Pflugers Arch - Eur J Physiol (2015) 467:931–943
DOI 10.1007/s00424-014-1655-3

INVITED REVIEW

Two-pore domain potassium channels: potential therapeutic targets for the treatment of pain

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Received: 16 September 2014 / Revised: 11 November 2014 / Accepted: 13 November 2014 / Published online: 26 November 2014

Abstract Recent evidence points to a pivotal contribution of a variety of different potassium channels, including two-pore domain potassium (K2P) channels, in chronic pain processing. Expression of several different K2P channel subunits has been detected in nociceptive dorsal root ganglion neurons and trigeminal ganglion neurons, in particular, TREK1, TREK2, TRESK, TRAAK, TASK3 and TWIK1 channels. Of these, the strongest body of evidence from functional studies highlights the importance of TREK1, TRESK and, recently, TREK2 channels. For example, TREK1 knockout mice are more sensitive than wild-type mice to a number of painful stimuli but less sensitive to morphine-induced analgesia. TRESK knockdown mice show behavioural evidence of increased pain and increased sensitivity to painful pressure. Importantly, familial migraine with aura is associated with a dominant-negative mutation in human TRESK channels. Thus, the functional up-regulation of K2P channel activity may be a useful strategy in the development of new therapies for the treatment of pain. Whilst there are few currently available compounds that selectively and directly enhance the activity of TRESK and TREK2 channels, recent advances have been made in terms of identifying compounds that activate TREK1 channels and in understanding how they might act on the channel. Large-scale bio-informatic approaches and the further development of databases of putative ligands, channel structures and putative ligand binding sites on these structures may form the basis for future experimental strategies to detect novel molecules acting to enhance K2P channel activity that would be useful in the treatment of pain.

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Keywords Pain · Two-pore domain potassium channel · TREK1 · TRESK · TREK2 · Nociceptive neuron

Introduction

In the periphery, a population of somatosensory neurons, often termed nociceptors, detect and mediate sensitivity to noxious painful stimuli. The cell bodies of nociceptors are located in the dorsal root ganglia (DRG) or trigeminal ganglia (TG). They possess a peripheral axonal branch that innervates target organs such as skin and muscle and a central axonal branch that innervates the spinal cord. The biophysical and molecular properties of nociceptors enable them to selectively detect and respond to potentially injurious stimuli [7, 8, 96].

There are two major classes of nociceptor, the Aδ fibres that are lightly myelinated and have intermediate cell body sizes (when compared to the larger cell bodies of Aβ fibres, a small proportion of which are also nociceptive) and C fibres that are unmyelinated and have smaller cell bodies [95]. C fibres can be subdivided, based on the expression of neuropeptides such as substance P, binding to the plant lectin IB4, or expression of thermosensitive TRP channels [96]. Acute localised pain is primarily transmitted by Aδ fibres, whilst diffuse pain, including itch, is mediated by C fibres (see [80]).

By sensing painful stimuli and initiating reactions to avoid them, nociceptors play an essential role in maintaining normal body function. Acute physiological pain is normally protective and is initiated by opening of sensory excitatory ion channels (such as TRPV1 channels and ASIC channels, Fig. 1) within nociceptive terminals in response to damaging (or potentially damaging) stimuli of sufficient strength [101]. However, persistent pain is maladaptive [102]. Furthermore, altered pain processing can contribute to hypersensitivity in patients. For example, sunburn produces temporary sensitisation of the affected area so that normally innocuous stimuli

such as light touch or warmth are perceived as painful (allodynia) or normally painful stimuli are exacerbated (hyperalgesia) [7]. Certain disease states such as arthritis and cancer can lead to intense and unremitting pain [7].

Why is there a need for new treatments for pain?

Existing first-line drugs for the treatment of chronic pain, including non-steroidal anti-inflammatory agents, such as aspirin, and opioids, such as morphine, do not alleviate pain completely and, in certain situations including neuropathic pain, do not work very well at all [67, 95]. Furthermore, each has the potential for associated problems, such as tolerance and addiction to opioids, particularly if used chronically.

Chronic pain conditions are often characterised by persistent over-excitability of peripheral nociceptors brought about by medium to long-term changes in ion channel organisation and activity [29, 95]. Recent evidence points to a pivotal contribution of a variety of different potassium channels in chronic pain processing [29, 95], including K2P channels (Fig. 1, see also [2, 67, 80]). In this review, we consider the evidence in support of a role of two-pore domain potassium (K2P) channels in pain processing and detail advances in K2P channel pharmacology, which offer hope of new therapeutic advances in the treatment of pain.

K2P channels

K2P channels underlie background, or leak, K currents, which play an important role in the regulation of the resting membrane potential and excitability of many mammalian neurons including somatosensory neurons (e.g. [2]). There are 15 K2P channel types that are often divided into 6 subfamilies (TWIK, TREK, TASK, TALK, THIK and TRESK) on the basis of their structural and functional properties [33, 36, 59]. The activity of K2P channels can be up- or down-regulated by a diverse array of pharmacological agents, e.g. [69], and physiological mediators [43, 74] and by a large number of neurotransmitter activated pathways [66]. In addition to a role in pain transmission, evidence is accumulating for the potential importance of targeting the activity of K2P channels in a number of therapeutic situations in the nervous system, including neuroprotection, depression, anaesthesia and epilepsy (e.g. [10, 43]).

