



Kent Academic Repository

McCoull, D., Veale, Emma L., Walsh, Yvonne, Byrom, L., Avkiran, T., Large, J.M., Vaitone, E., Gaffey, F., Jerman, J., Mathie, Alistair and others (2021) *Aprepitant is a novel, selective activator of the K2P channel TRAAK*. *Biochemical and Biophysical Research Communications*, 588 . pp. 41-46. ISSN 0006-291X.

Downloaded from

<https://kar.kent.ac.uk/92477/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1016/j.bbrc.2021.12.031>

This document version

Publisher pdf

DOI for this version

Licence for this version

CC BY (Attribution)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal*, Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).



Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Aprepitant is a novel, selective activator of the K2P channel TRAAK

D. McCoull^{a,*}, E.L. Veale^b, Y. Walsh^b, L. Byrom^a, T. Avkiran^a, J.M. Large^a, E. Vaitone^a, F. Gaffey^a, J. Jerman^a, A. Mathie^{b,c}, P.D. Wright^a^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^c School of Engineering, Arts, Science and Technology, University of Suffolk, Ipswich, IP4 1QJ, UK

ARTICLE INFO

Article history:

Received 9 December 2021

Accepted 12 December 2021

Available online 14 December 2021

Keywords:

K2P

TRAAK

KCNK4

Aprepitant

Pain

Screening

ABSTRACT

TRAAK (KCNK4, K2P4.1) is a mechanosensitive two-pore domain potassium (K2P) channel. Due to its expression within sensory neurons and genetic link to neuropathic pain it represents a promising potential target for novel analgesics. In common with many other channels in the wider K2P sub-family, there remains a paucity of small molecule pharmacological tools. Specifically, there is a lack of molecules selective for TRAAK over the other members of the TREK subfamily of K2P channels. We developed a thallium flux assay to allow high throughput screening of compounds and facilitate the identification of novel TRAAK activators. Using a library of ~1200 drug like molecules we identified Aprepitant as a small molecule activator of TRAAK. Aprepitant is an NK-1 antagonist used to treat nausea and vomiting. Close structural analogues of Aprepitant and a range of NK-1 antagonists were also selected or designed for purchase or brief chemical synthesis and screened for their ability to activate TRAAK. Electrophysiology experiments confirmed that Aprepitant activates both the 'long' and 'short' transcript variants of TRAAK. We also demonstrated that Aprepitant is selective and does not activate other members of the K2P superfamily. This work describes the development of a high throughput assay to identify potential TRAAK activators and subsequent identification and confirmation of the novel TRAAK activator Aprepitant. This discovery identifies a useful tool compound which can be used to further probe the function of TRAAK K2P channels.

© 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

There continues to be significant interest in examining and modulating the function of the 15 members of the two-pore domain potassium channel family (K2Ps) [1]. This has stemmed from a growing body of genetic and functional studies linking their known effects on establishing and maintaining resting membrane potential to pathophysiological conditions [2]. Concomitantly, a greater recent understanding of the structural characteristics [3] and expression profiles [4] of these channels is also driving interest in their potential as therapeutic targets, in particular with respect to pain-related phenotypes.

Among the three members of the TREK sub-family, the TRAAK channel (encoded by the KCN4 gene, also referred to as K_{2P}4.1) [5] is the most distinct in terms of structural homology [6], whilst

retaining some similarities in responses to certain lipids and to mechanical and thermal stimuli. It has been shown that TRAAK is expressed in dorsal root ganglia (DRG) and trigeminal ganglia (TG) neurons by measuring K2P mRNA abundance using RT-qPCR [7]. In normal rodent DRG neurons [7] it was reported that TRAAK mRNA was the second highest in abundance of the K2P family. This is in agreement with earlier in-situ hybridisation studies which showed TRAAK expression to be enriched in the DRG (human and rodent), and that TRAAK is predominantly expressed in the CNS (with little peripheral expression) [8]. TRAAK channels are highly expressed at the Nodes of Ranvier [9,10] where they are required for rapid action potential repolarisation [11].

