). TREK-1 was found to be insensitive to or very slightly inhibited by low concentrations (≤ 1 mM) of barium (Patel et al. 1998; Meadows et al. 2000; Zhou et al. 2009), although stronger inhibition had been described with equivalent and higher concentrations (Fink et al. 1996; Ma et al. 2011). Inhibition

of TREK-2 with ≥ 2 mM (Bang et al. 2000; Han et al. 2002; Ferroni et al. 2003; Kang et al. 2007; Xiao et al. 2009; Cadaveira-Mosquera et al. 2011) but not with ≤ 1 mM (Lesage et al. 2000b; Kim et al. 2005) has also been reported. Similarly Ba^{2+} was also found to block mouse (Fink et al. 1998) but not human (Lesage et al. 2000a; Ozaita and Vega-Saenz de Miera 2002) or rat (Han et al. 2003) TRAAK homologues. In summary, TREK channels can be inhibited by moderate millimolar concentrations of barium. However, complete dose-response relationships must be established to determine whether distinct TREK subunits or their orthologues exhibit different sensitivities. A putative competition between Ba^{2+} and extracellular K^+ , recently reported, should also be taken into account (see Ma et al. 2011).

There is also conflicting data regarding the effects of quinine and quinidine. While these alkaloids have been reported to inhibit TREK-1 (Patel et al. 1998; Meadows et al. 2000; Zhou et al. 2009; Seifert et al. 2009) and TREK-2 (Lesage et al. 2000b; Ferroni et al. 2003; Kucheryavych et al. 2009), other studies found no effects on TREK-1 (Fink et al. 1996; Koh et al. 2001), TREK-2 (Bang et al. 2000; Han et al. 2002; Cadaveira-Mosquera et al. 2011) and TRAAK channels (Lesage et al. 2000a; Ozaita and Vega-Saenz de Miera 2002).

The inorganic dye ruthenium red (RR) can interact with a large number of ion channels, including K2P. RR strongly blocks macroscopic currents via heterologously expressed TRAAK (Czirj  k and Enyedi 2002, 2006) but not TREK-1 channels (Czirj  k and Enyedi 2002). Besides, we recently reported that RR fails to block TREK-2-like native sympathetic currents (Cadaveira-Mosquera et al. 2011), suggesting RR as a useful tool with which to identify native TRAAK channels.

The antidepressant fluoxetine strongly inhibits TREK currents. Interestingly TREK-1-KO, but not TRAAK-KO, mice are much less sensitive to depression and stress than normal cohorts, and they exhibit a behavior similar to that of control mice treated with classical antidepressants (Heurteaux et al. 2006). As TREK-1-KO mice exhibit abnormally high dorsal raphe activity, the antidepressant phenotype has been attributed to the loss of this channel in serotonin-producing neurons (Bayliss and Barrett 2008). Fluoxetine and norfluoxetine strongly inhibit TREK-1 channels (Kennard et al. 2005; Heurteaux et al. 2006; Sandoz et al. 2011; Moha ou Maati et al. 2011; Eckert et al. 2011), while a range of serotonin reuptake inhibitors and antidepressants (including fluoxetine, paroxetine, sertraline, fluvoxamine, maprotiline, citalopram, mirtazapine and doxepin) block human TREK-1 currents at concentrations that do not affect TRAAK (Heurteaux et al. 2006; Eckert et al. 2011). TREK-2 channels are also inhibited by fluoxetine (Kang et al. 2008; Cadaveira-Mosquera et al. 2011), although clinically related drugs such as lamotrigine have no such effect (Kang et al. 2008). In summary, fluoxetine can be used to distinguish between TREK-1/2 and TRAAK channels. Furthermore, inhibiting TREK-1 may be a putative strategy to develop new antidepressants and other mood regulators (Gordon and Hen 2006) as recently demonstrated with the peptide spadin (Mazella et al. 2010; Moha ou Maati et al. 2011). In fact, it has been recently observed an association between human genetic variants in the TREK-1 locus and patient resistance to multiple antidepressant classes (Perlis et al. 2008).

Heterologously expressed human TREK-1 and TREK-2, but not TRAAK, macroscopic and inside-out currents have been reported to be inhibited by antipsychotic substances like fluphenazine, chlorpromazine, haloperidol, flupenthixol, loxapine, clozapine and pimozide but not by sulpiride and tiapride (Patel et al. 1998; Thümmel et al. 2007), suggesting that TREK-1 may also be related with other psychiatric disorders like schizophrenia.

2.3.2 *Channel Enhancers*

Volatile general anesthetics such as chloroform, cyclopropane, diethyl ether, halothane, isoflurane, nitrous oxide, sevoflurane and xenon reversibly activate currents (macroscopic or single-channel) via mouse and human TREK-1 channels, and they hyperpolarize COS cells expressing TREK-1 (Patel et al. 1998, 1999; Heurteaux et al. 2004; Franks and Honore 2004; Gruss et al. 2004a). Natively-expressed TREK-1-like channels in astrocytes and striatal neurons are also activated by halothane (Heurteaux et al. 2004; Seifert et al. 2009). The effect of volatile anesthetics has been attributed to their direct action, independent of second messengers (Patel et al. 1999). The strong resistance of TREK-1-KO mice to gaseous anesthetics such as chloroform, halothane, sevoflurane, desflurane and isoflurane may have important clinical relevance (Heurteaux et al. 2004) and indeed, TREK-1-KO and TRAAK-KO mice display thermal and mechanical hyperalgesia (Alloui et al. 2006; Noël et al. 2009). In contrast to general anesthetics, the barbiturate pentobarbital has no effect on macroscopic TREK-1 currents and it displays comparable anesthetic effects in KO and wt mice (Heurteaux et al. 2004). Macroscopic TREK-2 currents are also strongly activated by gaseous anesthetics such as chloroform, halothane and isoflurane (Lesage et al. 2000b; Gu et al. 2002) but not by bupivacaine or lidocaine (Bang et al. 2000; Han et al. 2002). On the contrary, TRAAK macroscopic currents from mouse and human are insensitive to volatile general anesthetics such as chloroform, diethyl ether, halothane and isoflurane (Patel et al. 1999; Lesage et al. 2000a). Taken together, these findings suggest that TREK channels may be potential targets for the development of new analgesics and anesthetics.

While macroscopic currents through heterologously expressed TREK-1 (Czirják and Enyedi 2006) and TREK-2 (Kim et al. 2005; Czirják and Enyedi 2006) channels are markedly enhanced by low micromolar concentrations (about 10 μM) of zinc (Zn^{2+}), human TREK-1 is inhibited by high (IC_{50} ca. 650 μM) Zn^{2+} concentrations (Gruss et al. 2004b). Species differences and in expression systems could underlie this discrepancy, although the concentrations used may also have contributed to the divergent outcomes. Macroscopic currents generated by the expression of TRAAK channels (Czirják and Enyedi 2006) are only slightly inhibited at much higher concentrations of Zn^{2+} . Moreover, activation by Zn^{2+} of native TREK-2-like macroscopic currents in sympathetic neurons has been recently demonstrated (Cadaveira-Mosquera et al. 2011). Zinc is an important component of a large number of proteins and it is also synaptically released from glutamatergic neurons (Frederickson

and Bush 2001). Thus, TREK channels have been proposed as possible targets for synaptically released zinc (Kim et al. 2005; Czirják and Enyedi 2006). A similar enhancement of TREK-1 and TREK-2 currents is induced by the mercuric ion (Hg^{2+}), having no effect on TRAAK currents (Czirják and Enyedi 2006). TREK-1 (Gruss et al. 2004b) and TREK-2 (Kim et al. 2005) macroscopic currents are also activated by low micromolar concentrations of copper (Cu^{2+}) applied to the extracellular side of the membrane. These divalent cations can be useful to distinguish native TRAAK from TREK-1/2 channels.