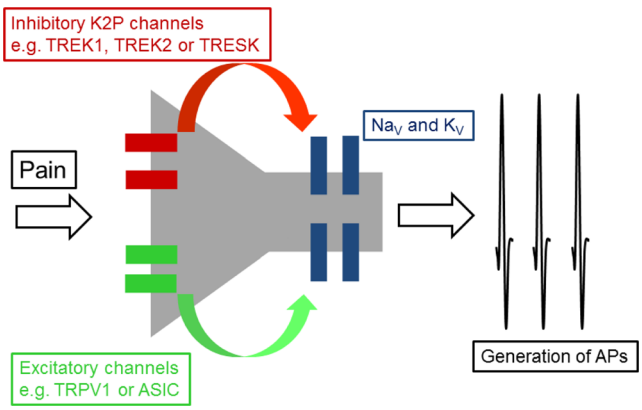

Fig. 1 Role of ion channels at the peripheral terminals of primary sensory neurons. A painful stimulus activates the sensory neuron through a variety of transducer channels such as TRPV1 or ASIC channels. Activation of these channels will depolarise the peripheral nerve terminal, activating voltage-gated Na channels and K channels. This leads to the generation of action potentials (*APs*). Activation of K2P channels (such as TREK1, TREK2 and TRESK channels), on the other hand, will hyperpolarise the peripheral nerve terminal and oppose AP generation. Adapted from [67]

Expression of K2P channels in somatosensory neurons

Expression of several different K2P channel subunits has been detected in nociceptive DRG and TG neurons (see Table 1, also [1, 2, 28, 48, 63, 65, 70, 81, 92, 104, 106]). There is less evidence available as to which of these channels is the most functionally important and what changes in expression levels (and function) may occur in chronic pain. Inflammation, including that found in conditions such as colitis, has been found to change expression levels of many of these channels [54, 65].

Functional evidence for importance of K2P channels in somatosensory neurons

The expression studies suggest that a number of K2P channels may have a role in regulating that activity of nociceptors. A recent study found that a cocktail of non-selective K2P channel blockers (lamotrigine, bupivacaine, doxapram and hydroxyl-α-sanshool) depolarised small DRG neurons by around 10 mV [30]. However, both M (K_V7) channel blockers and 4-aminopyridine produced a similar level of depolarization, suggesting that multiple K channel types contribute to the resting potential of these neurons [30]. The strongest body of evidence from functional studies highlights the importance of TREK1 and TRESK channels, with more recent studies revealing the additional potential importance of TREK2 channels.

TREK1 channels

TREK1 channels are expressed in both small- and medium-sized DRG neurons where they are co-localised with excitatory TRPV1 channels (Table 1, also [2, 25, 63, 92]).

Table 1 Expression of K2P channels in nociceptive somatosensory neurons

Channel	Expression	Other information	Reference
KCNK1 (TWIK1)	Rat and mouse DRGs (IH)	Restricted expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Large and medium rat DRGs (IH)	No overlap with TRPV1 or IB4. Decreased by neuropathic injury	[81] Pollema-Mays et al. 2013
KCNK2 (TREK1)	Small and medium mouse DRGs (IC)	–	[63] Maingret et al. 2000
	Rat and mouse DRGs (IH)	Restricted expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Small (C fibre) mouse DRGs (IH, IC)	Overlap with TRPV1	[2] Alloui et al. 2006
	Rat DRG (RT-PCR	–	[48] Kang and Kim 2006
	Mouse DRG (RT-PCR)	Medium expression	[28] Dobler et al. 2007
	Rat TG (31 %; IH)	Overlap with TRPV1, TRPM8	[104] Yamamoto et al. 2009
	Rat DRG (RT-PCR)	Expression unaltered by axotomy	[98] Tulleuda et al. 2011
	Rat DRG (RT-PCR)	Medium expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
KCNK3 (TASK1)	Rat and mouse DRGs (IH)	High expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Rat DRG (RT-PCR)	–	[48] Kang and Kim 2006
	Mouse DRG (RT-PCR)	Low expression	[28] Dobler et al. 2007
	Rat DRG (RT-PCR)	Expression unaltered by axotomy	[98] Tulleuda et al. 2011
	Small rat DRGs (IH)	Low expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
	Rat DRG (RT-PCR)	Overlap with TRPV1. Unaltered by neuropathic injury	[81] Pollema-Mays et al. 2013
KCNK4 (TRAAK)	Rat and mouse DRGs (IH)	High expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Rat DRG (RT-PCR)	–	[48] Kang and Kim 2006
	Mouse DRG (RT-PCR)	Low expression	[28] Dobler et al. 2007
	Rat TG (60 %; IH)	Overlap with TRPV1, TRPM8	[104] Yamamoto et al. 2009
	Rat DRG (RT-PCR)	Expression unaltered by axotomy	[98] Tulleuda et al. 2011
	Rat DRG (RT-PCR)	High expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
KCNK5 (TASK2)	Rat and mouse DRGs (IH)	High expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Mouse DRG (RT-PCR)	Low expression	[28] Dobler et al. 2007
	Rat DRG (RT-PCR, IC)	Low expression decreased further by 4 days inflammation	[65] Marsh et al. 2012
KCNK6 (TWIK2)	Rat DRG (RT-PCR)	High expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
KCNK9 (TASK3)	Rat and mouse DRGs (IH)	Restricted expression levels	[92] Talley et al. 2001
	Rat DRG (RT-PCR)	–	[48] Kang and Kim 2006
	Mouse DRG (RT-PCR)	Negligible expression	[28] Dobler et al. 2007
	Rat DRG (RT-PCR)	Low expression decreased further by 4 days inflammation	[65] Marsh et al. 2012
	Small (C fibre) rat DRGs (IH)	No overlap with TRPV1 or IB4. Unaltered by neuropathic injury	[81] Pollema-Mays et al. 2013
	Mouse DRG (RT-PCR, IC)	Highly enriched expression in TRPM8-positive neurons	[72] Morenilla-Palao et al. 2014
KCNK10 (TREK2)	Rat and mouse DRGs (IH)	Restricted expression levels	[92] Talley et al. 2001
	Human DRG (RT-PCR)	–	[70] Medhurst et al. 2001
	Rat DRG (RT-PCR)	–	[48] Kang and Kim 2006
	Rat TG (43 %; IH)	Overlap with TRPV1, TRPM8	[104] Yamamoto et al. 2009

