Biochemical and Biophysical Research Communications 520 (2019) 35–40

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pranlukast is a novel small molecule activator of the two-pore domain potassium channel TREK2

Paul D. Wright [a, *], David McCoull [a], Yvonne Walsh [b], Jonathan M. Large [a], Barbara W. Hadrys [a], Egle Gaurilcikaite [a], Lewis Byrom [a], Emma L. Veale [b], Jeff Jerman [a], Alistair Mathie [b]

[a] LifeArc, Accelerator Building, Open Innovation Campus, Stevenage, SG1 2FX, UK
[b] Medway School of Pharmacy, University of Greenwich and University of Kent, Anson Building, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK

ARTICLE INFO

Article history:
Received 6 September 2019
Accepted 21 September 2019
Available online 26 September 2019

Keywords:
TREK2
KCNK10
K2P
Activator
Pranlukast
Pain

ABSTRACT

TREK2 (KCNK10, K_{2P}10.1) is a two-pore domain potassium (K2P) channel and a potential target for the treatment of pain. Like the majority of the K2P superfamily, there is currently a lack of useful pharmacological tools to study TREK2. Here we present a strategy for identifying novel TREK2 activators. A cell-based thallium flux assay was developed and used to screen a library of drug-like molecules, from which we identified the CysLT1 antagonist Pranlukast as a novel activator of TREK2. This compound was selective for TREK2 versus TREK1 and showed no activity at TRAAK. Pranlukast was also screened against other members of the K2P superfamily. Several close analogues of Pranlukast and other CysLT1 antagonists were also tested for their ability to activate K2P channels. Consistent with previous work, structure activity relationships showed that subtle structural changes to these analogues completely attenuated the activation of TREK2, whereas for TREK1, analogues moved from activators to inhibitors. Pranlukast's activity was also confirmed using whole-cell patch clamp electrophysiology. Studies using mutant forms of TREK2 suggest Pranlukast does not bind in the K2P modulator pocket or the BL-1249 binding site. Pranlukast therefore represents a novel tool by which to study the mechanism of TREK2 activation.

1. Introduction

The KCNK gene family of two-pore domain potassium (K2P) channels contribute to background (or 'leak') potassium current, helping to establish and maintain resting membrane potential. The KCNK superfamily comprises 15 channels, which are further subdivided into six subfamilies: TREK, TASK, TALK, THIK, TWIK and TRESK [1]. Each K2P subunit contains two pore-forming loops which come together to form an active dimer. Functional and genetic evidence has implicated K2Ps in a diverse set of pathophysiologies [2]. Furthermore, K2P channels have been shown to be expressed in sensory neurons, making them potential targets for the treatment of pain by using small molecules to increase channel activity and reduce neuronal excitability [3].

The TREK subfamily (TWIK related K+ channel) comprises 3 members; TREK1 (KCNK2, K_{2P}2.1), TREK2 (KCNK10, K_{2P}10.1) and TRAAK (KCNK4, K_{2P}4.1). TREK2 and TREK1 share 79% sequence homology with each other, whereas TRAAK exhibits around 69% homology with the other TREK channels and exhibits different characteristics [4]. It has been shown in heterologous expressions systems that these channels can also form heterodimers with each other [4]. TREK channels are mechanosensitive [5] and are regulated by both temperature and pH [6]. All members of the TREK subfamily have also been shown to be expressed in rodent and human dorsal root ganglion (DRG) cells [7,8] and, combined with their potential role modulating neuronal excitability, have therefore been suggested as novel targets for the treatment of pain [3].

TREK2 has been shown to be selectively expressed on rat IB4+ C-nociceptors [9]. siRNA knockdown suggests that TREK2 is responsible for a −10mV hyperpolarization in these cells. In both small and medium sized rodent DRG neurons TREK2 was also shown to likely contribute most (69%) to the resting K^+ current [10]. This was shown to be greater than TRESK, TREK1 and TRAAK, despite the suggestion that TREK2 is expressed at lower levels in the DRG than TRESK and TRAAK [11]. Importantly, after CFA-induced inflammation, spontaneous foot lifting, a measure of

* Corresponding author.
E-mail address: Paul.wright@lifearc.org (P.D. Wright).

https://doi.org/10.1016/j.bbrc.2019.09.093

spontaneous pain, was increased in animals where TREK2 had been lowered by siRNA and in animals with naturally lower levels of TREK2 [9]. In chronic constriction injury models of neuropathic pain, an increase in TREK2 mRNA was also observed compared to non-injured animals [12].