Initial studies suggested that macroscopic currents from TREK-1 channels were insensitive to intracellular acidification by CO_2 bubbling (Fink et al. 1996). However, intracellular acidification using extracellular HCO_3^- or dinitrophenol (DNP) strongly increases heterologously expressed TREK-1 macroscopic outward currents (Maingret et al. 1999b; Duprat et al. 2000). Single-channel TREK-1 (Maingret et al. 1999b; Honore et al. 2002; Chemin et al. 2005b; Sandoz et al. 2006; Moha ou Maati et al. 2011) and TREK-2 (Bang et al. 2000; Lesage et al. 2000b; Kim et al. 2001b; Kang et al. 2007) currents are also enhanced by low pH levels in cell-attached (extracellular application of CO_2 , HCO_3^- or removal of NH_4Cl) and inside-out patches. Macroscopic and single-channel currents in natively expressed TREK-1-like channels from myocytes, supraoptic, DRG and striatal neurons (Enyeart et al. 2002; Tan et al. 2002; Han et al. 2003; Heurteaux et al. 2004; Kang et al. 2005; Chemin et al. 2005b; Alloui et al. 2006) and TREK-2-like channels from cerebellar, entorhinal, supraoptic and sympathetic neurons (Han et al. 2002, 2003; Kang et al. 2005, 2007; Deng et al. 2009; Cadaveira-Mosquera et al. 2011) are also strongly enhanced by intracellular acidification. Indeed, circumvallate and foliate taste buds express TREK-1 and TREK-2 channels, suggesting a role on the sour taste transduction (Richter et al. 2004).

Interestingly, extracellular acidification was recently proposed to strongly inhibit single-channels and macroscopic currents when human TREK-1 channels are expressed in oocytes, while the murine counterpart is much less sensitive (Cohen et al. 2008, 2009; Ma et al. 2011). This inhibition appears to involve two distinct temporal components, with an initial rapid inhibition (within seconds) due to direct protonation of the channel and a subsequent slower reduction (within minutes), probably involving proton-sensing G-protein receptors and a second messenger of the Gq-PLC pathway (Cohen et al. 2009). Note that both TREK-1 and TREK-2 channels have also been reported to be substantially insensitive to the acidification of the extracellular medium (Lesage et al. 2000b; Zhou et al. 2009).

Analysis of TRAAK currents has revealed an increase in macroscopic currents in response to alkalinization, however no effect is observed in response to either external or internal acidification (Fink et al. 1998; Maingret et al. 1999b). Recording of TRAAK channels in inside-out patches has further demonstrated the insensitivity of these channels to intracellular acidosis (Maingret et al. 1999b; Kim et al. 2001a), and their activation by intracellular alkalinization (Kim et al. 2001a; Han et al. 2003).

Coexpression of the A-kinase-anchoring protein (AKAP150) with TREK-1 and TREK-2, but not with TRAAK channels, strongly enhanced whole-cell macroscopic currents in COS cells (Sandoz et al. 2006). Whether this scaffolding protein interacts ubiquitously with TREK channels in native cells is still a matter of debate.

2.3.3 *Neuroprotectors*

In several heterologous systems (OO, COS, HEK, CHO), both single-channel and macroscopic currents from human and rodent TREK-1 are strongly and reversibly potentiated by arachidonic acid (AA) and other polyunsaturated free fatty acids (PUFAs), such as docosahexaenoate, oleate and linolenate (Patel et al. 1998; Maingret et al. 1999b, 2000b; Meadows et al. 2000; Honore et al. 2002; Sandoz et al. 2006; Mazella et al. 2010; Moha ou Maati et al. 2011). Natively expressed channels with properties resembling those of TREK-1 are also activated by AA in astrocytes (Seifert et al. 2009), myocytes (Kang et al. 2005; Li et al. 2006) and cerebellar (Lauritzen et al. 2000), supraoptic (Han et al. 2003), striatal (Heurteaux et al. 2004; Chemin et al. 2005a), hippocampal (Mazella et al. 2010) and DRG neurons (Alloui et al. 2006). By contrast, saturated fatty acids (SFA) such as arachidate, myristate, palmitate or stearate have no effect on macroscopic (Maingret et al. 2000b) or single-channel (Patel et al. 1998; see also Maingret et al. 2000a) TREK-1 currents. Notably, TREK-1-KO, but not TRAAK-KO, mice are extremely sensitive to kainic acid-induced epileptic seizures and to experimentally-induced global and spinal cord ischemia mortality (Heurteaux et al. 2004). Interestingly, expressed TREK-1 macroscopic currents are strongly activated by FCCP, an inductor of chemical ischemia (Honore et al. 2002) and moreover, the presence of TREK-1 channels in HEK cells protects them from OGD-induced ischemia (Moha ou Maati et al. 2011). Accordingly, enhancing TREK-1 currents by administration of PUFAs (linolenic) and lysophospholipids (lysophosphatidyl-choline), but not with saturated fatty acids (palmitic), elicits a strongly anti-ischemic effect in wild-type but not in TREK-1-KO mice (Lauritzen et al. 2000; Blondeau et al. 2002; Heurteaux et al. 2004). Based on these findings it is tempting to speculate that activation of TREK-1 channels may decrease neuronal excitability and enhance blood flow during cerebral ischemia, thereby diminishing brain damage (Blondeau et al. 2007; Bayliss and Barrett 2008).

Macroscopic (Lesage et al. 2000b; Gu et al. 2002) and single-channel (Bang et al. 2000; Kim et al. 2001b; Han et al. 2002; Kang et al. 2007) currents from heterologously expressed TREK-2 channels are also activated by low micromolar concentrations of unsaturated fatty acids (arachidonic, docosahexanoic, eicosapentaenoic, linolenic, linoleic, and oleic), yet not by saturated (elaidic, palmitic and stearic) fatty acids (Bang et al. 2000; Lesage et al. 2000b). Similarly, natively expressed TREK-2-like single-channels (in cerebellar granule and DRG neurons) and macroscopic currents (in sympathetic neurons and astrocytes) are activated by arachidonic and linolenic acids but not by the saturated AA-analog arachidic acid (Ferroni et al. 2003; Kang et al. 2005; Kucheryavykh et al. 2009; Cadaveira-Mosquera et al. 2011).

Significantly, the enhancement of mouse TRAAK currents by arachidonic acid (AA) and other unsaturated fatty acids led to the specific nomenclature of this channel. Micromolar concentrations of AA and other unsaturated fatty acids (docosahexaenoate, eicosapentaenoate, linoleate, oleate and linolenate) induce a strong, reversible and dose-dependent increase in the amplitude of the macroscopic and/or single-channel currents from heterologously expressed TRAAK channels (Fink et al.

1998; Maingret et al. 1999a, 1999b, 2000b; Lesage et al. 2000a; Meadows et al. 2001; Kim et al. 2001a; Han et al. 2003), while saturated fatty acids (myristate, palmitate, stearate and arachidate) again produce no such effects (Fink et al. 1998; Lesage et al. 2000a; Maingret et al. 2000b). TRAAK activation by AA develops slowly, resulting in an outwardly rectifying macroscopic current. Similar results have been reported for the mouse, rat and human orthologs. Members of the TREK subfamily are strongly activated by AA and other PUFAs in inside-out and outside-out patch configurations but not in cell-attached patches, suggesting a direct effect on the channel or on the lipid environment (for a review see Patel et al. 2001).

