Influence of the N Terminus on the Biophysical Properties and Pharmacology of TREK1 Potassium Channels

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ABSTRACT

TWIK-related K⁺ (TREK1) potassium channels are members of the two-pore domain potassium channel family and contribute to background potassium conductances in many cell types, where their activity can be regulated by a variety of physiologic and pharmacologic mediators. Fenamates such as FFA (flufenamic acid; 2-[[3-(trifluoromethyl)phenyl]amino]benzoic acid), MFA [mefenamic acid; 2-(2,3-dimethylphenyl)aminobenzoic acid], NFA [niflumic acid; 2-[[3-(trifluoromethyl)phenyl]amino]nicotinic acid], and diclofenac [2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid] and the related experimental drug BL-1249 [6-(5,6,7,8-tetrahydro-naphthalen-1-yl)-2-(1H-tetrazol-5-yl)-phenyl]amine] enhance the activity of TREK1 currents, and we show that BL-1249 is the most potent of these compounds. Alternative translation initiation produces a shorter, N terminus truncated form of TREK1 with a much reduced open probability and a proposed increased permeability to sodium compared with the longer form. We show that both forms of TREK1 can be activated by fenamates and that a number of mutations that affect TREK1 channel gating occlude the action of fenamates but only in the longer form of TREK1. Furthermore, fenamates produce a marked enhancement of current through the shorter, truncated form of TREK1 and reveal a K⁺-selective channel, like the long form. These results provide insight into the mechanism of TREK1 channel activation by fenamates, and, given the role of TREK1 channels in pain, they suggest a novel analgesic mechanism for these compounds.

Introduction

TREK1 (TWIK-related K, also K2p2.1, KCNK2) potassium channels are members of the two-pore domain potassium channel family (Enyedi and Czirjak, 2010) and contribute to background potassium conductances in many cell types, where they show activity over a wide range of voltages. Their activity can be up- or downregulated by a variety of physiologic and pharmacologic mediators (Honore, 2007; Noel et al., 2011). These regulatory mechanisms include membrane stretch, membrane depolarization, heat, and intracellular acidosis as well as changes in extracellular pH, arachidonic acid, and other polyunsaturated fatty acids. Activation of Gαq- and Gα12/13-coupled receptors and protein kinases, such as protein kinase C and protein kinase A, inhibit the activity of TREK1 channels (Enyedi and Czirjak, 2010). However, there is an enhancement of TREK1 activity in response to the activation of Gαi-coupled receptors including GABA₃ receptors (Cain et al., 2008; Sandoz et al., 2012). A number of clinically important drugs also affect the activity of TREK1, including the neuroprotective agent riluzole (Duprat et al., 2000), the antipsychotic agent chlorpromazine (Patel et al., 1998), and the antidepressant agent fluoxetine (Kennard et al., 2005).

TREK1 channels are expressed in sensory neurons, particularly in nociceptors (Alloui et al., 2006; Marsh et al., 2012), and they are also broadly distributed in the central nervous system (Fink et al., 1996; Talley et al., 2001; Aller and Wisden, 2008). A role for TREK1 in depression (Heurteaux et al., 2006), polymodal pain perception (Alloui et al., 2006; Noel et al., 2009), and diseases related to blood-brain barrier dysfunction (Bittner et al., 2013) has been proposed. A number of gaseous general anesthetic agents such as halothane (Patel et al., 1999), nitrous oxide, xenon, and cyclopropane, which are effective in the clinically relevant range (Gruss et al., 2004), also enhance the activity of TREK1 channels. Furthermore, in TREK1-deficient animals, the anesthetic efficiency of chloroform, halothane, sevoflurane, and desflurane is significantly reduced (Heurteaux et al., 2004), which is suggestive of the importance of TREK1 channels in mediating, at least in part, the effect of gaseous general anesthetic agents.
TREK1 knockout mice are more sensitive to painful heat sensations and a variety of other painful stimuli, including mechanical and inflammatory stimuli, compared with control animals (Alloui et al., 2006). This change in pain sensitivity of TREK1 knockout mice and the presence of TREK1 in sensory neurons, including dorsal root ganglia neurons, suggests that TREK1 channels may be a promising target for the development of new analgesics that act to enhance the activity of these channels (Alloui et al., 2006; Woolf and Ma, 2007; Mathie, 2010).