In mouse models, it has been shown that TRAAK KO mice have increased responses to mechanical and thermal stimuli [12]. In this study, it was also shown that TRAAK and TREK-1 channels reduce excitability of heat nociceptors, by acting as a counterbalance to depolarisation [12]. Similarly, there is growing evidence linking the TRAAK channel to the pain therapeutic space. At the wider genomic level, it was observed that polymorphisms were associated with

* Corresponding author.

E-mail address: david.mccoull@lifearc.org (D. McCoull).

neuropathic pain following surgery [13]. Thus, although less well validated than TREK-1 or TREK-2, the TRAAK channel is increasingly thought to represent an attractive target for the treatment of peripheral neuropathic pain.

Activation of TRAAK by small molecules has been described, chiefly by non-selective activators of the closely related TREK-1 and TREK-2 channels. Compounds such as C3001a [14], BL-1249 [15], Flufenamic acid [16], ML67-33 [17], DCPIB [18], 2-APB [19], Arachidonic acid [5] and a group of phospholipid modulators [20] have been reported to activate the TRAAK channel at a number of distinct binding sites. Hence, despite TRAAK's potential role in neuronal excitability and therefore its potential utility as a target for novel analgesics [21], there remains a requirement for selective small molecule activators of TRAAK with which to probe its function in more detail.

Our experience in target-class screening to identify small molecule activators of K2P channels, using a thallium flux assay [22] has resulted in the discovery and characterisation of selective activators of several channels in this family [23–25]. Here we describe a recent application of this work, which has identified an activator of the TRAAK channel with selectivity against the TREK-1 and TREK-2 sub-family members. Further, we have confirmed and characterised this activity in more detail, in the context of other K2P channel selectivity and whole cell patch clamp electrophysiology experiments.

2. Materials and methods

2.1. Thallium flux assays

Cells transiently expressing TRAAK (NM_033310.2) were generated using U-2 OS cells and TRAAK Baculovirus (BacMam) (5% v/v). For methods detailing transfection and thallium flux assay protocols see Ref. [22]. The TRAAK variant used for thallium flux studies corresponds to the 'short' variant described in whole-cell patch voltage clamp experiments. Channel activity in thallium flux experiments was defined as the rate of fluorescence increase, over the pre-addition baseline, between 15 and 25 s following thallium addition. For selectivity assays also see Ref. [22].

2.2. Whole-cell patch clamp electrophysiology

The methods used in this study for tissue culture and transfection of tsA201 cells have been described previously [26]. Briefly, for electrophysiological experiments, pcDNA3.1 was cloned with the gene of interest. These vectors, along with a green fluorescent protein (GFP) expressing vector, were transiently transfected into tsA201 cells using the calcium phosphate method. The cells were used for experiments the next day. Currents were recorded from tsA201 cells using whole-cell patch-clamp in the voltage clamp configuration. Cells were placed in the recording chamber filled with an external solution which contained (in mM): 145 NaCl, 2.5 KCl, 3 MgCl₂, 1 CaCl₂, and 10 HEPES (pH 7.4 using NaOH). Patch clamp glass pipettes were pulled from thin-walled borosilicate glass with resistances between 3 and 6 MΩ. The glass pipette internal solution consisted of (in mM): 150 KCl, 3 MgCl₂, 5 EGTA, and 10 HEPES (pH 7.4 using KOH) [24].

K2P currents were studied using a step-ramp voltage protocol [25] from a holding potential of –60 mV. Each sweep of the protocol lasted for 1.5 s, including sampling at the holding potential and sweeps were repeated once every 5 s. For analysis of outward current, we measured the difference current from steps between –40 mV and –80 mV. Currents were recorded using an Axoclamp 1D (Molecular Devices, Sunnyvale, CA, USA) amplifier and stored and analysed using pClamp 10.2 software (Molecular

Devices).