Lysophospholipids, such as lysophosphatidylcholine (LPC) and lysophosphatidylinositol (LPI), strongly enhance the heterologously expressed macroscopic TREK-1 (Maingret et al. 2000b), TREK-2 (Lesage et al. 2000b) and TRAAK (Maingret et al. 2000b) currents at low concentrations. Similar potentiation is induced by platelet-activating factor, but not by phosphatidylcholine, lysophosphatidic acid (LPA) or choline (Maingret et al. 2000b). The action of LPA seems to be complex, it has been reported to strongly inhibit macroscopic currents and to antagonize LPC-activated currents through TREK-1 channels expressed in oocytes (Cohen et al. 2009), but also to enhance expressed TREK-1, TREK-2 and TRAAK and native striatal TREK-1-like channels in inside-out patches but not in whole-cell, outside-out or cell-attached patches (Chemin et al. 2005a). Lysophospholipids, at least LPC, may influence TREK-1 channels via a second messenger, as LPC strongly activates the channel in cell-attached patches but not in excised-patches (Maingret et al. 2000b). A similar hypothesis (involving PLC) has been proposed for the inhibitory effect of LPA (Cohen et al. 2009).

The neuroprotective, anti-convulsive and anti-ischemic drug riluzole, currently used to treat amyotrophic lateral sclerosis (ALS), potentiates macroscopic currents through heterologously expressed mouse, rat and human TREK-1 (Duprat et al. 2000; Meadows et al. 2000; Moha ou Maati et al. 2011) and TREK-2 channels (Lesage et al. 2000b). This activating effect is transient, and is followed by current decline and ultimately, strong inhibition. Similar transient current activation has been described in natively expressed adrenocortical (Enyeart et al. 2002), sympathetic (Cadaveira-Mosquera et al. 2011) and nodose ganglion cells (Fernández-Fernández et al. 2011). Inclusion of riluzole in the pipette also increases the open probability of TREK-2 single-channels in cell-attached patches from SCG neurons (Cadaveira-Mosquera et al. 2011). Secondary TREK-1 inhibition has been attributed to the inhibition of PDA, which increases cAMP levels and provokes channel phosphorylation by PKA (Duprat et al. 2000). Interestingly, riluzole also induces a sustained increase in the open probability of single heterologous TREK-1 channels when recorded in excised outside-out channels, suggesting that the inhibition is through second messengers (Duprat et al. 2000). Macroscopic and single-channel currents from TRAAK channels are also strongly enhanced by riluzole (Fink et al. 1998; Duprat et al. 2000), but distinctively, the macroscopic current activation is sustained as long as the presence of riluzole is maintained (Duprat et al. 2000).

Notably, another neuroprotective agent, sipatrigine, but not lamotrigine, strongly inhibits hTREK-1 and hTRAAK currents in HEK cells (Meadows et al. 2001).

While the link between potassium channel inhibition and neuroprotection remains unclear, TREK-1 overexpression has recently been proposed as a marker for prostate cancer, and sipatrigine was shown to inhibit the proliferative effect of TREK-1 overexpression (Voloshyna et al. 2008).

In summary, several known nervous system protective factors strongly increase the activity of TREK channels, including the heterogeneous “neuroprotective agents”, unsaturated fatty acids and lysophospholipids. TREK channels are also robustly activated by disturbances associated with tissue damage, such as cell swelling, intracellular acidification and increases in the concentration of free fatty acids. The hyperpolarization induced by the activation of TREK channels is thought to reduce calcium influx through voltage-gated calcium channels and NMDA receptors, thereby acting as a neuroprotective signal. The enhancement of TREK currents by a large number of neuroprotective agents singles out this K2P subfamily as a promising target for the development of new neuroprotective strategies and drugs.

2.4 Mechanosensitivity

It is generally accepted that TREK channels are selectively activated by the convex curvature of the membrane. Accordingly, a reversible increase in the open probability of these channels has been reported in response to negative pressure applied to the external surface (or positive pressure to the internal surface), osmotic changes that induce cell swelling, shear stress and the application of crenating compounds. Although cytoskeletal integrity is not necessary to maintain mechanosensitivity, it appears to repress mechanical activation of TREK channels, probably by opposing to membrane stretch (Lesage and Lazdunski 2000).

The open probability of heterologously expressed members of the TREK subfamily, TREK-1 (Patel et al. 1998; Maingret et al. 1999b, 2000a, 2002; Koh et al. 2001; Honore et al. 2002, 2006; Moha ou Maati et al. 2011), TREK-2 (Bang et al. 2000; Lesage et al. 2000b; Kim et al. 2001b; Kang et al. 2007) and TRAAK (Maingret et al. 1999a, 1999b; Lesage et al. 2000a; Kim et al. 2001a; Han et al. 2003) is gradually increased when negative pressure is applied to the recording pipette in cell-attached and inside-out patches (Fig. 2.2a). Consistently, TRAAK activity is only enhanced by positive pressure in outside-out patches (Maingret et al. 1999a; Honore et al. 2006). Although more commonly studied in heterologous systems, natively expressed TREK-1-like (Tan et al. 2002; Han et al. 2003; Heurteaux et al. 2004; Kang et al. 2005; Chemin et al. 2005a; Alloui et al. 2006), TREK-2-like (Han et al. 2003; Kang et al. 2005, 2007) and TRAAK-like (Han et al. 2003) channels also exhibit this behavior in myocytes, DRG, supraoptic, cerebellar and striatal neurons. In general the open probability of TREK channels is very low at atmospheric pressure (Fig. 2.2a) and it increases with negative pressure in a dose-dependent manner, with half-maximal activation between -20 and -60 mmHg (Patel et al. 1998; Maingret et al. 1999a, 1999b; Bang et al. 2000; Kim et al. 2001a; see also Han et al. 2003).

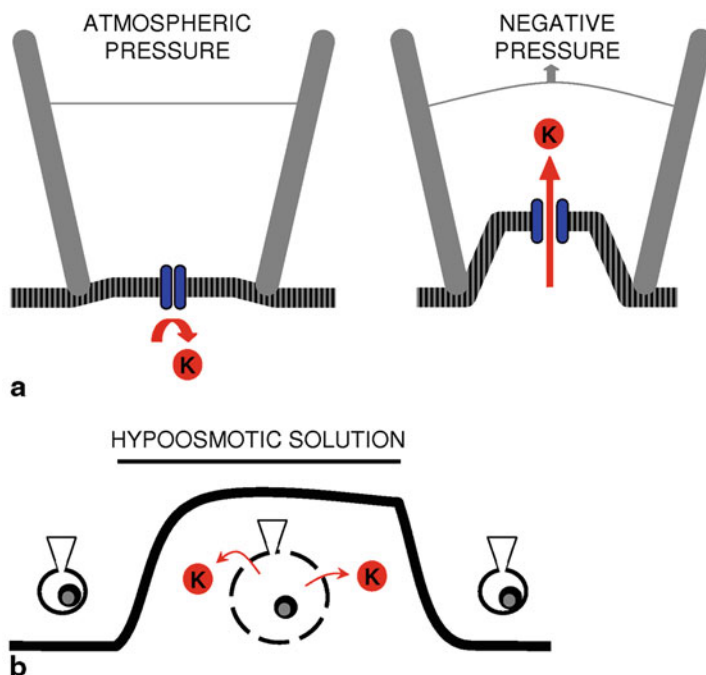


Fig. 2.2 TREK channels are mechanosensitive. In cell-attached or outside-out patches, the open probability of TREK channel is very low (**a**, *left*), while it increases greatly when negative pressure (suction) is applied to the recording pipette (**a**, *right*). Similarly, cell swelling induced by an extracellular solution of low osmolarity strongly increases the macroscopic whole-cell current through TREK channels (**b**). The data are not real but rather, they are based on our own experiments in sympathetic neurons where a change in osmolarity from 290–245 mOsm invoked an outward current of about 220 pA. (see Cadaveira-Mosquera et al. 2011)