Intriguingly, alternative translation initiation (ATI) of KCNK2 (TREK1) transcripts has been shown to produce a shorter form of TREK1 that is truncated at the N terminus. Both short and long forms appear to be expressed as proteins in both neurons (Thomas et al., 2008) and recombinant expression systems (Eckert et al., 2011). The alternative translation initiation codon is a methionine that immediately precedes the first transmembrane helix (M1) and results in a complete deletion of the intracellular N terminus of the channel. The truncated form gives rise to a current with a much reduced open probability and a proposed measurable permeability to sodium (Thomas et al., 2008).

Fenamate compounds, such as diclofenac [2-(2,6-dichlorophenyl)acetic acid], FFA (flufenamic acid; 2-([3-(trifluoromethyl)phenyl]amino)benzoic acid), MFA (mefenamic acid; 2-(2,3-dimethylphenyl)aminobenzoic acid), and NFA (niflumic acid; 2-[(3-trifluoromethyl)phenyl]aminonicotinic acid), are nonsteroidal anti-inflammatory drugs that are already used clinically in the treatment of pain. They have been shown to up- or downregulate the activity of a number of ion channels, including TREK1 where they act to enhance current (Takahira et al., 2005).

Our study characterized the mechanism of fenamate action on TREK1 channels. Our results reveal that the activity of both short and longer forms of TREK1 is enhanced by fenamates, but their action on the truncated form also reveals a K⁺ selective current. Their activatory effect is also enhanced in the truncated forms of TREK1. Furthermore, we show that gating mutations can occlude the effect of fenamates but only in the longer form of TREK1. The results provide an important insight into the mechanism of action of these compounds.

Materials and Methods

Cell Culture. We grew tsA201 cells (ECACC; Sigma-Aldrich, Gillingham, Dorset, UK), modified human embryonic kidney 293 cells, in a monolayer tissue culture flask maintained in a growth medium that was composed of 88% minimum essential media with Earle’s salts and 2 mM l-glutamine, 10% of heat-inactivated fetal bovine serum, 1% penicillin (10,000 units ml⁻¹) and streptomycin (10 mg ml⁻¹), and 1% nonessential amino acids. The cells were placed in an incubator at 37°C with a humidified atmosphere of 95% oxygen and 5% carbon dioxide. After 2 or 3 days, when the cells were 70 to 90% confluent, they were split and resuspended in a 4-well plate containing 13-mm diameter cover slips (poly-p-lysine–coated) in 0.5 ml of media, ready to be transfected the next day.

Transfection. For the electrophysiological experiments, pcDNA3.1 vector was cloned with the gene of interest: human TREK1 (hTREK1) or human TWIK-related arachidonic acid-stimulated K⁺ (hTRAAK), wild-type or mutated. This and a similar vector containing green fluorescent protein were incorporated into the cells (0.5 µg per well for each plasmid) using the calcium phosphate method. The cells were incubated for 6 to 12 hours at 37°C in 95% oxygen and 5% carbon dioxide. Then the cells were washed using a phosphate-buffered saline solution, and new media was added to each well. The cells were used for experiments after 24 hours.

Mutations and Truncations. Point mutations were introduced by site-directed mutagenesis into the TREK1 or TRAAK using the Quikchange kit (Stratagene, Amsterdam, The Netherlands), and all mutations were confirmed by direct sequencing.

Whole-Cell Patch-Clamp Electrophysiology. Currents were recorded using the whole-cell patch-clamp in a voltage clamp configuration in tsA201 cells transiently transfected with the channel of interest. The cover slip with the cells was placed in a recording chamber filled with an external medium composed of 145 mM NaCl, 2.5 mM KCl, 3 mM MgCl₂, 1 mM CaCl₂, and 10 mM HEPES (pH to 7.4, using NaOH). The internal medium used in the glass pipette comprised 150 mM KCl, 3 mM MgCl₂, 5 mM EGTA, and 10 mM HEPES (pH to 7.4, using KOH). Modulatory compounds were applied by bath perfusion at a rate of 4 to 5 ml min⁻¹. Complete exchange of bath solution occurred within 100 to 120 seconds. All data were collected at room temperature (19–22°C). The transfected cells were detected using a fluorescent microscope with UV light. The cells were voltage-clamped using an Axopatch 1D or Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) and low pass filtered at 5 kHz before sampling (2–10 kHz) and online capture.