Table 1 (continued)

Channel	Expression	Other information	Reference
	Rat DRG (RT-PCR)	Expression unaltered by axotomy	[98] Tulleuda et al. 2011
	Rat DRG (RT-PCR)	High expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
	Small (C fibre) rat DRGs (IC)	Overlap with IB4, decreased by axotomy	[1] Acosta et al. 2014
KCNK12 (THIK2)	Rat DRG (RT-PCR)	Medium expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
KCNK13 (THIK1)	Rat DRG (RT-PCR)	Low expression unaltered by 4 days inflammation	[65] Marsh et al. 2012
KCNK16 (TALK1)	Mouse DRG (RT-PCR)	Low expression	[28] Dobler et al. 2007
KCNK18 (TRESK)	Rat DRG (RT-PCR)	–	[48] Kang and Kim 2006
	Mouse DRG (RT-PCR, IH)	High expression	[28] Dobler et al. 2007
	Rat small and medium DRG (IH)	High expression	[106] Yoo et al. 2009
	Human and mouse DRG and TG (RT-PCR, IH, IC)	High expression relative to elsewhere	[55] Lafreniere et al. 2010
	Rat DRG (RT-PCR)	Expression decreased by axotomy	[98] Tulleuda et al. 2011
	Rat DRG (RT-PCR)	High expression unaltered by 4 days inflammation	[65] Marsh et al. 2012

IH immunohistochemistry, *IC* immunocytochemistry

Regulators of TREK1 channels include membrane stretch [77], membrane depolarisation [11, 34, 44, 77], heat [63], intracellular acidosis [44, 53], changes in extracellular pH [85], arachidonic acid and other polyunsaturated fatty acids (PUFAs) [56, 64]. Activation of Gαq- and Gαo-coupled receptors and protein kinases such as PKC and PKA [17, 34, 44, 73] inhibit the activity of TREK1 channels. However, there is an enhancement of TREK1 activity in response to the activation of Gαi-coupled receptors [15] including $GABA_B$ receptors and μ opioid receptors [27, 86]. A number of clinically useful drugs also affect the activity of TREK1 potassium channels including the neuroprotective agent riluzole [31], the antipsychotic agent chlorpromazine [77] and the antidepressant agent fluoxetine [49].

It is of interest that a number of gaseous general anaesthetic agents such as halothane [76], nitrous oxide, xenon and cyclopropane, which are effective in the clinically relevant range [37], enhance the activity of TREK1 channels. In TREK1-deficient animals, the anaesthetic efficiency of chloroform, halothane, sevoflurane and desoflurane was significantly reduced. Furthermore, their anesthetic action developed with delay, and the minimum alveolar concentration (MAC), which is required to suppress motor reactions to painful stimuli, was increased [41] all suggestive of the importance of TREK1 channels in the central nervous system (CNS), at least in part, mediating the effect of gaseous general anaesthetic agents.

The activity of TREK1 channels has a steep, but bell-shaped, temperature sensitivity making them potential sensors of painful heat stimuli [63, 75]. TREK1 knockout mice have been found to be more sensitive than wild-type mice to painful heat sensations near the threshold between non-painful and painful heat [2]. TREK1 channels have maximum open probability around normal body temperature and reduced open probability at warmer and colder temperatures. Therefore, at temperatures where noxious depolarising TRP channels are activated by heat (or cold), the opposing influence of hyperpolarising TREK1 channels will be diminished [75]. TREK1 deletion also resulted in allodynia, whilst inflammation-induced mechanical and thermal hyperalgesia was lower in TREK1 knockout mice than in wild-type mice [2]. Furthermore, two pain-associated signals, external acidosis and lysophosphatidic acid (LPA), known to rise during injury and inflammation, profoundly down-regulate human TREK1 channel activity [19]. More recently, it has been shown [57] that the TLR7-receptor agonist, imiquimod, enhances the excitability of DRG neurons, at least in part, through blocking TREK1 and TRAAK channels. Taken together, this evidence suggests that TREK1 is a channel involved in polymodal pain perception [2].