2.3. Compounds

Aprepitant was purchased from Tocris BioScience (United Kingdom). Fosaprepitant was purchased from BioTechne (United Kingdom). ent-Aprepitant, desfluoro Aprepitant, Aprepitant-M2 and Aprepitant-M3 were purchased from Carbosynth Ltd (United Kingdom). GR 203040, CP 99994, CP 122721, L-733,060, L-732,138 and L-760,735 were all purchased from BioTechne (United Kingdom). LA-TRAAK-1 to LA-TRAAK-6 were synthesised by adapting previously published synthetic procedures, as referenced in the main text. The TASK-3 inhibitor (compound 3 in Ref. [27]), was purchased from AKos (Germany).

3. Results

3.1. Identification of novel TRAAK activators

To address the lack of pharmacological tools to investigate this channel, we sought to develop a cell-based assay that would enable us to identify small molecule activators of TRAAK. Using the approach previously described in Ref. [22] a fluorescence-based thallium flux screen was developed. The key requirement in the development of these assays is to identify conditions which facilitate the identification of activators, that is, to confirm an appropriate level of channel (TRAAK) expression. To do this, cells were initially transduced with differing levels of human TRAAK BacMam. Fig. 1A shows that as the level of BacMam added (% v/v) increased, so did the rate of fluorescence increase within cells upon the addition of thallium. This was taken as confirming we observed an increase in cellular levels of TRAAK channel expression. A BacMam concentration of 5% was chosen as this was not the maximum, and thus facilitated the identification of activators. In other words, the expression level was appropriate to allow sufficient dynamic range to measure an increase in signal [22]. It should also be noted that, for all concentrations of BacMam tested, the magnitude of the TRAAK signal observed in the thallium flux assay was low compared to other members of the TREK family [25]; this is perhaps reflective of the native TRAAK biology and the channel's open probability [28].

Using knowledge gained from previous screens, we were able to use our 'target-class' [22] approach to rapidly adapt conditions to generate a high throughput assay. This involved building on previous observations of K2P screens and using existing in-house automation methods and liquid handling techniques. A library of ~1200 drug-like molecules was screened which led to the identification of a robust and novel activator – Aprepitant (Fig. 1B and see Supplementary Material for structure). This compound is used clinically to prevent chemotherapy-induced nausea and vomiting (CINV) [29,30]. A second activator from this library was also identified, Fosaprepitant – a pro-drug of Aprepitant and a highly similar structure. Both these molecules act as antagonists at the G-Protein coupled receptor NK-1, a member of the Tachykinin receptor family. Neither compound showed an increase in fluorescence in non-transduced U-2 OS cells (data not shown).

We then compared the activity of Aprepitant to other tool compounds which have previously been demonstrated to show activity at TRAAK or are known activators of the closely related TREK-1 or TREK-2 channels - BL-1249, Prostaglandin F_{2α}, GI-530159, Phorbol-2-myristate, TPA, ML-402 and ML-67-33 (Fig. 1C). Of these only G-531059, at high concentrations, showed a robust activation of TRAAK. EC₅₀ determinations were also difficult due to the lack of defined maximal asymptote. Interestingly, GI-530159 has previously been shown to not possess TRAAK activator