There is overall a lack of specific pharmacological tools to probe endogenous K2P channel function and TREK2 is equally underserved. Several TREK1 activators show activity at TREK2 including arachidonic acid, docosahexaenoic and linoleic acids, lysophosphatidylcholine [13], Flufenamic acid (FFA) [14], BL-1249 [15,16], ML67-33 [17], ML335 and ML402 [18] and GI-530159 [19]. Screening of a bioactive lipid library identified 11-deoxy prostaglandin F2α as an activator of TREK2 but an inhibitor of TREK1 [20]. This compound was also shown to reduce Ca^{2+} influx in C-fiber primary DRG cells [20].

Here we describe the development of a thallium based TREK2 assay to facilitate the discovery of channel activators, which led to the discovery of Pranlukast, a leukotriene receptor antagonist, as a relatively selective TREK2 activator. Pranlukast's activity was confirmed in whole-cell patch clamp electrophysiology and a subset of Pranlukast analogues were also screened for activity on K2P channels.

2. Materials and methods

2.1. Thallium flux assays

Cells transiently expressing TREK2 (NP_612191, NM_138318.2) were generated by using U-2 OS cells and TREK2 BacMam (2% v/v) solution (SB Drug Discovery, UK). For methods detailing thallium flux assays see Ref. [21]. Channel activity was defined as the increase in fluorescence over baseline between 13 and 19 s, after thallium addition. For selectivity assays also see Ref. [21].

2.2. Whole-cell patch clamp electrophysiology

Most of the methods used here have been described previously [15,22]. pcDNA3.1 was cloned with the gene of interest. To generate N-terminally truncated mutants a deletion mutagenesis strategy was utilized. All constructs, along with a green fluorescent protein (GFP) expressing vector, were transiently transfected into tsA201 cells using the calcium phosphate method and used for experimentation the next day. Currents were recorded using whole-cell patch clamp in the voltage clamp configuration in an external solution containing (in mM): 145 NaCl, 2.5 KCl, 3 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES (pH 7.4 using NaOH) and internal solution containing (in mM): 150 KCl, 3 $MgCl_2$, 5 EGTA, and 10 HEPES (pH 7.4 using KOH). External solutions and compounds were superfused at a rate of 4–5 mL min^{-1}. Complete exchange of solution occurred within 100–120 s. Patch clamp glass pipettes were pulled from thin walled borosilicate glass (GC150TF; Harvard Apparatus, Edenbridge, UK) and the resistance was measured between 3 and 6 MΩ.

K2P currents were studied using a step-ramp voltage protocol starting at a holding potential of −60 mV . The cells were then hyperpolarised from −60 mV to −80 mV for 100 ms , held at −40 mV for 500 ms, and then stepped down to −120 mV for 100 ms before beginning the voltage ramp to +20 mV for 500 ms. After the ramp, the cells were held at −80 mV for 100 ms before resuming to −60 mV holding potential. The protocol lasts for 1.5 s , including sampling at the holding potential and repeated once every 5 s. For analysis of outward current, we measured the current between −40 mV and −80 mV steps. Currents were recorded using Axopatch 200 (Molecular Devices, Sunnyvale, CA, USA) and analysed using pClamp 10.2 software (Molecular Devices).

2.3. Compounds

Pranlukast was purchased from Sigma. Cinalukast, Montelukast, MK-571, FPL-55712, SR-2640 purchased from Tocris. LY-171883 purchased from Cayman Chemical. Zafirlukast purchased from Selleck Chemicals. LA-TREK2-1, LA-TREK2-2 and LA-TREK2-3 were synthesised at LifeArc. All compounds were dissolved in 100% DMSO.

2.4. Statistics and analysis

For thallium flux assays data are mean ± standard deviation ($n \geq 3$ independent experiments) unless stated. Compound response curves were iteratively fitted to a four-parameter logistic model using Graphpad Prism v7.01 (Graphpad, USA). For electrophysiological experiments, data are expressed as mean values with a range of 95% Confidence Intervals. Statistical comparisons were made using paired or unpaired t tests in Graphpad Prism 6.0. Absolute P values are given where appropriate and differences were considered significant at the $P \leq 0.05$ level.

3. Results

3.1. Development of a thallium-based assay to identify activators of TREK2

Given the role of TREK2 in defining excitability within the DRG, it is likely that an activator of TREK2 would be therapeutically beneficial for the treatment of pain. We therefore sought to develop a cell-based assay to identify small molecules which selectively activate TREK2. Fluorescence-based thallium screens offer a way to measure the activity of potassium channels in a high-throughput manner. We have previously described the use of BacMam and the importance of channel expression level in the identification of activators [21,23] and successfully used this approach to identify novel activators of TRESK – Cloxyquin [23] and TASK3 – Terbinafine [21].