Both TREK1- and TRAAK-deficient mice (as well as double knockouts) show greater basal sensitivity to mechanical stimuli and heat, and, in response to chronic inflammation, they present both mechanical and thermal hypersensitivity [2, 75]. Interestingly, only double TREK1 and TRAAK knockout mice show increased cold sensitivity in response to nerve injury [75]. It has been suggested that oxaliplatin-induced cold hypersensitivity may be due to reduced TREK1 and TRAAK expression [26].

A recent study has suggested that the pain-relieving actions of morphine may be linked to TREK1 channel activity [27]. Morphine, acting through μ opioid receptors, was found to enhance TREK1 current directly. Moreover, TREK1 knockout

animals showed significantly less morphine-induced analgesia than WT animals, but there was no difference in the three main adverse effects produced by morphine (constipation, respiratory depression and dependence) between WT and KO animals. This suggests that direct activation of TREK1 by morphine acting downstream from the μ opioid receptor induces strong analgesic effects without underlying any of the adverse side effects of morphine [27].

One important consideration, however, if targeting enhancement of TREK1 channel activity in the treatment of pain, is the observation that a number of drugs used in the treatment of depression, such as fluoxetine, are inhibitors of TREK1 channels (e.g. [49]). Furthermore, TREK1 knockout mice appeared to be resistant to "depression" in several established models, which tested depression-like behaviour [42]. Thus, enhancing TREK1 channel activity in the CNS may have the unintended side effect of exacerbating or inducing depression in some patients.

TRESK channels

TRESK channels are present in DRGs from both mice and humans (Table 1, also [28, 48, 88]). It has been shown that DRG neurons from TRESK knockdown mice require less current injection to elicit action potentials [28] and blocking TRESK channels by pharmacological means is pro-algesic [98]. Similarly, overexpression of TRESK channels, via intrathecal delivery of adenovirus constructs, inhibits capsaicin mediated substance P release from DRG neurons [108] and relieves neuropathic pain syndromes induced by peripheral nerve injury [107]. It has also been observed that overexpression of TRESK channels reduces the excitability of TG neurons [39]. Rat sciatic nerve axotomy results in decreased TRESK expression that is correlated with an induced hyperexcitability of L4–L5 DRG neurons and the development of neuropathic pain [98]. These authors also showed behavioural evidence of increased pain and increased sensitivity to painful pressure after TRESK knockdown in vivo [98].

The potential importance of CNS TRESK channels in pain associated with migraine was highlighted by the observation that familial migraine with aura is associated with a dominant-negative mutation (splice variant F139WfsX24) in TRESK channels [55]. The mutant channel has a two-base-pair deletion in its C-terminus, which causes a frame shift, resulting in a 162-amino-acid premature truncation of its C-terminus. The truncated channel, when expressed on its own in a heterologous expression system, showed no functionality under physiological conditions. When it was co-expressed with WT TRESK channels, F139WfsX24 acted as a dominant-negative, reducing WT TRESK channel expression at the plasma membrane in both recombinant systems and trigeminal ganglion neurons resulting in hypersensitivity of the latter [60]. However, it is important to note that other missense mutations in TRESK channels, including one which resulted in complete lack of channel function (C110R), did not show a correlation with the presence of migraine in patients ([3], see also [40, 62]).

It has been suggested that the primary active ingredient from Szechaun peppers (hydroxy- α-sanshool) excites a subset of capsaicin-sensitive sensory neurons by inhibiting TRESK channels [9]. Application of hydroxy-α-sanshool to sensory neuron peripheral terminals activates Aδ fibres and a subset of slowly conducting C fibres [58]. However, these authors propose that the compound acts on a distinct subset of primary afferent capsaicin-sensitive, mustard oil-insensitive sensory neurons, which may not be involved in pain sensation [58]. Similarly, the synthetic alkylamide IBA activates certain spinal neurons that receive convergent input from mechanoreceptors and nociceptors [89], and there is in vivo electrophysiological evidence of C-nociceptor activation following IBA injection in the rat hindpaw [98].

This action on TRESK channels is thought to underlie the distinctive numbing effect induced by this natural, widely used analgesic. However, this is, at first sight, a paradoxical observation given that TRESK channel activity is inhibited rather than enhanced. It is possible that the tingling paresthesia induced by these compounds eventually leads to desensitisation of the excited neurons and a numbing of the sensation of pain. Alternatively, the actions of these compounds in producing numbing anaesthesia may be mediated through a combination of actions including block of Na channels such as $Na_V1.7$ [97].