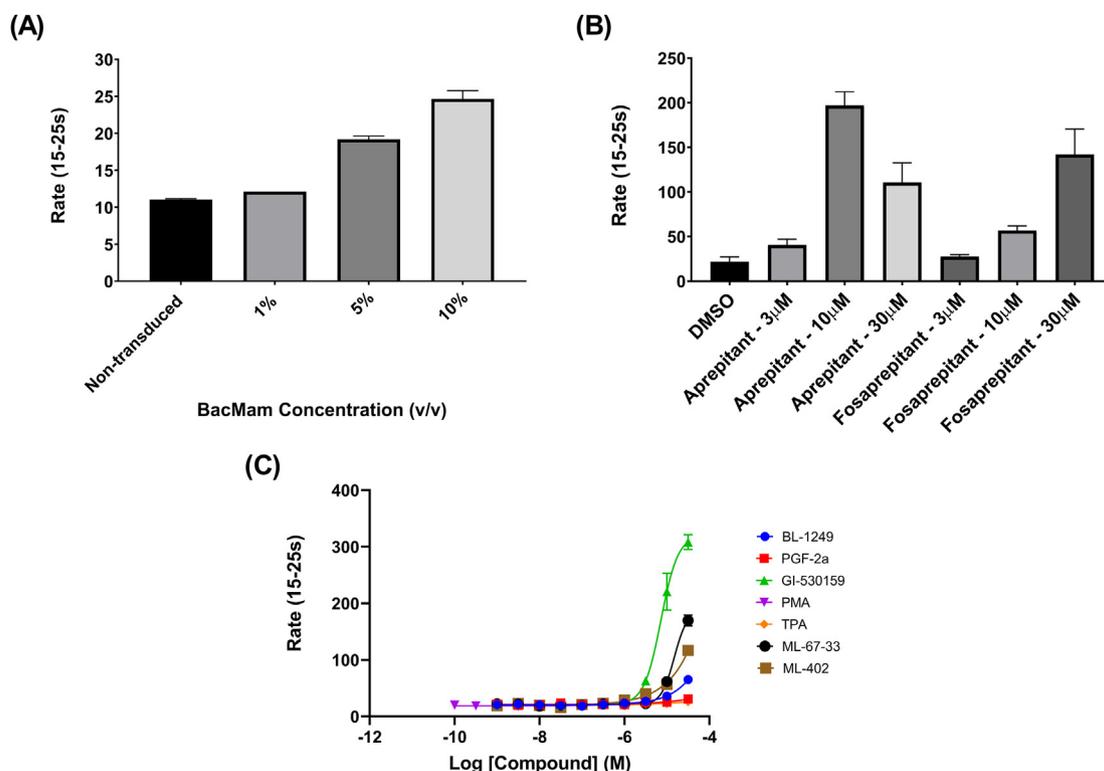


Fig. 1. Using thallium flux to identify activators of TRAAK. (A) Titration of TRAAK BacMam. As BacMam levels increase (% v/v) there is an increase in the rate of cellular fluorescence after the addition of thallium. This is representative of increased TRAAK channel activity. (B) TRAAK activity is increased after addition of Aprepitant or Fosaprepitant. Bar graphs display average rate of fluorescence increase after the addition of thallium in the presence of DMSO, Aprepitant and Fosaprepitant ($n \geq 3$, \pm standard deviation). (C) Pharmacology of TREK family activators using thallium flux to measure activation of TRAAK. Data shows compound response curves of BL-1249, 11-deoxy prostaglandin F₂ α (PGF-2 α), GI-530159, phorbol-2-myristate (PMA), tetrapentylammonium (TPA), ML-402 and ML-67-33. All data show rate of fluorescence increase between 15 and 25 s $n \geq 3$, \pm standard deviation.

activity [31]. This difference may be linked to the use of a different cellular background between the studies or to our use of thallium flux and not electrophysiology to study GI-530159's activity. In addition, given that we only saw robust activation at concentrations greater than or equal to 10 μ M and the authors of [31] used a maximum of 10 μ M, this observation may be explained by subtle shifts in potency due to assay conditions, the isoform of TRAAK channel studied, liquid handling techniques or a combination of these differences.