Initially we sought to define the transduction parameters to enable a system suitable for screening. Increasing amounts of BacMam were added to U-2 OS cells and upon the addition of thallium, the rate of increase in cellular fluorescence then used as a surrogate measure of channel activity. Fig. 1A confirms that increased BacMam correlated with increased channel function. Additional parameters including dye loading conditions, thallium concentration and cell number were also analysed to ensure maximal assay performance (data not shown). To confirm the signal was TREK2 mediated, we investigated the pharmacology of published [15,19,20] TREK2 modulators: BL-1249 ($pEC50 = 6.0$ (±0.3)), GI-530159 ($pEC50 = 5.5$ (±0.3)) and 11-deoxy prostaglandin F2α ($pEC50 = 5.3$ (±0.2)) were all confirmed as TREK2 activators. In line with previous data [24] phorbol-12-myristate-13-acetate (PMA) showed robust inhibition of the TREK2 signal whilst TPA had no effect on channel activity (Fig. 1B). For the purpose of potency determinations curve fitting maxima asymptotes were constrained for 11-deoxy prostaglandin F2α and GI-530159 as appropriate.

A library of approximately 1000 structurally diverse small molecule compounds was then screened to identify novel activators of TREK2. Activity was defined relative to the PMA inhibited wells (0%) and DMSO/vehicle containing wells (100%) and a small number of compounds were identified that increased the rate of fluorescence >3 standards deviations above the control response. Putative hits were re-screened against non-transduced cells to remove assay interferers (compounds which increase fluorescence signal independent of TREK2). Pranlukast, a cysteinyl leukotriene

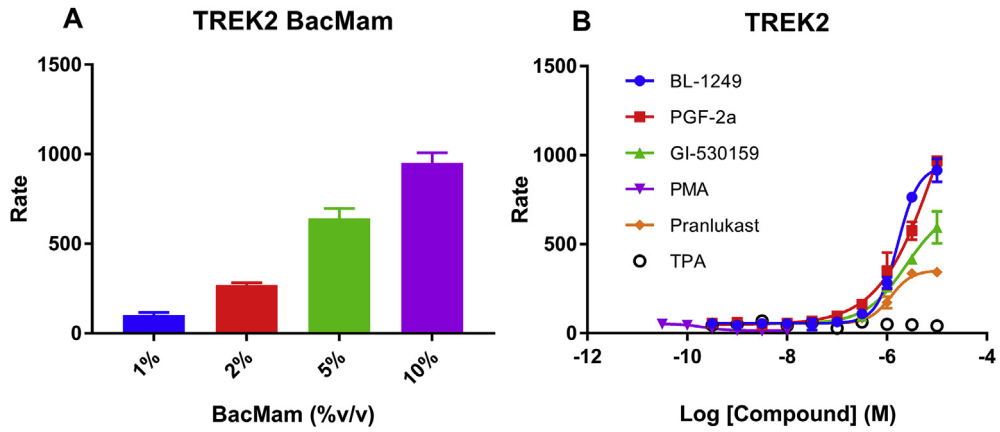

Fig. 1. Development of a thallium flux assay to measure TREK2 activity. (A) Titration of BacMam. As BacMam levels increase (%v/v present in media), there is an increase in the rate of fluorescence after the addition of thallium. This is proportional to increased TREK2 activity in the assay. (B) Pharmacology of TREK2 activators. Exemplar data showing activity of BL-1249, 11-deoxy prostaglandin F2α (PGF-2a), GI-530159, PMA, Pranlukast and TPA. All data show rate of fluorescence increases between 13 and 19 s, error bars denote S.E.M, n ≥ 2.

receptor-1 antagonist, was identified as a robust activator of TREK2 with a pEC50 of 5.8 (±0.1), Fig. 1B. The magnitude of the Pranlukast response was consistently lower than that of the putative TREK1 opener, BL-1249. The maximal rate of fluorescence increase was 54 (±9) % of that observed with BL-1249. Our thallium flux assay is typically run with a 30-min pre-incubation of compound before the thallium addition. For Pranlukast we also ran our assay with no pre-incubation i.e. thallium was added immediately after Pranlukast. Using this paradigm, the magnitude of Pranlukast was nearly identical to that of BL-1249 whilst the pEC50 remained consistent.