To study the potassium leak current, a “step-ramp” voltage protocol was used. For the step component of the protocol, cells were hyperpolarized from a holding voltage of −60 to −80 mV for 100 milliseconds then stepped to −40 mV for 500 milliseconds. For the ramp, cells were then stepped to −120 mV for 100 milliseconds, followed by a 500-millisecond voltage ramp to +20 mV and a step back to −80 mV for another 100 milliseconds, before being returned to the holding voltage of −60 mV. This protocol was composed of sweeps lasting 1.5 seconds (including sampling at the holding voltage) and was repeated once every 5 seconds. An example of the typical current response seen for wild-type TREK1 channels to this protocol is illustrated in Fig. 1B. For analysis of outward current, we measured the current difference between the −80 and −40 mV steps. The current-voltage graphs were obtained from the ramp change in voltage between −120 and +20 mV. The currents obtained with the imposed voltage protocol were recorded and analyzed using pCLAMP 10.2 software (Molecular Devices) and Microsoft Excel (Redmond, WA). For each cell, the pA was normalized to the cell capacitance (pF).

Data Analysis. Data are expressed as mean ± S.E.M., and n represents the number of cells used for the experiment. The statistical analyses used either Student’s t test or a one-way analysis of variance with the post hoc Dunnett’s multiple comparisons test, using GraphPad Prism 6.02 (GraphPad Software, San Diego, CA). For the t test, P < 0.05 was considered statistically significant for differences between means. For Dunnett’s test, P < 0.05 was considered statistically significantly different (confidence interval >95% for the difference between the two compared means).

Chemicals. All fine chemicals were purchased from Sigma-Aldrich.

Homology Modeling. The homology model of hTREK1 (UniProtKB/Swiss-Prot ID: O95069-2, isoform 2) was created using Modeler 9v8 (http://salilab.org/modeller/) (Sali and Blundell, 1993) using the human TRAAK structure (PDB ID 4i9w) as template. ClustalW (http://www.clustal.org/clustal2/) (Higgins et al., 1996) was used to align the TRAAK and TREK1 sequences.

Results

Enhancement of TREK1 Current by Fenamates. A number of fenamate compounds, such as FFA, NFA, MFA, and diclofenac, enhance the activity of wild-type TREK1 channels (see also Takahira et al., 2005). Figure 1 shows the percentage of enhancement observed after bath application of 100 µM of each compound. The degree of enhancement is greatest with FFA (250% ± 35%, n = 17) and is least with
diclofenac (37% ± 6%, n = 11). BL-1249 [(5,6,7,8-tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine], another fenamate-like structure and a putative activator of TREK1-like currents in human bladder myocytes (Tertyshnikova et al., 2005), also activated recombinantly expressed TREK1. Interestingly, BL-1249 was around 30 to 100 times more potent than FFA, with 1 μM producing a 130% ± 17% (n = 8) enhancement (Fig. 1) and 3 μM producing a 414% ± 110% (n = 3) enhancement. BL-1249 and each of the fenamates are nonselective cyclooxygenase (COX) inhibitors. COX inhibition can lead to an increase in intracellular arachidonic acid. Because external application of arachidonic acid is also known to stimulate
TREK1 channels (Patel et al., 1998), it was therefore important to determine whether the enhancement of current is mediated through an increased concentration of arachidonic acid. Ibuprofen is a nonselective COX inhibitor, but it had little effect on wild-type TREK1 current, with 100 μM producing only a 10% ± 4% (n = 9) enhancement of current under the same conditions that BL-1249 and the other fenamates enhanced TREK1 current (Fig. 2). Furthermore, the COX-1 selective nonsteroidal anti-inflammatory drug indomethacin (100 μM) caused a small but significant decrease in TREK1 current with a reversible inhibition of 24% ± 1% (n = 3) (Fig. 2).