TRESK channel currents are enhanced by activation of the calcium/calmodulin-dependent phosphatase, calcineurin, following activation of Gαq-coupled receptors, such as M3 muscarinic receptors, and a rise in intracellular calcium [22, 23]. Calcineurin has been shown to interact directly with both an NFAT-like "PQIIIS" docking motif in the large intracellular loop between transmembrane domains 2 and 3 in the human TRESK channel [20] and a second "LQLP" docking site in the same intracellular loop [21]. It is of interest that transplant patients taking the immunosuppressants (and calcineurin inhibitors) cyclosporin A and tacrolimus (FK506) have been seen to suffer an increase in drug-associated headaches and calcineurin-inhibitor induced pain syndrome (CIPS) [45]. It has been suggested that agonists of calcineurin (and therefore activators of TRESK channels) may provide a potential new treatment for pain ([45], also see below), although it is not known what the overall effect of non-selective activation of calcineurin might be.

TREK2 channels

Despite the wealth of evidence pointing to the importance of TREK1 and TRESK channels in pain pathways, above, some

studies suggest that other K2P channels may also be important. In both small- and medium-sized DRG neurons, single-channel and whole-cell patch recordings suggested that, in addition to TRESK channels, TREK2 channels were most likely to underlie the majority of background current present in these cells [28, 48]. TREK2 channels have been shown to be selectively expressed in IB4 binding C nociceptors [1]. These channels contribute to the resting membrane potential of these neurons since small-interfering RNA (siRNA) against TREK2 depolarised the neurons by 10 mV. Interestingly, axotomy decreased TREK2 expression levels. The authors proposed that TREK2 expression might act to limit pathological pain [1]. Furthermore, TREK2 channels are temperature sensitive [47], and it has recently been shown that TREK2 channels regulate the perception of warm and cool temperature in mice by regulating the firing activity of certain sensory C fibres, which detect such moderate temperature changes [78].

TWIK1 and TASK3 channels

A study focussing on expression levels of TASK1, TASK3 and TWIK1 channels in DRG neurons found much higher levels of TWIK1 channels, which decreased dramatically over 1–4 weeks following neuropathic surgery, suggesting an involvement of TWIK1 in the maintenance of pain [81]. It is of some interest that TWIK1 channels have recently been suggested to form heterodimeric channels with TREK1 when they are co-expressed, in at least some cell types [46].

Although TASK3 channels have low overall levels of expression in DRG neurons and there is little evidence to show a role for them in general pain transduction, a recent study has suggested that these channels are highly enriched in TRPM8 positive neurons [72]. Since TASK3 knockout mice were found to have a hypersensitivity to cold stimuli, this suggests that TASK3 channels may have an important role in the transduction of painful cold stimuli [72].

Functional up-regulation of K2P channel activity in the treatment of pain

The evidence above suggests that up-regulation of K2P channel activity and particularly up-regulation of TREK1, TREK2 and TRESK channel activity may be a useful strategy in the development of new therapies for the treatment of pain (see also [29, 67, 95, 101]). Activation of postsynaptic TREK1, TREK2 or TRESK channels would hyperpolarise the membrane of sensory neurons and depress neuronal activity in the pain pathway, thus countering excitation by depolarising noxious stimuli, activating sensory excitatory ion channels such as TRPV1 or ASIC channels (Fig. 1). Furthermore, enhanced activity of TREK1, TREK2 and TRESK channels located presynaptically will limit excitatory neurotransmitter release.

Two other features of K2P channels are important if they are to be considered useful therapeutic targets. Firstly, although generally widely expressed in many tissues, which may lead to issues with on-target side effects, each K2P channel has a distinct distribution pattern in the body. Secondly, whilst K2P channels are structurally similar to each other they are far from identical. It is possible to identify unique regions of each of these channels, which may provide opportunities for rational drug design (see also [67]).

Functional up-regulation of TREK and TRESK channels

Until relatively recently, there has been a lack of availability of selective pharmacologically active compounds that are known to enhance the activity of K2P channels such as TREK1, TREK2 and TRESK. Indeed, there are relatively few selective agents available that inhibit the activity of K2P channels. However a number of recent advances have been made in this area, particularly in terms of compounds that activate TREK1 channels.

The activity of TREK1 channels is also enhanced by a number of physiological mediators, so, firstly, these physiological regulations and the mechanisms that underlie them will be considered since this might provide useful information as to how drugs that enhance TREK1 current might act. A number of amino acids in TREK1 channels have been identified as being important for the regulation of channel gating. For example, E306, located close to the interface between the fourth transmembrane domain (TM4) and the intracellular C terminus of TREK1, is a key amino acid in transducing channel gating following the action of agents including intracellular acidification, polyunsaturated fatty acids (such as linoleic acid), arachidonic acid, heat and general anaesthetic agents, which enhance the activity of TREK1 channels [44, 56, 63, 87]. Mutations of this amino acid to an A or a G (E306A, E306G) are gain of function mutations, which mimic intracellular acidosis [44, 49]. This is also the pathway through which activation of Gαi-coupled receptors, such as the μ opioid receptor act to enhance the activity of TREK1 channels [27].