3.2. *Selectivity and structure activity relationship studies of Pranlukast*

To investigate the selectivity of Pranlukast as an activator of TREK2 we measured its pharmacology against a range of other K2P channels. Thallium flux assays were developed to allow profiling against other members of the K2P superfamily (Table 1). No activity could be observed against TASK3, TASK2 and THIK1. The activity observed with TREK1 and TRESK highlights the difficulty in comparing relative selectivity of activating molecules. Regarding TREK1, a near identical pEC50 of 5.7 (±0.2) was observed for Pranlukast. However, in order to account for differences between each test system, e.g. how thallium flux compares to channel activity, it is perhaps better to compare the activity of Pranlukast to that of other known TREK2 activators, i.e. 'benchmarking' relative activity. As described above, both BL-1249 and GI-530159 are shown to be active at TREK1 and TREK2. BL-1249 has been described as displaying equal potency at both TREK1 and TREK2 [25]. Pranlukast is either equipotent (BL-1249) or more potent (GI-530159) than these standards at TREK2 but is markedly less potent than both at TREK1. It should also be noted that in our thallium flux assays BL-1249 is more active at TREK1 (pEC50 = 6.6 (±0.3)) than TREK2 (pEC50 = 6.0 (±0.3)). In the absence of a native ligand it is impossible to completely rationalize differences in Pranlukast activity between TREK1 and TREK2 but the data is suggestive of selectivity for TREK2 when compared to TREK1 and is in line with observations that activity of activators is not easily compared by pEC50/potency alone. At TRAAK Pranlukast displayed no significant activation (data not shown). Pranlukast showed a near identical pEC50 of 5.6 (±0.2) at TRESK, however it was significantly less potent than other TRESK activators (PMA pEC50 = 8.6 (±0.1)) and showed a lower magnitude of response. This data highlights the need to use multiple tool compounds when investigating native channels responsible for a physiological response and that rank order of potencies is important in the absence of compounds showing complete selectivity.

To investigate structure activity relationships of Pranlukast's effects at TREK2 other commercially available CysLT1 receptor antagonists were purchased and assayed (Table 1) based on the

Table 1
Activity of Pranlukast, TREK tool compounds, Pranlukast analogues and CysLT1 antagonists at multiple K2P channels. All compounds were measured using thallium flux assay and rate of fluorescence increase. Compounds were defined as inactive where no curve fit was possible. Compounds listed as pEC50 < 5.0 did not activate at 50% at the top concentration used. All compounds screened n ≥ 4 independent experiments.

	TREK1	TREK2	TRESK	TASK-3	TASK2	THIK1	NT
Pranlukast	Activator (pEC50 = 5.7 ± 0.2)	Activator (pEC50 = 5.8 ± 0.1)	Activator (pEC50 = 5.6 ± 0.2)	Activator (pEC50 < 5.0)	Inactive	Inactive	Inactive
BL-1249	Activator (pEC50 = 6.6 ± 0.3)	Activator (pEC50 = 6.0 ± 0.3)	Activator (pEC50 = 5.5 ± 0.6)	Inactive	Inactive	Inactive	Inactive
GI-530159	Activator (pEC50 = 6.7 ± 0.1)	Activator (pEC50 = 5.5 ± 0.3)	Activator (pEC50 = 5.1 ± 0.6)	Inactive	Inactive	Inactive	Inactive
PGF2α	Inhibitor	Activator (pEC50 = 5.3 ± 0.2)	Inactive	Inactive	Inactive	Inactive	Inactive
PMA	Inhibitor	Inhibitor	Activator (pEC50 = 8.6 ± 0.1)	Inactive	Inactive	Inactive	Inactive
Zafirlukast	Inhibitor	Activator (pEC50 < 5.0)	Activator (pEC50 < 5.0)	Activator (pEC50 = 5.6 ± 0.1)	ND	ND	ND
Cinalukast	Inactive	Inactive	Inactive	Activator (pEC50 < 5.0)	Inactive	Inactive	Inactive
Montelukast	Inhibitor	Inactive	Activator (pEC50 < 5.0)	Inactive	ND	ND	ND
MK-571	Inhibitor	Inactive	Inactive	Inactive	ND	ND	ND
FPL 55712	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
SR 2640	Activator (pEC50 < 5.0)	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
LY171883	Inhibitor	Inactive	Inactive	Inactive	ND	ND	ND
LA-TREK2-1	Activator (pEC50 = 5.3 ± 0.2)	Activator (pEC50 < 5.0)	Activator (pEC50 < 5.0)	Inactive	Inactive	Inactive	Inactive
LA-TREK2-2	Inactive	Activator (pEC50 < 5.0)	Activator (pEC50 < 5.0)	Inactive	Inactive	Inactive	Inactive
LA-TREK2-3	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

pharmacophore. All bore a carboxylic acid motif or an isostere thereof, and if activity were observed, might be expected to bind at a similar site to Pranlukast itself. A small number of close analogues of Pranlukast were also designed and synthesised to complement the commercial compounds. These were chosen to explore the shortening or removal of the lipophilic carboxamide side chain (LA-TREK2-1, LA-TREK2-2 and LA-TREK2-3), with a view to determining minimal structural requirements for activity. Structures are shown in supplementary material. None of the analogues tested showed an increase in activity at TREK2 compared to Pranlukast. Only Zafirlukast, LA-TREK2-1, and LA-TREK2-2 retained any ability to activate TREK2 and in each case at much lower potencies (pEC50 > 5). Interestingly, several analogues tested were inhibitors of TREK1. Zafirlukast was an inhibitor of TREK1 and an activator of TREK2. Montelukast, LY171883, MK-571 were inhibitors of TREK1 but inactive at TREK2. This data was in line with previous observations suggesting very small changes to structures can have profound effects on activity at K2P channels, and that compounds within a chemical series can be activators or inhibitors.