3.2. Exploring compound SAR

As described, Aprepitant (S1, see supplementary material for chemical structures of all compounds S1 – S18) is an orally active NK-1 receptor antagonist, given to alleviate post-operative and chemotherapy induced emesis [29,30]. The more soluble pro-drug Fosaprepitant (S2) is an effective intravenous alternative. We first purchased and screened commercially available close analogues and metabolites of Aprepitant (S3 – S6, supplementary material) among which we found additional active compounds in the form of ent-Aprepitant (S3), desfluoro Aprepitant (S4) and the metabolite Aprepitant M3 (S6), although (S6) showed a lower but reproducible activation (Table 1). These data again highlighted 'tight' SAR space which has been previously associated with activators of K2P channels of different chemotypes and binding sites. We also purchased a small number of additional NK-1 antagonists (S7 – S12), to probe for any link to activity around the well-described NK-1 pharmacophore. Many are structurally related to Aprepitant, by virtue of their saturated 6-membered ring core structures which allow other chemical substituents to occupy similar positions in 3D

Table 1

Activity of Aprepitant analogues and NK-1 antagonists. Activity defined as maximum % activity observed relative to 30 μ M GI-530159. For all data activity is shown at 10 μ M, except for Fosaprepitant, L-732,138 and L-760,735 for which activity is shown at 30 μ M. L-760,735 also showed an effect on non-transduced cells. Compounds which showed less than 20% activity of 30 μ M GI-530159 were defined as inactive. Data is $n \geq 3$, \pm standard deviation. For structures of compounds please see Supplementary Material.

Compound name and number	Activity at TRAAK
Aprepitant (S1)	52.4 \pm 5.9
Fosaprepitant (S2)	53.0 \pm 6.6
ent-Aprepitant (S3)	56.3 \pm 7.9
desfluoro Aprepitant (S4)	54.2 \pm 5.1
Aprepitant M2 (S5)	INACTIVE
Aprepitant M3 (S6)	22.9 \pm 1.2
GR 203040 (S7)	INACTIVE
CP 99994 (S8)	INACTIVE
CP 122721 (S9)	INACTIVE
L-733,060 (S10)	INACTIVE
L-732,138 (S11)	21.7 \pm 1.5
L-760,735 (S12)	30.3 \pm 2.3
LA-TRAAK-1 (S13)	INACTIVE
LA-TRAAK-2 (S14)	INACTIVE
LA-TRAAK-3 (S15)	INACTIVE
LA-TRAAK-4 (S16)	INACTIVE
LA-TRAAK-5 (S17)	INACTIVE
LA-TRAAK-6 (S18)	INACTIVE

space as those on the Aprepitant molecule. Where the core structure is non-cyclic (as in S11), the positions of the substituents still hold broadly to this pharmacophore. When S7 – S12 were screened using the thallium flux assay, only two compounds (S11 and S12) showed activity at TRAAK, again at lower but reproducible levels.

These two compounds were deemed to be the most closely related structures to Aprepitant itself, so would perhaps be more likely to maintain some activity. A small number of close analogues of Aprepitant (S13 – S18) were also selected and synthesised by modifying previous procedures [32]; this was with a view to assessing possible divergence from known NK-1 structure activity relationships. These analogues were chosen to probe the impact of differing stereochemistry at the key chiral centres or the alteration of the morpholine N-substituent, to attempt to locate further active analogues of this chemotype. In some cases, these more subtle structural changes were hoped to maintain activity within a narrower chemical SAR space. However, none of S13 – S18 were found to show any activity upon screening against TRAAK. Taken together, these initial data suggest that the binding site for TRAAK activation is not shared with the site for NK-1 antagonism, although likely further exemplar compounds would need to be identified and screened to prove this empirically.

3.3. Selectivity of Aprepitant

To confirm the utility of Aprepitant as tool activator of TRAAK we next sought to investigate its selectivity against other members of the K2P superfamily. Due to differing intrinsic properties of each channel being tested and different sensitivity to activation for each assay, the comparison of activation across channels is complex, and it is important to include pharmacological tools for each channel being analysed. This is challenging when appropriate and selective

tools do not exist for many K2P channels. Aprepitant did not show significant activation of any channel screened. Importantly this includes the closely related TREK-1 and TREK-2 channels. As Fig. 2 shows, activity was only just above baseline and marginal compared to known TREK activators. Interestingly, Aprepitant showed some inhibition at a subset of other channels. At TRESK, partial inhibition (compared to TPA) was observed. At TASK-2, Aprepitant inhibited the channel but was less potent than TPA. Given the lack of selectivity of TPA and the potential for non-specific reductions in signal in the thallium flux assay, these observations will need to be further investigated and confirmed using electrophysiology. Taken together, however, this data is highly suggestive that Aprepitant does not activate other K2P channels.