Macroscopic currents through heterologously expressed TREK-1 channels are reversibly modulated by membrane stretching (Fig. 2.2b). When extracellular osmolarity is increased (cell shrinking) the amplitude is reduced, while decreases in osmolarity (cell swelling) increase current amplitude (Patel et al. 1998; see also Maingret et al. 2000a). TREK-1 macroscopic currents can also be activated by laminar shear stress induced by increasing the speed of bath perfusion (Patel et al. 1998) or by cell elongation using a micromanipulator (Koh et al. 2001). Similar effects have been described for native TREK-1-like (Li et al. 2006) and TREK-2-like (Ferroni et al. 2003; Cadaveira-Mosquera et al. 2011) macroscopic currents.

The degree of activation by negative pressure appears to be greater at positive potentials (Patel et al. 1998; Maingret et al. 1999a; Lesage et al. 2000b; Kim et al. 2001a). Moreover, a synergistic effect of mechanical activation combined with activation by variations in AA and pH has been described for TREK-1, TREK-2 and TRAAK channels (Maingret et al. 1999b; Kim et al. 2001a, 2001b; Honore et al. 2002). Interestingly, TREK-1 and TRAAK, but not TREK-2 currents

evoked by negative pressure undergo strong desensitization within a 100 ms time-frame, an effect prevented by acidic pH values or in the presence of AA (Honore et al. 2006).

Inhibition of macroscopic and single-channel TREK-1 (Maingret et al. 2000b), TREK-2-like (Ferroni et al. 2003) and TRAAK (Maingret et al. 1999a) currents evoked by mechanical stimuli and other activators has been described using stretch-sensitive channel blockers such as amiloride and gadolinium (Gd^{3+}). Surprisingly, insensitivity of both TREK-1 (Fink et al. 1996) and TREK-2 (Bang et al. 2000) channels to 100 μM Gd^{3+} has also been reported. While the reason for these conflicting results remains unclear, Gd^{3+} may more effectively block evoked rather than background TREK currents.

Crenators are anionic and neutral amphipathic molecules that insert preferentially into the external membrane leaflet, altering membrane curvature (Hao et al. 2009). Several such compounds, including trinitrophenol (TNP) and lysolecithin, can activate TREK-1 (Patel et al. 1998) and TRAAK (Maingret et al. 1999a) macroscopic currents. By contrast, TREK-1 and TREK-2 currents are inhibited by “cup-formers” such as chlorpromazine (CPZ) and tetracaine, cationic amphipathic molecules that insert preferentially into the internal membrane leaflet (Patel et al. 1998; Maingret et al. 2000b; Chemin et al. 2005a; Honore et al. 2006). These results suggest that TREK channels are activated by the expansion of the membrane, consistent with their strong activation by negative pressure applied through the recording pipette or upon induction of cell swelling.

An interesting question is whether mechanical stimulation acts via the cell cytoskeleton. Mechanical activation of TREK-1 and TRAAK channels persists when cytoskeleton-disrupting agents are applied to inside-out patches (e.g., colchicine, latrunculin A or cytochalasin D), and they even activate the channels in cell-attached patches (Patel et al. 1998; Maingret et al. 1999a; Lauritzen et al. 2005; Honore et al. 2006). Together with the enhanced activation by negative pressure in excised versus cell-attached patches (Maingret et al. 1999a; Bang et al. 2000; Lauritzen et al. 2005; Honore et al. 2006; Moha ou Maati et al. 2011), these findings suggest that the cytoskeleton exerts a continuous negative regulatory effect on TREK channels, even though mechanical stimulation seems to be directly transmitted by the deformation of the lipid bilayer.

Similarly, it remains unknown whether TREK channel mechanosensitivity plays a major role in neuronal function in the CNS, where they are widely expressed (Fink et al. 1998; Reyes et al. 2000; Meadows et al. 2000; Lesage et al. 2000b; Talley et al. 2001; Hervieu et al. 2001; Medhurst et al. 2001; Gu et al. 2002). However, this sensitivity is thought to play a general role in controlling cell shape and volume, as well as cone motility and elongation (Maingret et al. 1999a; Reyes et al. 2000; Lauritzen et al. 2005).

TREK channels expressed in somatosensory DRG neurons of the peripheral nervous system (PNS) (Talley et al. 2001; Medhurst et al. 2001; Kang and Kim 2006), and mechanosensitive neurons innervating the bladder and colon (La et al. 2011) are involved in the detection and transduction of skin and organ deformation, and even in mechanically-evoked painful stimuli (Maingret et al. 2000a; Kang and Kim 2006;

Yamamoto et al. 2009). In fact, TREK-1-KO, TRAAK-KO and TREK-1/TRAAK-KO mice all exhibit significant mechanical hypersensitivity to stimulation with von Frey hairs (Alloui et al. 2006; Noël et al. 2009).

To date, the expression of these channels in the autonomic nervous system has received little attention. However, we recently described strong expression of TREK-1 channels in the visceral afferents of the mouse nodose ganglion (Fernández-Fernández et al. 2011; Cadaveira-Mosquera et al. 2012) and TREK-2 in motor neurons of the mouse superior cervical ganglia (Cadaveira-Mosquera et al. 2011, 2012), although all three members were expressed in both ganglia. Expression of TREK-1 and TRAAK has also been reported in the rat nodose ganglion (Zhao et al. 2010) and a role for these channels has been proposed in gastrointestinal acid sensation (Holzer 2011). TRAAK channels were also recently identified in the terminals of mechanosensitive vagal afferents innervating the lungs (Lembrechts et al. 2011). Given the capacity of several internal organs to change size and form, these channels may play a key role in transmitting mechanical information from them to the central nervous system, but also in regulating some vagal reflexes.

Some TREK channels (mainly TREK-1 but not TRAAK) are also strongly expressed in the smooth muscle fibers of the visceral organs that are subjected to deformation, such as the lung, uterus, stomach, intestine, colon and bladder (Reyes et al. 1998; Koh et al. 2001). In fact, the stretch-activated potassium channels found in colonic myocytes may be TREK-1 channels (Koh et al. 2001). TREK-1 has also been described in the smooth muscle cells of pulmonary blood vessels (Lembrechts et al. 2011).

TREK-1 (Fink et al. 1996; Li et al. 2006) but not TREK-2 or TRAAK (Li et al. 2006) channels are well expressed in the heart (for a review see Gurney and Manoury 2009) and TREK-1 activation during heart contraction may contribute to cardiomyocyte repolarization and hyperpolarization, preventing the occurrence of ventricular extrasystoles (Patel et al. 1998; Tan et al. 2002; Li et al. 2006). Intracellular acidosis and cell swelling occurs during heart ischemia, which will activate TREK-1 channels and induce protective hyperpolarization. Finally, TREK-1 has been proposed to participate in the regulation of the heart by β -adrenergic stimulation (Terrenoire et al. 2001).