**Gating Mutations Interfere with Fenamate Enhancement of TREK1 Current.** A number of amino acids in TREK1 have been identified as being important for the regulation of channel gating. For example, E306 in the intracellular C terminus of TREK1 is a key amino acid in transducing channel gating after the action of agents such as pH(i), polyunsaturated fatty acids, arachidonic acid, anesthetic gases, and heat, which modulate the activity of TREK1 (Maingret et al., 2000a,b; Honoré et al., 2002; Gruss et al., 2004; Sandoz et al., 2006). Mutation of this amino acid (e.g., E306A, E306G) produces a gain of function phenotype that mimics intracellular acidosis (Honoré et al., 2002; Kennard et al., 2005), and it is difficult to further up- or downregulate the channel.

More recently, several amino acids at the extracellular end of transmembrane domain 4 (M4), close to the selectivity filter, have also been shown to give rise to a gain of function and interfere with channel gating. In particular, mutation W275S blunts regulation by both external and internal regulators such as extracellular and intracellular pH changes, heat, and arachidonic acid (Bagriantsev et al., 2011, 2012).

Figure 3 shows the effect of both of these mutations on the activation of TREK1 by FFA (100 μM) and BL-1249 (1 μM). For both mutations, the degree of enhancement by FFA and BL-1249 was significantly reduced compared with wild-type TREK1. This suggests that these compounds interfere with this gating pathway, or, alternatively, that this gating pathway, when fully activated, occludes the action of fenamates.

**M1, M2, and M4 Mutations in TREK1 Affect Fenamate Activation.** The recent experiments of Bagriantsev et al. (2011, 2012), Piechotta et al. (2011), and Rapedius et al. (2012) suggest that TREK1 channels may not gate directly at the lower bundle-crossing like many other classic tetrameric K+ channels (Cohen et al., 2009; Mathie et al., 2010). Instead, it appears that most if not all regulators of TREK1 activity produce their effect by altering gating at the selectivity filter of the channel, regardless of where in the channel they interact. Thus, the effect of a number of regulators that act via the intracellular C terminus (C terminus domain) of the channel.
channel, such as heat, intracellular acidification, stretch, arachidonic acid, and activation of Gαi-coupled receptors, must somehow be transduced to the selectivity filter, and the pore-lining M4 helix is thought to play an important role in this transduction due to its direct attachment to the regulatory C terminus domain.

In several other K+ channels, the equivalent region to M4 has been suggested to be important for the binding of regulatory molecules. For example, in human ether a-go-go-related gene channels, this region appears to be involved in the binding of both channel activators and inhibitors (Hosaka et al., 2007; Perry et al., 2010; Garg et al., 2011). We thus investigated whether this region of TREK1 might be important for the binding of fenamates and/or gating of the channel.

Using a previous structural model of TREK1 originally based upon KvAP (Piechotta et al., 2011), we identified a number of mutations in M1/M2/M4 that had a profound effect upon fenamate activation of the longer form of TREK1 and that initially suggested a putative binding site (not shown). Three mutations in particular, W44A, L174A, and Y284A, substantially reduced activation by both FFA (Fig. 4) and BL-1249 (Supplemental Fig. 1) whereas mutation of several adjacent amino acids had no effect. However, a subsequent homology model based on the more recent crystal structure of TRAAK (Brohawn et al., 2013) suggests that these residues do not cluster together (Fig. 4). The influence of these mutations on current density is shown in Supplemental Fig. 2. It is therefore more likely that these mutations act in a similar way to the E306A mutation by influencing TREK1 gating and thereby the efficacy of fenamate activation.