3.4. Whole-cell voltage-clamp recordings of the effect of Aprepitant on human TRAAK channel currents

Two isoforms of human (h) TRAAK were studied using whole-cell patch-clamp electrophysiology. These were a “short” form (the normal isoform used in studies of recombinant channels and that used for the thallium flux assays above - 393 aa, GenBank: AF259500) and a “long” form (419 aa, GenBank: AF259501), where the differences in length are due to differences in the N terminus that precedes the first transmembrane domain of the channel [33]. The effect of Aprepitant (10 μ M) was investigated on short TRAAK and long TRAAK channel currents in whole-cell patch-clamp recordings both by incubating cells in the compound and by acute

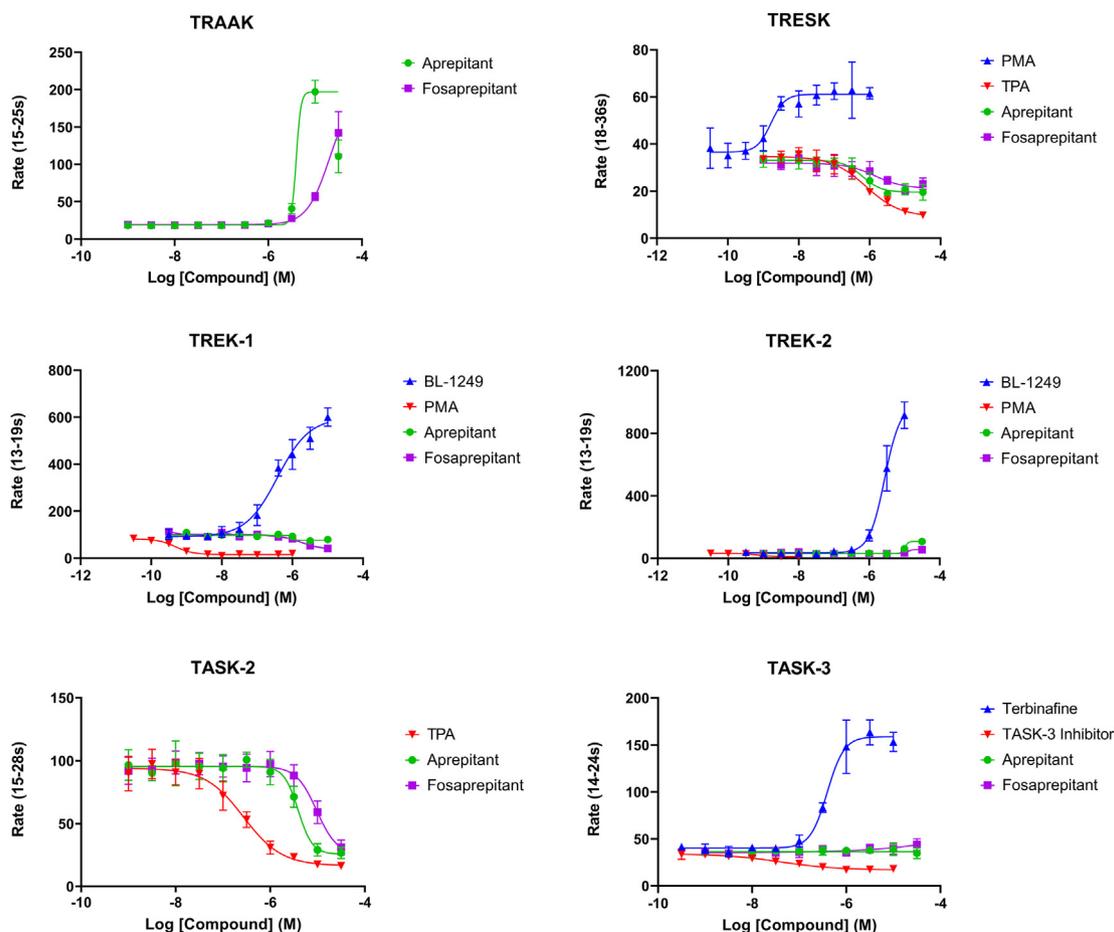


Fig. 2. Selectivity of Aprepitant at K2P channels. Aprepitant was screened at multiple K2P channels using thallium flux. Where available tool activators and inhibitors were also screened. Exemplar curves show rate of fluorescence increase ($n \geq 2$, \pm standard deviation).