2.5 Thermosensitivity

TREK-1 channels are thought to act as cold-sensors at the level of peripheral sensory and central hypothalamic neurons, arguing that low temperature would close them and hence depolarize the neurons increasing their excitability (Maingret et al. 2000a; Viana et al. 2002). Nonetheless, all three members of the TREK subfamily exhibit a similar degree of sensitivity to temperature changes, suggesting that TREK channels may be involved in temperature regulation and thermnociception (Kang et al. 2005; Pongs 2009).

While heterologously expressed TREK-1 (Maingret et al. 2000a; Kang et al. 2005), TREK-2 (Kang et al. 2005) and TRAAK (Kang et al. 2005) single-channels show weak activity at room temperature when recorded in cell-attached patches, their open probability (NPo) strongly and progressively increases as bath temperatures are raised (approximately 10-fold/10 °C). Interestingly, activation of all three channels by increasing temperature is dependent on cell integrity and it disappears in excised patches, suggesting the participation of an intracellular second messenger in temperature transduction (Maingret et al. 2000a; Kang et al. 2005; Honore 2007). Similarly, all TREK channels are activated by negative pressure, AA and intracellular pH modifications when recorded at 37 °C in either cell-attached or inside-out patches (Kang et al. 2005). The increase in temperature strongly potentiates the response of TREK-1 channels to membrane stretching, again revealing a synergistic effect of several stimuli on TREK channel activity (Maingret et al. 2000a). Natively expressed TREK-1, TREK-2 and TRAAK-like channels are also temperature-dependent when recorded in myocytes, astrocytes, cerebellar, entorhinal and DRG neurons (Kang et al. 2005; Deng et al. 2009; Kucheryavykh et al. 2009).

Heterologously expressed TREK-1, TREK-2 and TRAAK channels generate small outwardly rectifying macroscopic potassium currents at room temperature (22–24 °C), which are quickly, strongly and reversibly enhanced by progressive increases in temperature to 37 °C (Maingret et al. 2000a; Kang et al. 2005). In fact, current through TREK-1 channels is essentially absent at 12 °C (Maingret et al. 2000a). Interestingly, the current enhancement coupled with increased temperature is strongly attenuated by increasing extracellular osmolarity and by cAMP administration (CPT-cAMP, 0.5 mM), probably via PKA activation (Maingret et al. 2000a).

In general TREK channels are often reported to have a low open probability at rest and in the absence of any stimulus, and hence only a mild contribution to maintain the resting membrane potential should be expected. However, as the majority of these studies were carried out at room temperature (22–24 °C), the role of TREK channels in cellular behavior was probably underestimated. For greater understanding of this role, further experiments should be carried out at temperatures closer to physiological body temperature (32–37 °C: (see Lotshaw 2007).

TREK channels are strongly expressed in a large number of sensory neurons involved in thermal and thermosensitive sensory transduction, including the dorsal root ganglion (Maingret et al. 2000a; Meadows et al. 2001; Talley et al. 2001; Medhurst et al. 2001; Kang et al. 2005; Alloui et al. 2006), trigeminal (Hervieu et al. 2001; Yamamoto et al. 2009) and autonomic vagal (Zhao et al. 2010; Fernández-Fernández et al. 2011; Cadaveira-Mosquera et al. 2012) sensory neurons. In fact, TREK-1-KO and TREK-1/TRAAK-KO mice exhibit heat hyperalgesia, probably due to the increased sensitivity of small DRG neurons and C-fibers from KO mice to noxious heat (Alloui et al. 2006; Noël et al. 2009). Double but not single KO strains, also exhibit cold-hyperalgesia (Noël et al. 2009). Finally, TREK-1 channels are strongly expressed in hypothalamic regions classically involved in regulating body temperature (Maingret et al. 2000a).

2.6 G-Protein Modulation

The primary structure of TREK proteins reveals several potential phosphorylation sites for protein kinase C (PKC), PKA and protein kinase G (PKG) in the cytoplasmic C-terminus (Fink et al. 1996, 1998; Patel et al. 1998; Bang et al. 2000; Lesage et al. 2000b; Koh et al. 2001; Murbartian et al. 2005), suggesting putative modulation of TREK channels by the activation of G protein-coupled receptors (GPCRs). It was recently proposed that under most experimental conditions, basal G protein activity mediates the constant down regulation of TREK-1 (Cohen et al. 2009), and perhaps TREK-2, channels (Xiao et al. 2009).

Macroscopic currents from heterologously expressed TREK-1 (Fink et al. 1996; Patel et al. 1998; Duprat et al. 2000; Maingret et al. 2000b; Enyeart et al. 2002; Honore et al. 2002; Murbartian et al. 2005; Alloui et al. 2006) and TREK-2 (Bang et al. 2000; Lesage et al. 2000b; Gu et al. 2002), but not TRAAK (Fink et al. 1998) channels, are consistently inhibited by increasing intracellular cAMP levels with IBMX + forskolin, or CPT-cAMP. Stimulation of co-expressed Gs-coupled 5-HT₄ receptors also inhibits macroscopic TREK-1 (Patel et al. 1998) and TREK-2 (Lesage et al. 2000b) currents through the activation of adenylate cyclase (AC).

By contrast, activation of the co-expressed Gi-coupled mGluR2 and GABA_B receptors augment TREK-2 currents (Lesage et al. 2000b; Deng et al. 2009). In fact, the hyperpolarization and associated decrease in neuronal excitability induced by norepinephrine and GABA in neurons from the entorhinal cortex has been attributed to the stimulation of native Gi-coupled receptors, and the consequent activation of TREK-2-like channels by inhibition of the AC-cAMP-PKA pathway (Deng et al. 2009; Xiao et al. 2009). The modulation of TREK-2 channels in the entorhinal cortex seems to be related with spatial learning in rats (Deng et al. 2009).

The role of the cGMP pathway is more complex, as stimulation of PKG using sodium nitroprusside (SNP) or 8-Br-cGMP only increases TREK-1 macroscopic currents when using the perforated (but not the ruptured) patch-clamp technique (Koh et al. 2001). However, both SNP and 8-Br-cGMP increase the open probability of TREK-1 channels in cell-attached patches (Koh et al. 2001).

Expressed TREK-1 and TREK-2 channel currents are also inhibited by activating co-expressed Gq-coupled group I glutamate mGluR1 (Lesage et al. 2000b; Chemin et al. 2003; Sandoz et al. 2011), 5HT_{2c}R (Sandoz et al. 2011), THR1 (Murbartian et al. 2005), Orx1R (Murbartian et al. 2005) and muscarinic M1 (Lopes et al. 2005) and M3 (Kang et al. 2006, 2008) receptors (see Fig. 2.3), in whole-cell and cell-attached patches but not in outside-out patches (Kang et al. 2006). This inhibition requires the activation of phospholipase C (PLC), and while it is insensitive to pertussis and cholera toxins, it is suppressed by the PLC inhibitor U73122 (Chemin et al. 2003; Kang et al. 2006). PLC inhibition also greatly increases human TREK-1 currents when these channels are expressed in oocytes (Segal-Hayoun et al. 2010). However, the next steps in this pathway remain unclear, with little agreement on the second messenger involved.