**TRAAK Isoforms and Their Regulation by Fenamates.** To investigate this further, we considered the regulation of a related K2P channel (TRAAK) by FFA. Two isoforms of human TRAAK were studied: a short form (393 aa) and longer form (419 aa), where the differences in length are due to differences in the N terminus that precedes M1. Transcripts for both isoforms have been reported, but their relative abundance and/or importance is not known (Ozaita and Vega-Saenz de Miera, 2002). For both isoforms, the basal current is small, but substantial enhancement of current by FFA is seen in both cases. M4 is highly conserved in TRAAK and TREK1; however, in direct contrast to mutation of Y284

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**Fig. 3.** Reduced activation of mutated, gain of function TREK1 currents by fenamates. (A and B) Histogram of percentage enhancement of current by FFA (A) and BL-1249 (1 μM) (B) for wild-type TREK1 and TREK1_W275S and TREK1_E306A. (C) Left: representative time course for enhancement by BL-1249 (1 μM) of current through TREK1_W275S channels. Inset: currents evoked by the step-ramp voltage protocol recorded through TREK1_W275S channels in the absence or presence of BL-1249 (1 μM). Right: current-voltage relationships for TREK1_W275S channels in the absence (black) or presence (gray) of BL-1249 (1 μM). (D) Same as C for TREK1_E306A channels.
in TREK1, mutation of the equivalent residue in either the long (Fig. 5) or short isoform of TRAAK did not influence enhancement by FFA.

**TREK1 Isoforms and Their Regulation by Fenamates.**

ATI produces an isoform of TREK1 truncated at a similar position at the N terminus to the short form of TRAAK. To study these different versions of TREK1 in isolation, a point mutation (isoleucine for methionine) was introduced (M42I) that blocks ATI to give only the long form of the channel. To isolate the shorter, truncated form of the channel, the sequence encoding the first 41 amino acids was physically removed from the expression construct (Veale et al., 2010).

All compounds that were shown to enhance the activity of wild-type TREK1 (BL-1249, FFA, NFA, MFA, and diclofenac) also enhanced the activity of the long form of the channel (TREK1_M42I), as shown in Fig. 6. There were no statistically significant differences in the degree of enhancement observed.

By contrast, the current seen in control solutions through the N terminus deleted form of TREK1 (TREK1_DN) was very small in normal external K⁺ (2.5 mM), to the extent that it was not clear whether there was any significant expression of the channel (see Supplemental Fig. 3). Current through TREK1_DN channels had an amplitude of just 4 ± 1 pA/pF (n = 12) compared with 48 ± 4 pA/pF (n = 14) for wild-type TREK1, and a measured reversal potential of −47 ± 5 mV (n = 14) compared with −85 ± 1 mV (n = 27) for wild-type TREK1 under the same conditions. However, changing the external K⁺ concentration to 25 and 147.5 mM gave measurable current (Supplemental Fig. 3), particularly when compared with untransfected cells, allowing confidence that although the current was small, it was present and detectable.

Application of FFA, MFA, NFA, and BL-1249 (but not diclofenac) gave rise to a large increase in current through the short form of the channel (Fig. 7). These fenamate-enhanced currents had a reversal potential close to wild-type TREK1 currents, showing that in the presence of these compounds the current was K⁺ selective. The huge percentage increase in current in normal external K⁺ was not simply a result of there being negligible current to begin with. In 147.5 external K⁺, there is significant current through TREK1_DN (219 ± 32 pA, n = 13, at −80 mV), and this was enhanced by over 5000% (5566% ± 1633%, n = 8) in the presence of 100 μM FFA (Supplemental Fig. 3). Thus, it is likely that the large percentage of increase seen is due to the initial low open probability of TREK1_DN.

In direct contrast, truncation of the TREK1 C terminus (TREK1_DC, deletion of all amino acids after T322) also reduces the basal current to levels similar to that seen with TREK1_DN (6 ± 1 pA/pF, n = 11; see also Kennard et al., 2005), but this is enhanced by FFA proportionally similar to wild-type TREK1 (259% ± 29%, n = 11; see Fig. 4). Because TREK1_DC has been proposed to represent the C terminus of the channel in its dephosphorylated form, compared with a mixture of phosphorylated and dephosphorylated channels present in wild-type TREK1 (Honèr et al., 2002; Kennard et al., 2005), this suggests that the action of FFA does not depend on the phosphorylation state of the C terminus.
The gain of function E306A also enhanced current through TREK1ΔN (Fig. 8). Interestingly, the reversal potential of this enhanced current was exactly the same as the reversal potential for wild-type TREK1 (~85 ± 1 mV, n = 16), and FFA was able to further enhance the current through TREK1ΔN(E306A) (Fig. 8).