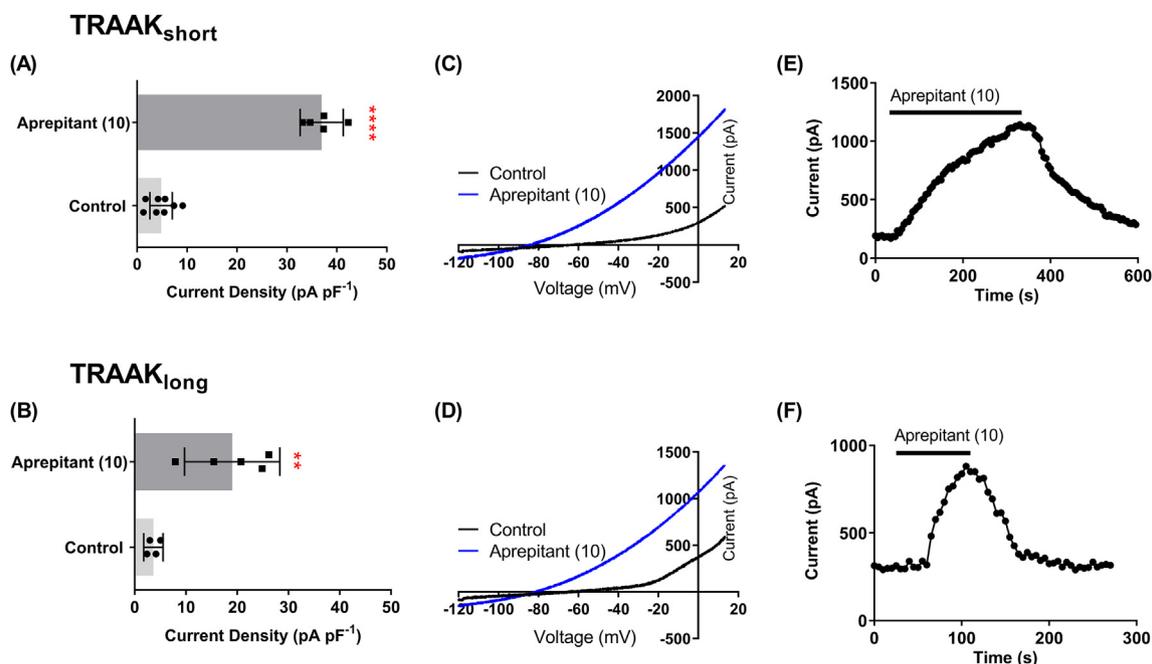


Fig. 3. Enhancement of TRAAK channel currents by Aprepitant (10 μM). Current density (measured as the difference current between -40 and -80 mV, see methods) for cells transfected with the short form (A) and the long form (B) of TRAAK in the presence and absence of Aprepitant (10 μM). The middle panels show representative current-voltage relationships for currents through the short (C) and long (D) forms of TRAAK in the absence (black trace) and presence (blue trace) of Aprepitant (10 μM). Acute application of Aprepitant (10 μM) to individual cells also enhances current through both the short (E) and long (F) forms of TRAAK. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

perfusion of the compound whilst recording from individual cells.