Macroscopic whole-cell TREK-1 currents are potently inhibited by ACh in oocytes co-expressing M1 receptors (Lopes et al. 2005). PIP₂ applied to inside-out

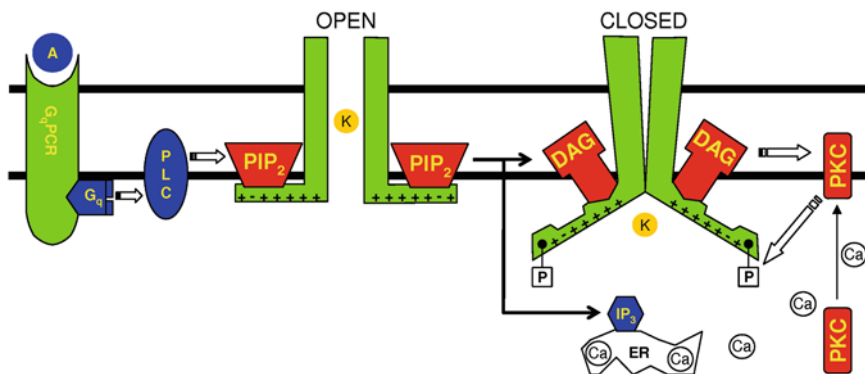


Fig. 2.3 TREK-1 and TREK-2 but not TRAAK are inhibited by the activation of Gq protein-coupled receptors. Agonists (A) of Gq protein-coupled receptors (*Gq-PCR*) activate Gq proteins, which in turn stimulate phospholipase C (*PLC*). *PLC* breaks down phosphatidylinositol-bisphosphate (*PIP*₂) to produce inositol-triphosphate (*IP*₃) and diacylglycerol (*DAG*). *IP*₃ migrates to the endoplasmic reticulum (*ER*) to induce calcium (*Ca*) release, while *DAG* activates protein kinase C (*PKC*), which in turn phosphorylates (-P) TREK channels. The identity of the elements in this cascade that directly mediate TREK channel inhibition remains unclear. Of the three mechanisms proposed (in red), detachment of *PIP*₂, direct interaction with *DAG* and/or phosphorylation by *PKC*, the first appears the most plausible

macropatches strongly enhances the activity of heterologously expressed TREK-1 channels, while poly-lysine (a competitor of *PIP*₂) and wortmannin (a blocker of PI 4-kinases that impedes *PIP*₂ replenishment) inhibit their activity, suggesting that *PIP*₂ depletion by *PLC* underlies the agonist-induced inhibition of TREK-1 (Lopes et al. 2005; Chemin et al. 2005b, 2007). Findings from our laboratory show that native TREK-2-like channels expressed in sympathetic neurons are inhibited by agonists in a *PIP*₂-dependent manner, via activation of Gq proteins (Reboreda et al. 2010; Rivas-Ramírez et al. 2011). However, addition of antibodies against *PIP*₂, blockade of its replenishment with wortmannin, or the use of *PIP*₂ depletion systems were also reported not to affect TREK1/2 macroscopic currents (Chemin et al. 2003; Kang et al. 2006; Sandoz et al. 2011). Confusingly, also *PIP* or even *PI* may interact with TREK channels so that *PIP*₂ depletion may not be sufficient to see a strong current inhibition (Chemin et al. 2005b; Segal-Hayoun et al. 2010; Sandoz et al. 2011).

Administration of *DOG*, a membrane-permeable analog of *DAG*, rapidly inhibits TREK-1/2 macroscopic currents, while impermeable *DAG*-analogs (*SAG* and *SLG*) also inhibit AA-evoked single-channel TREK-1 and TREK-2 currents in inside-out patches. Hence, *DAG* may directly inhibit TREK channels without the participation of *PKC* (Chemin et al. 2003). However, *DAG* or its impermeable *DAG*-analogs also fail to substantially affect TREK-1 and TREK-2 currents in inside-out macropatches (Lopes et al. 2005; Kang et al. 2006).

Phosphorylation by *PKC* has been proposed to mediate the modulation of TREK channels by Gq proteins, based on the inhibition of TREK-1/2 currents by the *PKC*

activators PMA and PDBu (Fink et al. 1996; Maingret et al. 2000b; Gu et al. 2002; Murbartian et al. 2005; Kang et al. 2006). Moreover, the PKC inhibitor bisindolylmaleimide (BIS) reportedly attenuates the inhibition of TREK-1/2 by TRH, ACh and OrxA (Murbartian et al. 2005; Kang et al. 2006). However, elsewhere no such effect on TREK-1/2 currents was observed with the PKC activators PMA and PDBu or PKC inhibition with staurosporine, calphostin-C or a PKC peptide inhibitor (Bang et al. 2000; Lesage et al. 2000b; Chemin et al. 2003), or on their inhibition by glutamate, ACh or serotonin (Chemin et al. 2003; Lopes et al. 2005; Sandoz et al. 2011). Similarly, modulation of intracellular calcium and IP₃ levels has no effect on TREK1/2 currents (Lesage et al. 2000b; Chemin et al. 2003; Kang et al. 2006) or their inhibition by glutamate or ACh (Chemin et al. 2003; Lopes et al. 2005).

There is however a good agreement in that TRAAK channels are not modulated through Gq-protein coupled receptors, given the insensitivity of TRAAK expressed currents to the activation of co-expressed mGluR1 (Chemin et al. 2003) and M1 (Lopes et al. 2005) receptors. Neither changes in the internal calcium concentration (IP₃, EGTA) nor activation of PKC (PMA) affect TRAAK currents (Fink et al. 1998), yet application of PIP₂ to inside-out macropatches does activate TRAAK channels, an effect dependent on prior mechanical stimulation (Lopes et al. 2005).

In summary, the modulation of TREK-1/2 channels by the activation of Gq-coupled receptors is far from being understood. While several elements of the PLC cascade (mainly PIP₂, DAG and PKC; see Fig. 2.3) have been proposed to influence the activity of these channels, there is also evidence to the contrary. Currently, the PIP₂ depletion theory appears to be the most attractive (see also Suh and Hille 2005; Lotshaw 2007; Suh and Hille 2007). Accordingly, it has been proposed that the final mechanism involves the dissociation of the TREK-1 polybasic C-terminal from the plasma membrane (see Fig. 2.3) when membrane PIP₂ is broken down (Chemin 2005; Sandoz 2011; Honore 2007).

2.7 Distribution/Expression

A good number of the important functions ascribed to K2P channels come from the extensive distribution of these channels in the mammalian body. The widespread expression of TREK channels both in and beyond the nervous system has been described in the mouse (Fink et al. 1996, 1998; Reyes et al. 2000), rat (Bang et al. 2000; Talley et al. 2001; Hervieu et al. 2001; Gu et al. 2002) and human (Meadows et al. 2000; Lesage et al. 2000a, 2000b; Meadows et al. 2001; Medhurst et al. 2001). The expression of TREK channels (in the form of mRNA, protein or channel current) has been assessed using a wide variety of techniques including RT-PCR, qRT-PCR, immunocytochemistry, immunohistochemistry, *in situ* hybridisation, Northern blot, Western blot and electrophysiology.

The interpretation of expression data is hampered by several limitations. Firstly, not all techniques are equally appropriate for quantitative analysis and secondly, expression levels are generally represented in qualitative or comparative terms, limiting

comparison between studies. In addition, the levels of TREK channel expression may differ between species (see Table 2.1), although almost identical distributions have been reported for all TREK subfamily members in the mouse and rat CNS (Talley et al. 2001). Therefore, the data summarized below and in Table 2.1 should be regarded as orientative, and the reader is encouraged to read the original articles for specific details. To avoid unnecessary complexity, expression data is grouped into three main categories: strong (S), which includes medium, strong and very strong expression; weak (W), including what authors reported as weak or very weak expression; and absent (A), which refers to absence or failure to detect expression. Where discrepancies exist, more than one of these labels is assigned to a given region in Table 2.1, although it should be noted that some of these discrepancies may have arisen through my own interpretation of other authors' data.