More recently, several amino acids at the extracellular end of TM4, close to the selectivity filter of the channel, have also been shown to give rise to a gain of function and interfere with channel gating (Fig. 2). In particular, mutation W275S of TREK1 channels blunts regulation by both external and internal regulators such as extracellular and intracellular pH changes, heat and arachidonic acid [5, 6]. Indeed, a number of experiments suggest that TREK1 channels may not possess a functionally important bundle crossing or activation gate [18, 68] but, instead, that many regulators of TREK1 activity

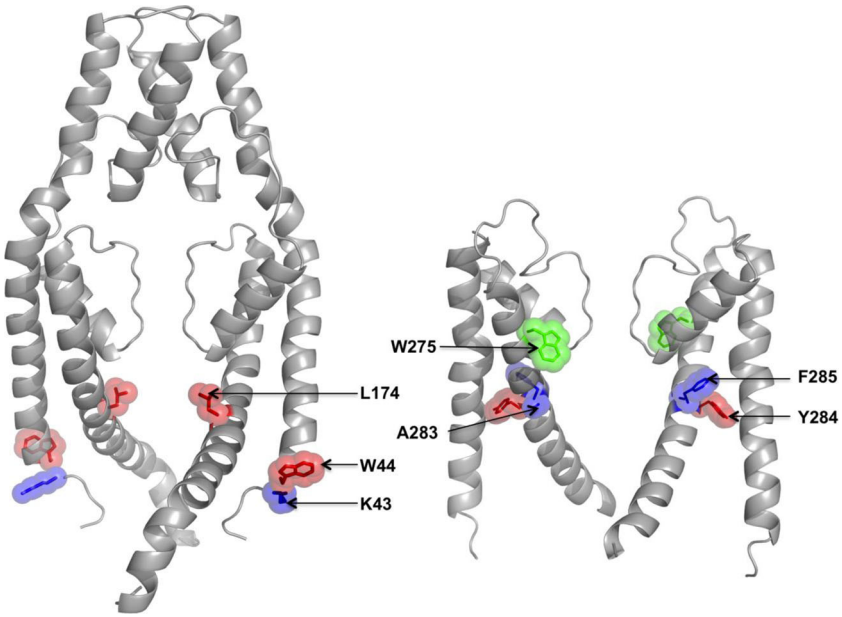

Fig. 2 Structure of TREK1 channel. Homology model of hTREK1 (UniProtKB/Swiss-Prot ID: O95069-2, isoform 2) based on hTRAAK crystal structure (PDB ID: 3UM7). For clarity, TM3 (M3) and TM4 (M4) are rotated through 90° and separated from TM1 (M1) and TM2 (M2). Mutated amino acids are illustrated in colour. W275 (gain of function mutation to S) in *green*, W44, L174, Y284 (mutated to A reduces fenamate effectiveness) in *red*. K43, A283 and F285 in *blue* do not reduce the effect of fenamates when mutated

produce their effect by altering gating at the selectivity filter of the channel, regardless of where on the channel they bind [5, 6, 79, 83]. Thus, the effect of those regulators, which act on the intracellular C-terminus of the channel, must be transduced to the selectivity filter gate close to the extracellular side of the membrane. The TM4 region of the channel is thought to play an important role in this transduction [5].

These data are all consistent with the existing structural information about K2P channels arising from the solved crystal structures of TWIK1 and TRAAK, which capture these channels in an "open" conformation [12, 13, 71].

Pharmacological enhancement of TREK1 channel activity

A number of pharmacologically active compounds enhance the activity of TREK1 channels (see Table 2 for details). The properties of these compounds in relation to TREK1 enhancement are detailed below, but it is important to remember that most, if not all, of these compounds are not selective for TREK1 channels and many have actions on a range of other ion channels and even other proteins, leading to potential off-target side effects and unwanted actions.

DEPC

The histidine modifying agent diethylpyrocarbonate (DEPC) has been shown to prevent inhibition of TREK1 by extracellular protons, which act by binding to an extracellular histidine residue, H126 [85]. DEPC occludes the binding of hydrogen ions to this residue, thus leading to a greatly increased current density at physiological extracellular pH. The irreversible modification by DEPC followed a relatively slow time course that required application for several minutes for the maximal effect to be observed [see 100].

Riluzole

Riluzole, a neuroprotective agent used in the treatment of amylotrophic lateral sclerosis, produces a transient enhancement of TREK1 current. Activation of TREK1 channels was proposed to be mediated by a direct action of the compound on the channel, since it persisted in excised patch recordings [31]. However, riluzole subsequently induced a sustained inhibition of TREK1 current due to activation of protein kinase A [30]. As such, the overall effect of this compound in vivo is difficult to predict, although riluzole has been shown to hyperpolarise DRG neurons [30] and have strong anti-nociceptive activity [84].

Caffeic acid esters

Another group of compounds that enhance the activity of TREK1 channels are caffeic acid esters (such as cinnamyl 1,3,4-dihydroxy-α-cyanocinnamate (CDC) and caffeic acid phenylethyl ester (CAPE)). It has been suggested that caffeic acid derivatives bind to an external site to produce their effects on TREK1 channels, since activity was retained when the compounds were applied externally in outside-out patch recordings [24]. Recently, a range of substituted caffeic acid esters based on a hybrid of CDC and CAPE have been developed, the most promising of which (compound 12U)