3.3. Characterisation of Pranlukast on TREK channel currents using whole-cell patch clamp electrophysiology

The effect of Pranlukast (3 μM) was investigated on WT TREK1 and TREK2 channel currents in whole-cell recordings. Exemplar traces for each are shown in Fig. 2. Fig. 2A shows the time course for activation of TREK1 by Pranlukast and Fig. 2B individual current traces in the presence and absence of Pranlukast. Fig. 2C shows the time course for activation of TREK2 by Pranlukast and Fig. 2D individual current traces in the presence and absence of the compound. The combined data for all cells is shown in Fig. 3. For TREK2, Pranlukast (3 μM) evoked a 228.6% [95% CI: 162.0–295.2, n = 30] enhancement of current. The size of the current was significantly larger in the presence than absence of Pranlukast ($p < 0.0001$, paired *t*-test, Fig. 3). For TREK1, a 66.4% [95% CI: 20.7–112.1, n = 17] enhancement of current was observed with Pranlukast, which was again significant ($p = 0.0093$, paired *t*-test, Fig. 3). Importantly, the degree of enhancement of TREK2 current by Pranlukast was significantly larger than that seen for TREK1 current ($p = 0.0011$, *t*-test). In agreement with the thallium flux data, this is highly suggestive that Pranlukast shows a selective activation for TREK2 compared to TREK1.

Alternative translation initiation (ATI) gives rise to more than one form of both TREK1 and TREK2 [26,27]. For TREK1 there are two ATI-isoforms. The shorter form lacking 41 amino acids of the N terminus [28]. This shorter form of TREK1 has different properties compared to the longer form, with a smaller current density, decreased potassium (K) selectivity and increased sensitivity to some activators including FFA and BL-1249 [26,27]. In this study, the current density for the short form of TREK1 was 2.7 pA/pF [95% CI: 1.8–3.6, n = 10] compared to 32.8 pA/pF [95% CI: 25.4–40.1, n = 26] for the longer form of TREK1. Activation of the short form of TREK1 by Pranlukast was just 17.6% [95% CI: −24.4–59.7, n = 7] and the current size was not significantly different in the presence compared to the absence of Pranlukast ($p = 0.387$, paired *t*-test, Fig. 3). For TREK2, there are three isoforms [27] with variable