2.7.1 Strong Expression

Since their initial identification, all three TREK channels (TREK-1, TREK-2 and TRAAK) have been seen to be strongly expressed in the brain of the three most commonly studied species (mouse, rat and human: see Table 2.1), specifically in the amygdala, basal ganglia, cortex, dorsal root ganglia and hippocampus. TREK-1 channels have been also reported to be well expressed in the brain, cortex, hippocampus, hypothalamus and DRG of all three species. It is notable that there is almost no expression data for TREK-2 in the mouse, (but see Aller and Wisden 2008). However, TREK-2 channels show a good level of expression in rat and human amygdala, brain, cerebellum, hippocampus, thalamus, DRG, pancreas, spleen and testis. Also in the three species, TRAAK has been shown to be well expressed in amygdala, brain, cortex and hippocampus (Fink et al. 1996, 1998; Patel et al. 1999; Bang et al. 2000; Reyes et al. 2000; Meadows et al. 2000, 2001; Lauritzen et al. 2000; Lesage et al. 2000a, 2000b; Maingret et al. 2000a; Talley et al. 2001; Hervieu et al. 2001; Medhurst et al. 2001; Kim et al. 2001a; Gu et al. 2002; Dobler et al. 2007; Putzke et al. 2007; Aller and Wisden 2008; La et al. 2011).

2.7.2 Weak Expression

Structures in which TREK-1 data have shown a weak expression in at least one of the species and strong expression has not been reported in any of them are: brain stem, globus pallidus, habenula, pons, supraoptic, SCG, trigeminal ganglion, pancreas, pituitary gland, prostate, testis, thymus and uterus. The same type of weak expression was reported for TREK-2 in arcuate, interpeduncular, red nucleus, spinal cord, nodose ganglion, trigeminal ganglion, heart, liver, lung, pituitary gland, placenta, prostate, skeletal muscle, stomach, thymus and ventricle. For TRAAK in arcuate, astrocytes, colliculus, interpeduncular, medulla, solitary nucleus, substantia nigra,

Table 2.1 Tissue distribution of TREK channels. Data were compiled from studies that used any technical approach permitting the reasonable quantification of mRNA, protein or ionic current. The category labeled strong (S) refers to medium, strong and very strong expression levels, weak (W) refers to weak and very weak levels and absent (A) describes absent or undetectable expression

	TREK-1				TREK-2				TRAAK			
	m	r	h		m	r	h		m	r	h	
<i>Central nervous system</i>												
Accumbens ^{12,14,23–25,34}		SA	SW			A	S			W		S
Amygdala ^{9,10,12,14,20,23–25,34,34}	W	S	SW			SW	SA		S	S		SW
Arcuate nucleus ^{12,14,34}		S				W				W		
Basal ganglia ^{9,10,12,14,20,23–25,29,34}	W	S	S			S	S		S			S
Brain ^{2,9,10,12,15,19,20,23,25,27,28,31}	S	S	SW		S	S	S		S	S		S
Brain stem ^{9,10,29}	W								SW			
Caudate ^{1,9,10,12,14,20,24,25,29}	W	SW	S		W	A	S		SW			S
Cerebellum ^{1,2,9,10,12,14,18,20,23–25,29,33,34}	SW	W	W		S	SA	S		SW	W		WA
Cerebellum (nuclei) ^{9,10,12,29}	W	A				A			S			
Cochlear nuclei ^{14,29,34}		SA				A			S	A		
Colliculus ^{1,14}		S							W			
Cortex ^{1,9,10,12,14,20,23,25,29,34}	S	SW	S			W	S		SW	S		S
Cortex (frontal) ^{14,20,24,25}		S	SW				SW					S
Cortex (temporal) ^{1,20,25}			SW				S		W			S
Cortex (occipital) ^{20,25}			SW				S					S
Cortex (parietal) ¹⁴		S										
Cortex (entorhinal) ^{1,6,10,38}		W				S						
Cortex (piriform) ^{9,10,12,14,34}	S	S				S				S		
Dorsal motor vagus ¹²		S				S						
Facial nucleus ^{12,14}		S				S						
Gigantocellular nucleus ¹²		A							SW			
Glia ^{5,10}	A	A							S			
Glia (astrocytes) ^{8,11,32,42}	S	WA				S						
Globus pallidus ^{9,12,14,24,34}	W	WA				A			W	A		S

Table 2.1 (continued)

	TREK-1			TREK-2			TRAAK		
	m	r	h	m	r	h	m	r	h
Habenula ^{9,10,12,34}	W	WA			SW		S	A	
Hippocampus ^{1,9,10,12,14,18,18,20,23–25,29,34}	SW	S	S	W	SWA	S	SW	S	S
Hypoglossus ¹²		S			S				
Hypothalamus ^{9,10,12,14,22–25,29,33,34}	SW	S	SW		WA	S	SW	W	W
Interpeduncular nucleus ^{12,14,34}		S			WA			WA	
Locus coeruleus ^{12,24,34}		A			SA			A	SW
Medulla ^{2,9,10,20,24}	W		S		A	S	W		W
Midbrain ^{2,9,14}	W	S			A				
Oculomotor ¹⁴		S							
Olfactory bulb ^{9,10,12,29,34}	S	SW			S		S	W	
Olive ¹⁴		S							
Olive (inferior) ¹²		S			A				
Pons ^{2,9,10,12,29,34}	W	A			SWA		SW		
Putamen ^{1,9,10,12,14,20,24,25,29}	W	SW	S	W	A	S	SW		S
Raphe ^{12,14,34}		S			SW			A	
Red nucleus ^{14,34}		SA			W			A	
Septum ^{14,34}		SA			A			A	
Solitary nucleus ^{12,34}		S			SWA			WA	
Spinal cord ^{2,10,14,20,21,23–25,29,34}		SW	S		WA	WA	S	W	WA
Striatum ^{24,29,34}		S			A		S	W	S
Subiculum ^{1,10,14,29}	S	S					S		
Subthalamic nucleus ^{14,34}		SA			A			W	
Substantia nigra ^{9,10,14,20,23–25,34}	W	SA	SW		A	S	W	A	W
Supraoptic ^{12,13,34}		W			SW			W	
Thalamus ^{1,2,9,10,12,14,20,23–25,29,34}	W	SW	S	W	SWA	S	SW	W	SW
Trigeminal N. (motor) ¹²		S			S				
Tigeminal N. (spinal) ^{12,14,29,34}		SW			SW		S	W	
Trigeminal N. (principal) ¹⁴		S							
Vestibular nuclei ^{1,14,29,34}		SWA			A		S	A	

Table 2.1 (continued)