A number of the other mutations that altered the effectiveness of FFA on the longer form wild-type TREK1 (L174A, Y284A, W275S) also altered both the current density and the K⁺ selectivity of the shorter TREK1ΔN; however, one other mutation (W44A), close to the methionine start codon in TREK1ΔN, notably did not. These data are illustrated in Fig. 8. However, for all mutations, the current could still be enhanced further by FFA.

The data are summarized as absolute current density measurements for the mutated channels in the presence and absence of FFA (Fig. 8). In this way, it can be seen that both mutations and fenamates increase the size of the current, but the size of the effect they produce depends, to a large extent, on the initial current levels (and most likely channel open probability). Thus, the fenamates can induce a massive percentage increase in current through TREK1ΔN, especially where the initial current is very low. By contrast, the fenamates have little further enhancement of the gain of function E306A mutation in wild-type TREK1, where current (and open probability) is already large (Fig. 3). Thus, the different manipulations that enhance TREK1 current density appear to act synergistically and reach saturation (Fig. 8). It is of particular interest that the effects of N terminus truncation are largely overcome by gain of function mutations (E306A, W275S, L174A, and Y284A), by fenamate-mediated enhancement, or by both.

**Discussion**

The fenamate group of compounds enhances the activity of TREK1 and TRAAK channels, with FFA being the most potent and diclofenac the least potent among the four clinically used compounds tested. By contrast, FFA has relatively little effect on the related K2P channel TASK3 (Veale et al., 2014). The relative effectiveness of FFA, MFA, and NFA on TREK1 is consistent with that found previously by Takahira et al. (2005). However, the experimental fenamate BL-1249 is around 30 to 100 times more potent than FFA.

Fenamates are known to enhance current through a variety of K⁺ channels, including human ether a-go-go-related gene (Fernandez et al., 2008) and KCa₇ channels (Parrugia et al., 1993). They also enhance current through KCNQ/Kv7, but for these channels the potency sequence is rather different to that found for TREK1 channels; diclofenac is more potent than FFA, MFA, or NFA (Peretz et al., 2005, 2007), suggesting either differences in the binding site between the two channels or the mechanism of fenamate activation.

Fenamates also both activate and inhibit the Na-dependent Slo2.1 (KCa₄.2) channels through binding to distinct sites on the channel (Garg and Sanguinetti, 2012). In these channels, MFA and diclofenac were more potent activators than FFA and NFA. The lack of effect of indomethacin and ibuprofen on TREK1 suggests that the fenamates produce their effect by binding directly to the channel rather than by an indirect action through alterations in arachidonic acid levels after inhibition of COX enzymes.

Our initial studies, based on an earlier model of TREK1 that used KvAP as a structural template, proposed a number of amino acids that may contribute to a binding site for FFA and BL-1249 (Cao et al., 2010). However, more accurate templates have indicated that these residues (W44, L174, and Y284) were too far apart to comprise a binding site and that these mutations may simply interfere with channel gating and thereby affect ligand efficacy, as appears to be the case for the other gain of function mutations E306A (Maingret et al., 2000a,b; Honoré et al., 2002; Sandoz et al., 2006) and W275S (Bagriantsev et al., 2011, 2012). Our ongoing experiments suggest that mutation of Y284 also occludes the action of other regulators of TREK1 gating, such as alterations in extracellular pH. To directly address this issue, we have studied a related K2P channel (TRAAK) that is also enhanced by fenamates and by the use of the N terminus truncated form of TREK1 (TREK1ΔN), which has a very small basal current. For TRAAK channels, the mutations equivalent to Y284 in TREK1 did not alter the effectiveness of FFA or BL1249, showing that these compounds must still bind to TRAAK.
However, it also shows that there must be subtle differences in the gating mechanisms between TREK1 and TRAAK because, despite their homology, they are differentially affected by this mutation.