Table 2 Pharmacological enhancers of TREK1 channel activity

Compound	Enhancement of recombinant TREK1 channels	Other information	Reference
Riluzole	100 μM gives ~2-fold increase	Transient enhancement followed by inhibition through activation of PKA Also enhances TRAAK	[31] Duprat et al. 2000
DEPC	0.1 % gives ~3-fold increase (at pH 7.4)	Stops H ions binding to H126 and inhibiting current	[85] Sandoz et al. 2009 [100] Veale et al. 2010
CAPE	40 μM gives ~6-fold increase	Acts extracellularly	[24] Danthi et al. 2004
CDC	10 μM gives ~6-fold increase	Acts extracellularly	[24] Danthi et al. 2004
	20 μM gives 2.64-fold increase		[84] Rodrigues et al. 2014
Compound 12U (CDC analogue)	20 μM gives 2.87-fold increase		[84] Rodrigues et al. 2014
Flufenamic acid	100 μM gives ~3–4-fold increase	Other fenamates (such as niflumic acid, mefenamic acid) activate TREK1 but are less potent	[91] Takahira et al. 2005
		Also enhances TREK2 and TRAAK	[99] Veale et al. 2014
BL-1249	EC_{50} of 1 μM which gives ~2-fold increase		[16] Cao et al. 2010
	Maximum ~4-fold increase (100 μM)		[99] Veale et al. 2014
ML67-33	EC_{50} of 36 μM in oocytes or 10 μM in HEK293 cells, which gives ~7-fold increase Maximum ~11-fold increase (100 μM)	Most potent of a series of dihydroacridine analogues Also enhances TREK2 and TRAAK	[4] Bagriantsev et al. 2013

both enhances the activity of TREK1 channels and displays potent analgesic activity in vivo [84].

Fenamates

Fenamate compounds such as flufenamic acid, mefenamic acid, niflumic acid and diclofenac are non-steroidal anti-inflammatory drugs (NSAIDs), already used clinically in the treatment of pain. These compounds have been shown to up- or down-regulate the activity of a number of ion channels [38], including TREK1 channels, where they act to enhance current [91, 99]. BL-1249, another fenamate-like structure and a putative activator of TREK1-like currents in human bladder myocytes [93] also activated TREK1 channels (Fig. 3, also [16, 99]). The enhancement of TREK1 current by fenamates is occluded by mutations E306A and W275S (and several other mutations, which alter channel gating, see Figs. 2 and 3), which also occlude enhancement by many of the physiological enhancers of TREK1 current described above. Whilst this gives some information as to how these compounds act, it does not reveal information about where on the TREK1 channels they bind.

TREK1 channels can exist in two forms following alternative translation initiation [105]. Each of these forms is expressed as proteins in both neurons [94] and expression systems [32]. The alternative translation initiation codon is 42 amino acids upstream from the first methionine in the sequence at the N-terminus of the channel. The N-terminus truncated form gives rise to a current with a much reduced open probability and a collapsed selectivity filter with both a reduced potassium selectivity [61, 74, 94, 100] and a measurable permeability to sodium [94]. Treatment with both DEPC and fenamates, substantially enhances current through the N-terminus truncated form of the channel, and, interestingly, measurements of the reversal potential in the presence of these compounds reveal that the enhanced currents have become highly K selective [99, 100]. Thus, the action of these compounds on the N-terminus truncated form of TREK1 would act to hyperpolarise and decrease the excitability of the neurons they were expressed in, complementing action on the longer form of TREK1.

ML67-33 and related compounds

Using a yeast hybrid screening assay, a recent study [4] was able to identify two new inhibitors and, importantly, five new activators of TREK1 channels from a pool of 106,281 small molecules. The dihydroacridine analogue (ML67-33) could selectively and directly activate the family of temperature sensitive, stretch-activated K2P channels, which includes TREK1, TREK2 and TRAAK. Using a selectivity filter-based gating mutant of the P1 pore helix (G137I) and an intracellular C-terminal domain phosphorylation triple glycine mutant (I292G, G293, D294G), it was shown that ML67-33 activated TREK1, via the selectivity filter-based gate, by a distinct pathway from the intracellularly acting activators of