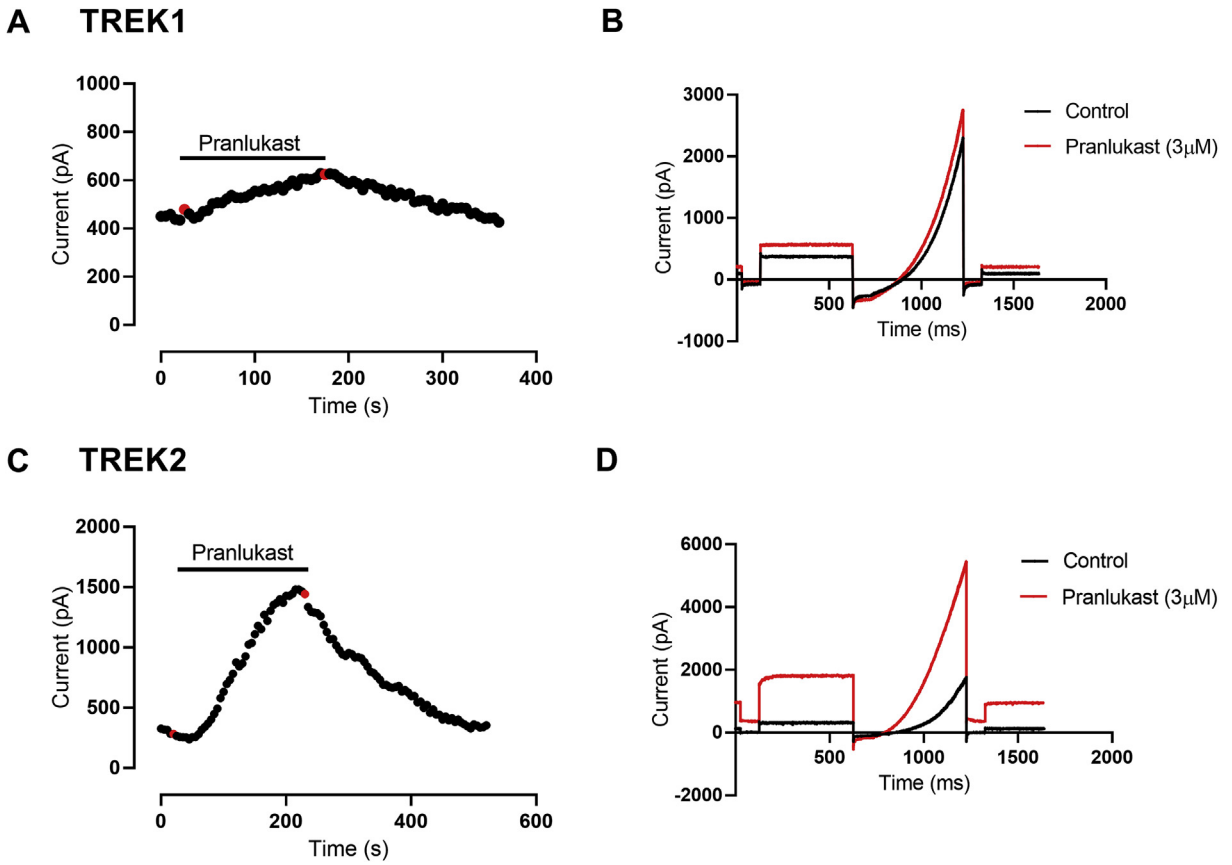

Fig. 2. A) Time course of enhancement of TREK1 wildtype (WT) current by Pranlukast (3 μM). (B) Currents recorded through TREK1 WT channels in control solution (black) and Pranlukast (3 μM) (red). (C) Time course of enhancement of TREK2 WT current by Pranlukast (3 μM). D) Currents recorded through TREK2 WT channels in (black) and Pranlukast (3 μM) (red). Currents were evoked by the step-ramp voltage protocol, as detailed in the Methods. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

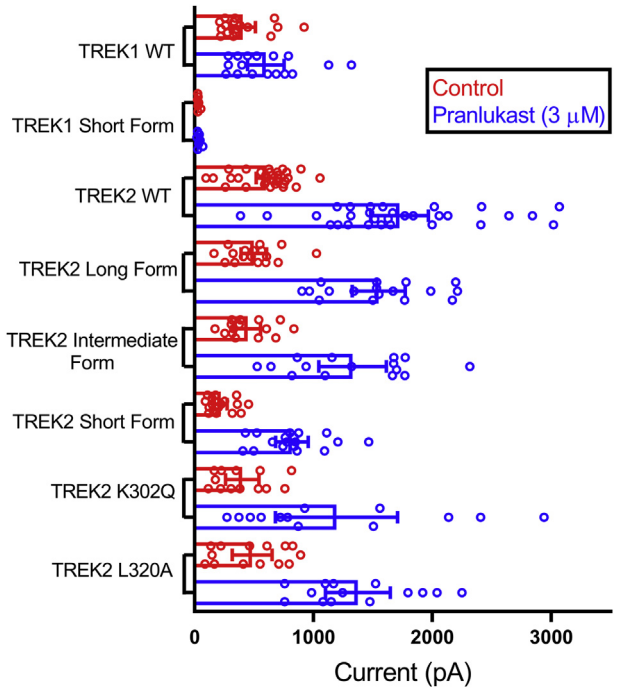

Fig. 3. Effect of Pranlukast (3 μM) on outward current (pA) measured as the difference between at −40 and −80 mV for TREK1 WT, TREK2 WT and variants of TREK1 and TREK2. Individual cell currents are shown before (red symbols) and after (blue symbols) the application of Pranlukast. Mean data is shown by the box, and the error bars represent 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

lengths of N terminus. Although the ion selectivity of these forms is not altered (unlike TREK1), they do show a differential single channel conductance profile and altered sensitivity to the inhibitory compound carvedilol [27,29]. The current density for the short form of TREK2 was 21.6 pA/pF [95% CI: 16.5–26.7, n = 27] compared to 41.6 pA/pF [95% CI: 31.7–51.5, n = 23] for the intermediate form and 49.3 pA/pF [95% CI: 36.7–61.2, n = 23] for the full-length form of TREK2. Activation of the short form of TREK2 by Pranlukast was 338.4% [95% CI: 240.9–435.9, n = 18], the intermediate form was 217.8% [95% CI: 160.0–275.6, n = 16] and the long form was 277.5% [95% CI: 159.7–395.3, n = 17]. For each isoform, the size of current was significantly larger in the presence than absence of Pranlukast ($p < 0.0001$ in each case, paired t tests, Fig. 3) and the degree of enhancement was similar to WT TREK2 (Fig. 3).