Furthermore, channels with equivalent mutations in TREK1ΔN (see below) were also still enhanced by FFA and other fenamates, showing that binding of these compounds is still able to occur in these mutant channels. Thus, although we cannot completely discount the contribution of the mutated residues to the binding site of fenamates, these mutations are more likely to be explained by alterations in channel gating than drug binding. Therefore, TREK1ΔN may be helpful in future studies aimed at identification of agonist binding sites on TREK1 as well as understanding the mechanisms by which these drugs influence gating at the selectivity filter.

TREK1 can exist in two forms after alternative translation initiation. Each of these forms is expressed as proteins in both neurons (Thomas et al., 2008) and recombinant expression systems (Eckert et al., 2011). Thomas et al. (2008) describe the N terminus truncated form of TREK1 (TREK1ΔN) as being regionally and developmentally regulated in a number of different regions of the rat central nervous system. Both forms are expressed in COS-7 cells (Thomas et al., 2008) but predominantly the long form is found in human embryonic
kidney 293 cells (Ma et al., 2011). For the related K2P channel TREK2, which also undergoes ATI (Simkin et al., 2008), the degree of ATI has been shown to be tissue specific (Staudacher et al., 2011) through differential regulation of mRNA translation. In addition to a number of splice variants that have recently been identified in TREK1 (Veale et al., 2010; Rinné et al., 2013), these ATI variants may also play an important role in those tissues in which they are expressed.

TREK1ΔN has been described as a constitutively “non-conductive” variant, with a “collapsed selectivity filter” (Ma et al., 2011) that is permeable to sodium under normal physiologic conditions, leading to membrane depolarization when it is active in neurons (Thomas et al., 2008). Furthermore, TREK1ΔN has been shown to copurify with the longer form (Thomas et al., 2008), so heteromers may also exist.

In this study, we have shown that current through TREK1ΔN is markedly enhanced by fenamates. Interestingly, measurements of the reversal potential in the presence of these compounds reveal that these enhanced currents are highly K⁺ selective. Similarly, gain of function mutations that influence channel gating (E306A, W275S, L174A, Y284A) not only increase channel current but also reveal a K⁺ selective current.

Given an equal level of expression of both forms of the channel, the long form will dominate under control conditions because its open probability appears far greater than TREK1ΔN. However, this would not be the case after
fenamate activation (or after gain of function mutations), which produces a substantial increase in the apparent open probability of TREK1. Because the action of fenamates (and gain of function mutations) reveals a K⁺-selective conductance, fenamate action on TREK1 would amplify its effect on any coexpressed longer forms of TREK1 to produce an increased hyperpolarization and decreased excitability of the cell membrane. Heteromeric combinations of the longer and short forms of the channel also would behave in the same manner in the presence of fenamates.

The involvement of TREK1 in pain (Alloui et al., 2006; Noel et al., 2009) suggests that compounds that enhance their activity, such as the fenamates described herein, would be of considerable value as lead compounds for potential new analgesics targeting this channel. Activation of postsynaptic TREK1 channels would hyperpolarize the membrane of central neurons and depress neuronal activity in the pain pathway, thus counteracting excitatory stimulation by increased neurotransmission. Furthermore, enhanced activity of TREK1 channels located presynaptically will limit excitatory neurotransmitter release.

Fig. 8. Activation of mutated TREK1ΔN currents by FFA. (A) Histogram of zero current potential (left) and current density (right) for wild-type TREK1 and various mutant channels in the absence or presence of FFA (100 μM). (B) Left: representative time course for enhancement by FFA (100 μM) of current through TREK1ΔN_Y284A channels. Right: current-voltage relationships for TREK1ΔN_Y284A channels in the absence (black) or presence (gray) of FFA (100 μM). (C) Same as B for TREK1ΔN_E306A channels.

Authorship Contributions

Participated in research design: Mathie, Stevens, Tucker, Veale, Cao, Omoto.
Conducted experiments: Veale, Bajaria, Al-Moubarak.
Performed data analysis: Veale, Bajaria, Al-Moubarak, Mathie.
Wrote or contributed to the writing of the manuscript: Mathie, Veale, Tucker, Stevens.

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