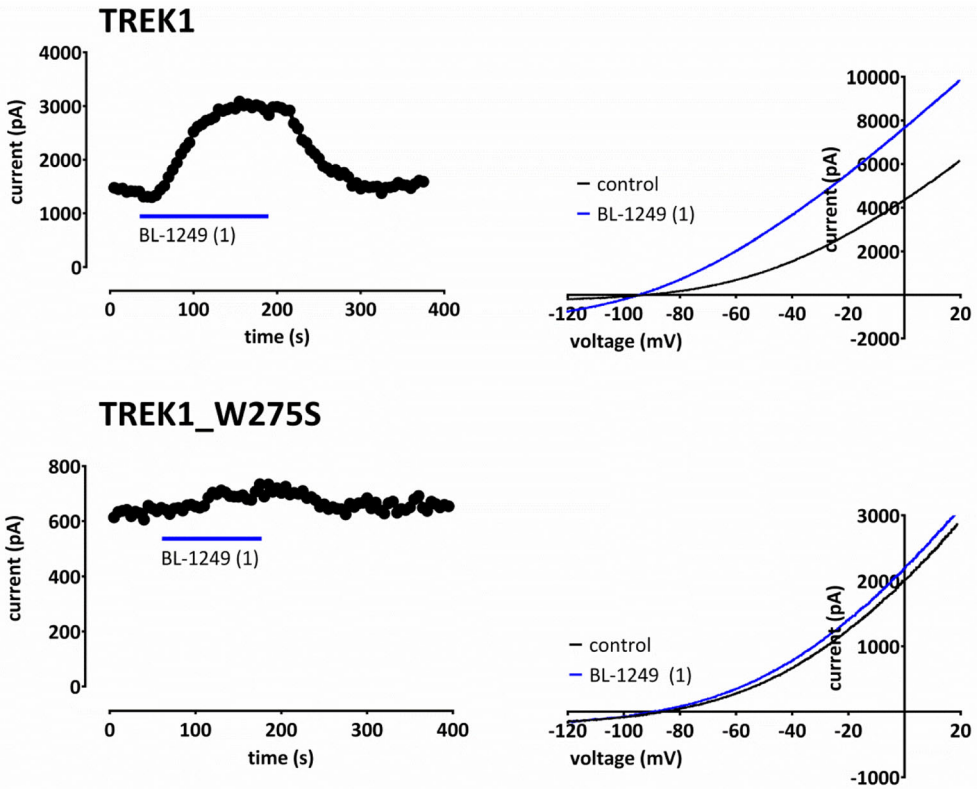

Fig. 3 Reduced activation of mutated (W275S) TREK1 currents by BL-1249. **a** Representative time course for enhancement by BL-1249 (1 μM) of current through WT TREK1 channels. **b** Currents recorded through WT TREK1 channels in the absence and presence of BL-1249 (1 μM). **c** Representative time course for enhancement by BL-1249 (1 μM) of current through TREK1_W275S channels. **d** Currents recorded through TREK1_W275S channels in the absence and presence of BL-1249 (1 μM). Adapted from [99]

TREK1 and the fenamate compounds described above and that the C-terminus of the channel was not involved [4].

Regulation of TREK2 channels

Whilst most compounds that activate TREK1 also activate TREK2, it should be noted that it is possible to differentially regulate the two channels. Extracellular acidification, for example, inhibits the activity of TREK1 channels but enhances the activity of TREK2 channels. Surprisingly, these opposing effects are mediated through a single conserved histidine residue in the two channels ([85], see above). It has been reported that TREK2 channels can be activated by a family of sulfonate chalcones [51]. By contrast, related sulphonamide chalcones inhibited these channels. It is not known whether these compounds have similar actions on TREK1 channels. TREK2 channel activity has also been shown to be markedly enhanced by the flavonoids, baicalein and wogonin, although it is not clear whether these compounds act directly or indirectly on the channels, or both [50].

Pharmacological enhancement of TRESK channel activity

Whilst there are several promising possibilities for compounds that activate TREK1 channels, there is much less information available, at present, regarding compounds that might activate TRESK channels or even how such regulatory compounds might act. One approach, albeit a rather non-selective one, might be to develop compounds that mimic physiological activation of TRESK either by raising intracellular calcium (see above) or by stimulating protein kinase C [82]. The antimicrobial agent, cloxyquin, has been shown to enhance the activity of TRESK channels [103]. This action is not thought to be a direct action on the channel, rather it is proposed that cloxyquin acts either to raise intracellular calcium levels or activate protein kinase C. Similarly, a recent high throughput library of pharmacologically active compounds (LOPAC) screen identified eight activators of TRESK channels, but all of these were proposed to be working indirectly to enhance current, again either through mobilising intracellular calcium or stimulating protein kinase C [14]. This is in contrast to several inhibitors identified in the same study, such as loratidine, which were proposed to act directly on the channel on the basis of point mutations on the TRESK channel (F145A, F352A), which attenuated the inhibition by loratidine. These mutations were also shown to attenuate inhibition of TRESK channels by lidocaine, quinine and propafenone in an earlier study [52], perhaps by interfering with TRESK channel gating.

At present, therefore, there is a distinct lack of identified molecules, which are known to bind directly to TRESK channels and activate them. A recent pharmaco-informatics

approach has identified a number of residues on the TRESK channel, which may be involved in ligand binding. These residues are located near the extracellular face of TMs 1, 2 and 4 based on a putative structural model of the TRESK channel. Additionally, these authors suggested several compounds, which might bind to this site and be useful in the treatment of migraine [90]. Whilst this approach provides an interesting potential start point for further experimental studies, it is not known whether the suggested compounds will indeed bind to the TRESK channel nor, if they do, whether they will enhance channel activity, block channel activity or have no effect.

Nevertheless, despite these caveats, it seems likely that large-scale bio-informatic approaches and the further development of databases of putative ligands, channel structures and putative ligand binding sites on these structures, such as those being developed by the SuperPain database [35], will provide important information. This can be used as a basis for future experimental strategies for the detection of novel molecules acting to enhance K2P channel activity that would be useful in the treatment of pain.

Acknowledgments This work was supported by the Biotechnology and Biological Sciences Research Council (UK) [BB/J000930/1] and Pfizer. Thanks to Ehab Al-Moubarak for homology modelling of the TREK1 channel on TRAAK (Fig. 2) and to Madeleine Mathie for help with Fig. 1.

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