From the crystal structure of TREK2 bound to the inhibitory compound norfluoxetine, a number of amino acids (AA) were identified as contact points for this compound including Leucine (L) 320 in the TM4 region [16]. The same AA on TREK1 has recently been shown to contribute to the binding site for the activator BL-1249 [30] and mutation of this AA has been shown to reduce the effectiveness of both compounds. We therefore mutated this AA (L320A) on TREK2 to determine, whether it contributed to the effectiveness of Pranlukast. Activation of TREK2_L320A by Pranlukast was 350.4% [95% CI: 129.4–571.5, n = 14], similar to that seen for WT TREK2 channels. The size of current was significantly larger in the presence than absence of Pranlukast ($p < 0.0001$, paired *t*-test, Fig. 3).

Two other pharmacological activators of TREK channels, ML335 and ML402 have been shown to act as molecular wedges to stabilise the channel in an open conformation by binding to a novel cryptic binding site [18]. Mutation of a lysine residue in TREK1, adjacent to the selectivity filter of the channel, has been shown to abolish the effect of these compounds. We mutated the equivalent residue in TREK2 (K302Q) to determine whether activation by Pranlukast was occurring due to a similar mechanism. Activation of TREK2_K302Q by Pranlukast was 257.3% [95% CI: 75.0–439.6, n = 13], again similar to that seen for WT TREK2. The size of current was significantly larger in the presence than absence of Pranlukast ($p = 0.0037$, paired *t*-test, Fig. 3).

This work confirms that Pranlukast is an activator of TREK2 and is suggestive it does not bind at either of the two described binding sites. It is interesting to note that a subset of Pranlukast analogues that are activators of TREK2 are inhibitors of TREK1. This is similar to the mode of action seen with 11-deoxy prostaglandin F2α, which is suggested to act in a similar way to extracellular protons [20]. Whilst it is clear further work using the known variants of TREK2 will be required to identify a binding modality of Pranlukast, it provides an interesting tool for further defining the different modes of activation described for TREK2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.09.093.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.09.093.

References

[1] E. Honore, The neuronal background K2P channels: focus on TREK1, Nat. Rev. Neurosci. 8 (2007) 251–261.
[2] V. Renigunta, G. Schlichthorl, J. Daut, Much more than a leak: structure and function of K(2)p-channels, Pflueg. Arch. Eur. J. Physiol. 467 (2015) 867–894.
[3] C. Tsantoulas, S.B. McMahon, Opening paths to novel analgesics: the role of potassium channels in chronic pain, Trends Neurosci. 37 (2014) 146–158.
[4] M. Lengyel, G. Czirjak, P. Enyedi, Formation of functional heterodimers by TREK-1 and TREK-2 two-pore domain potassium channel subunits, J. Biol. Chem. 291 (2016) 13649–13661.
[5] F. Maingret, A.J. Patel, F. Lesage, et al., Mechano- or acid stimulation, two interactive modes of activation of the TREK-1 potassium channel, J. Biol. Chem. 274 (1999) 26691–26696.
[6] F. Maingret, I. Lauritzen, A.J. Patel, et al., TREK-1 is a heat-activated background K(+) channel, EMBO J. 19 (2000) 2483–2491.
[7] E.M. Talley, G. Solorzano, Q. Lei, et al., Cns distribution of members of the two-pore-domain (KCNK) potassium channel family, J. Neurosci. 21 (2001) 7491–7505.
[8] A.D. Medhurst, G. Rennie, C.G. Chapman, et al., Distribution analysis of human two pore domain potassium channels in tissues of the central nervous system and periphery, Mol. Brain Res. 86 (2001) 101–114.
[9] C. Acosta, L. Djouhri, R. Watkins, et al., TREK2 expressed selectively in IB4-binding C-fiber nociceptors hyperpolarizes their membrane potentials and limits spontaneous pain, J. Neurosci. 34 (2014) 1494–1509.
[10] D. Kang, D. Kim, TREK-2 (K2P10.1) and TRESK (K2P18.1) are major background K+ channels in dorsal root ganglion neurons, Am. J. Physiol. Cell Physiol. 291 (2006) C138–C146.
[11] B. Marsh, C. Acosta, L. Djouhri, et al., Leak K(+) channel mRNAs in dorsal root ganglia: relation to inflammation and spontaneous pain behaviour, Mol. Cell. Neurosci. 49 (2012) 375–386.
[12] H.J. Han, S.W. Lee, G.T. Kim, et al., Enhanced expression of TREK-1 is related with chronic constriction injury of neuropathic pain mouse model in dorsal root ganglion, Biomol. Ther. (Seoul) 24 (2016) 252–259.
[13] F. Lesage, C. Terrenoire, G. Romey, et al., Human TREK2, a 2P domain mechano-sensitive K+ channel with multiple regulations by polyunsaturated fatty acids, lysophospholipids, and Gs, Gi, and Gq protein-coupled receptors, J. Biol. Chem. 275 (2000) 28398–28405.
[14] M. Takahira, M. Sakurai, N. Sakurada, et al., Fenamates and diltiazem modulate lipid-sensitive mechano-gated 2P domain K(+) channels, Pflueg. Arch. Eur. J. Physiol. 451 (2005) 474–478.
[15] E.L. Veale, E. Al-Moubarak, N. Bajaria, et al., Influence of the N terminus on the biophysical properties and pharmacology of TREK1 potassium channels, Mol. Pharmacol. 85 (2014) 671–681.
[16] Y.Y. Dong, A.C. Pike, A. Mackenzie at al, K2P channel gating mechanisms revealed by structures of TREK-2 and a complex with Prozac, Science 347 (2015) 1256–1259.
[17] S.N. Bagriantsev, K.H. Ang, A. Gallardo-Godoy, et al., A high-throughput functional screen identifies small molecule regulators of temperature- and