	TREK-1			TREK-2			TRAAK		
	m	r	h	m	r	h	m	r	h
<i>Peripheral somatic and autonomic nervous system</i>									
Carotid body ⁴⁰		S	S						
DRG ^{7,17,22,24,25,34}	S	S		S	S	S	SW	S	SW
Fibroblast ^{26,40}	A	A							
Glia ²⁶	A	A							
Nodose ganglion ^{4,41}	S	W		W			W	W	
Retina ^{10,29}							S		
Satellite cells ^{4,5}	A			A			A		
Superior cervical ganglion ^{4,5}	W			S			W		
Trigeminal ganglion ^{33,39}		W			WA			W	
Vestibular ganglion ²⁶	S	S		A			A		
<i>Non neuronal organs and tissues</i>									
Adipose tissue ²⁴									A
Bladder ¹⁰							A		
Bone ²⁴									A
Cartilage ²⁴									A
Colon ^{2,10,15,19,20}			A			S			A
Heart ^{2,9,10,12,15,19,20,23–25,28,35,36}	S	S	A		A	WA	A	A	A
Heart (atrium) ^{2,10,15}					WA		A	A	A
Heart (ventricle) ^{2,10,15,28,35}		S			WA		A	A	
Intestine ^{2,10,15,19,20,23–25}			SW		A		A	A	WA
Kidney ^{2,9,10,12,15,19,20,23–25}	S		SW		A	S	A	A	WA
Liver ^{2,9,10,12,15,19,20,23–25,25}	A		A		A	WA	A	S	A
Lung ^{2,9,10,12,15,19,20,23–25,25}	S		A		A	WA	A	W	A
Ovary ^{19,20}			S			A	A		A
Pancreas ^{2,12,15,19,20,23–25}			WA		S	SW		S	A
Pituitary gland ^{23–25}			W			W			WA
Placenta ^{2,10,12,19,20,23–25}		A	A		A	WA	A		SW

Table 2.1 (continued)

	TREK-1			TREK-2			TRAAK		
	m	r	h	m	r	h	m	r	h
Prostate ^{19,20,23–25,37}			WA			W			WA
Salivary gland ¹⁰									
Skeletal muscle ^{2,9,10,12,15,19,20,23–25}	S		WA		A	WA	A	S	A
Smooth muscle (basilar artery) ³	S	S							
Smooth muscle (bladder) ¹⁶	S			A			A		
Smooth muscle (carotid artery) ³	A	A							
Smooth muscle (intestine) ¹⁶	S			A			A		
Smooth muscle (portal vein) ¹⁶	S			A			A		
Smooth muscle (pulmonary artery) ¹⁶	S			S			A		
Smooth muscle (stomach) ¹⁶	S			S			A		
Smooth muscle (uterus) ¹⁶	S			A			A		
Spleen ^{2,15,19,20,23–25}			A		S	SA		A	A
Stomach ^{2,10,15,23–25}			S		A	W	A	W	A
Taste buds ³⁰	S			S					
Testis ^{2,10,15,19,20,23,25}			W		S	SW	A	S	SA
Thymus ^{10,19,20}			W			W	A		WA
Uterus ^{10,23,25}			W			A	A		A

m = mouse, **r** = rat, **h** = human 1. Aller and Wisden [2008](#); 2. Bang et al. [2000](#); 3. Blondeau et al. [2007](#); 4. Cadaveira-Mosquera et al. [2012](#); 5. Cadaveira-Mosquera et al. [2011](#); 6. Deng et al. [2009](#); 7. Dobler et al. [2007](#); 8. Ferroni et al. [2003](#); 9. Fink et al. [1996](#); 10. Fink et al. [1998](#); 11. Gnatenco et al. [2002](#); 12. Gu et al. [2002](#); 13. Han et al. [2003](#); 14. Hervieu et al. [2001](#); 15. Kim et al. [2001](#); 16. Koh et al. [2001](#); 17. La et al. [2011](#); 18. Lauritzen et al. [2000](#); 19. Lesage et al. [2000](#); 20. Lesage et al. [2000](#); 21. Maingret et al. [1999](#); 22. Maingret et al. [2000](#); 23. Meadows et al. [2000](#); 24. Meadows et al. [2001](#); 25. Medhurst et al. [2001](#); 26. Nicolas et al. [2004](#); 27. Patel et al. [1999](#); 28. Putzke et al. [2007](#); 29. Reyes et al. [2000](#); 30. Richter et al. [2004](#); 31. Richter et al. [2004](#); 32. Seifert et al. [2009](#); 33. Simkin et al. [2008](#); 34. Talley et al. [2001](#); 35. Tan et al. [2002](#); 36. Terrenoire et al. [2001](#); 37. Voloshyna et al. [2008](#); 38. Xiao et al. [2009](#); 39. Yamamoto et al. [2009](#); 40. Yamamoto and Taniguchi [2006](#); 41. Zhao et al. [2010](#); 42. Zhou et al. [2009](#)

subthalamic, supraoptic, nodose ganglion, SCG, trigeminal ganglion, intestine, kidney, lung, pituitary gland, prostate, stomach and thymus (Fink et al. 1996, 1998; Maingret et al. 1999a; Bang et al. 2000; Reyes et al. 2000; Meadows et al. 2000, 2001; Lauritzen et al. 2000; Lesage et al. 2000a, 2000b; Talley et al. 2001; Hervieu et al. 2001; Terrenoire et al. 2001; Medhurst et al. 2001; Kim et al. 2001a; Gu et al. 2002; Tan et al. 2002; Gnatenco et al. 2002; Han et al. 2003; Ferroni et al. 2003; Putzke et al. 2007; Voloshyna et al. 2008; Yamamoto et al. 2009; Zhou et al. 2009; Seifert et al. 2009; Zhao et al. 2010; Cadaveira-Mosquera et al. 2011, 2012).

2.7.3 *No Expression*

TREK-1 was reported to be absent in the gigantocellular nucleus, glial cells, locus coeruleus, fibroblasts, satellite cells, colon, liver, placenta and spleen. No TREK-2 expression has been reported in deep cerebellar nuclei, cochlear nuclei, globus pallidus, midbrain, inferior olive, septum, striatum, subthalamic nucleus, vestibular nuclei, satellite cells, vestibular ganglia, ovary and uterus. TRAAK is absent from the raphe, red nucleus, septum, satellite cells, vestibular ganglia, adipose tissue, bladder, bone, cartilage, colon, heart, ovary, salivary gland, smooth muscle, spleen and uterus (Fink et al. 1996, 1998; Bang et al. 2000; Reyes et al. 2000, 2001; Meadows et al. 2000; Lesage et al. 2000a, 2000b; Talley et al. 2001; Hervieu et al. 2001; Terrenoire et al. 2001; Medhurst et al. 2001; Koh et al. 2001; Kim et al. 2001a; Gu et al. 2002; Tan et al. 2002; Nicolas et al. 2004; Yamamoto and Taniguchi 2006; Putzke et al. 2007; Cadaveira-Mosquera et al. 2011, 2012).

2.8 Conclusion and Perspectives

TREK channels generate neuronal leak potassium currents throughout the nervous system and as such, these channels are well positioned to regulate the resting activity and excitability of the entire nervous system. Although considered background channels, they are modulated by a wide variety of physiological and pathological stimuli, including membrane potential, mechanical deformation and temperature. Together with their strong reactivity to clinically important drugs, including neuroprotectors, mood modulators, anesthetics and neurotransmitters, these properties have increased interest in TREK channels as putative targets for the development of new pharmacotherapeutic compounds. In addition, the study of TREK channels has taught us to approach leakage conductances with an open mind, as they do not always obey all the classical Hodgkin-Huxley rules. The discovery of more selective modulators of these channels should aid further understanding of their physiological and pathophysiological roles in the nervous system. Moreover, an improved and more consistent means of quantifying the distribution and expression of these channels would help clarify their true relevance in the organism.

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