mechano-sensitive K2P channels, ACS Chem. Biol. 8 (2013) 1841–1851.
[18] M. Lolicato, C. Arrigoni, T. Mori, et al., K2P2.1 (TREK-1)-activator complexes reveal a cryptic selectivity filter binding site, Nature 547 (2017) 364–368.
[19] A.J.C. Loucif, P.P. Saintot, J. Liu, et al., GI-530159, a novel, selective, mechanosensitive two-pore-domain potassium (K2P) channel opener, reduces rat dorsal root ganglion neuron excitability, Br. J. Pharmacol. 175 (2018) 2272–2283.
[20] P.K. Dadi, N.C. Vierra, E. Days, et al., Selective small molecule activators of TREK-2 channels stimulate dorsal root ganglion c-fiber nociceptor two-pore-domain potassium channel currents and limit calcium influx, ACS Chem. Neurosci. 8 (2017) 558–568.
[21] P.D. Wright, E.L. Veale, D. McCoull, et al., Terbinafine is a novel and selective activator of the two-pore domain potassium channel TASK3, Biochem. Biophys. Res. Commun. 493 (2017) 444–450.
[22] E.L. Veale, A. Mathie, Aristolochic acid, a plant extract used in the treatment of pain and linked to Balkan endemic nephropathy, is a regulator of K2P channels, Br. J. Pharmacol. 173 (2016) 1639–1652.
[23] P.D. Wright, G. Weir, J. Cartland, et al., Cloxyquin (5-chloroquinolin-8-ol) is an activator of the two-pore domain potassium channel TRESK, Biochem. Biophys. Res. Commun. 441 (2013) 463–468.
[24] W. Gu, G. Schlichthorl, J.R. Hirsch, et al., Expression pattern and functional characteristics of two novel splice variants of the two-pore-domain potassium channel TREK-2, J Physiol. 539 (2002) 657–668.
[25] L. Pope, C. Arrigoni, H. Lou, et al., Protein and chemical determinants of BL-1249 action and selectivity for K2P channels, ACS Chem. Neurosci. 9 (2018) 3153–3165.
[26] D. Thomas, L.D. Plant, C.M. Wilkens, et al., Alternative translation initiation in rat brain yields K2P2.1 potassium channels permeable to sodium, Neuron 58 (2008) 859–870.
[27] D. Simkin, E.J. Cavanaugh, D. Kim, Control of the single channel conductance of K2P10.1 (TREK-2) by the amino-terminus: role of alternative translation initiation, J Physiol. 586 (2008) 5651–5663.
[28] E.L. Veale, K.A. Rees, A. Mathie, et al., Dominant negative effects of a non-conducting TREK1 splice variant expressed in brain, J. Biol. Chem. 285 (2010) 29295–29304.
[29] J. Kisselbach, C. Seyler, P.A. Schweizer, et al., Modulation of K2P 2.1 and K2P 10.1 K(+) channel sensitivity to carvedilol by alternative mRNA translation initiation, Br. J. Pharmacol. 171 (2014) 5182–5194.
[30] M. Schewe, H. Sun, U. Mert, et al., A pharmacological master key mechanism that unlocks the selectivity filter gate in K(+) channels, Science 363 (2019) 875–880.