Fig. 3A and C shows that incubation with Aprepitant (10 μM) significantly ($P < 0.0001$, two-tailed, unpaired t -test) enhances current through the short form of the TRAAK channel by 7.7-fold, from 4.8 pA pF⁻¹ ($n = 8$, 95% CI: 3.0–6.7) in the absence of the compound to 37.0 pA pF⁻¹ ($n = 5$, 95% CI: 34.0–40.0) in its presence. Fig. 3B and D shows that incubation with Aprepitant (10 μM) also significantly ($P < 0.01$) enhances current through the long form of the TRAAK channel by 5.3-fold (slightly less than that seen for the short form), from 3.6 pA pF⁻¹ ($n = 4$, 95% CI: 2.6–4.7) in the absence of the compound to 19.0 pA pF⁻¹ ($n = 5$, 95% CI: 12.5–25.7) in its presence.

Perfusion of the compound to individual cells (Fig. 3E and F) confirmed the observations above that Aprepitant enhances current through both forms of the channel. However, as with incubation, Aprepitant was slightly more effective at enhancing current through the short form of the channel. Aprepitant (10 μM) enhanced current through the short form of the TRAAK channel by 599% of the control current ($n = 3$, 95% CI: 409–789), whilst Aprepitant (30 μM) enhanced current through the long form of the TRAAK channel by 183% of the control current ($n = 5$, 95% CI: 121–245). In contrast to the results with TRAAK channels, Aprepitant (10 μM) had no significant ($p < 0.05$) effect using a two tailed, unpaired t -test on non-transfected cells and did not enhance current through either hTREK-1 or hTREK-2 channels. For hTREK-1, current was 40.3 pA pF⁻¹ ($n = 5$, 95% CI: 16.3–64.3) in the absence of the compound and 30.9 pA pF⁻¹ ($n = 5$, 95% CI: 24.0–37.8) in its presence. For hTREK-2, current was 40.0 pA pF⁻¹ ($n = 7$, 95% CI: 28.7–51.2) in the absence of the compound and 32.1 pA pF⁻¹ ($n = 10$, 95% CI: 23.8–40.3) in its presence.

In conclusion, Aprepitant is a novel activator of TRAAK, which shows selectivity for TRAAK over other K₂P channels, identified through a novel thallium flux screen and subsequently confirmed through whole-cell manual patch-clamp electrophysiology. As

such, Aprepitant is a useful tool compound for identifying TRAAK channels in native systems and may form the basis for the synthesis of future selective activators of TRAAK channels as potential therapeutic agents in the treatment of pain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2021.12.031>.

References

- [1] S. Feliciangeli, F.C. Chatelain, D. Bichet, et al., The family of K₂P channels: salient structural and functional properties, *J. Physiol.* 12 (2015) 2587–2603.
- [2] V. Renigunta, G. Schlichterl, 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] A.M. Natale, P.E. Deal, D.L. Minor Jr., Structural insights into the mechanisms and pharmacology of K₂P potassium channels, *J. Mol. Biol.* 433 (2021) 166995.
- [4] 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.
- [5] M. Fink, F. Lesage, F. Duprat, et al., A neuronal two P domain K⁺ channel stimulated by arachidonic acid and polyunsaturated fatty acids, *EMBO J.* 17 (1998), 3297–3208.
- [6] S.G. Brochwaj, J. del Mármol, R. MacKinnon, Crystal structure of the human K₂P TRAAK, a lipid- and mechano-sensitive K⁺ ion channel, *Science* 335 (2012) 436–441.
- [7] 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.
- [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, *Brain Res. Mol. Brain Res.* 86 (2001) 101–114.
- [9] S.G. Brochwaj, W. Wang, A. Handler, et al., The mechanosensitive ion channel

- TRAAK is localized to the mammalian node of ranvier, *Elife* 8 (2019), e50403.
- [10] J.R. Schwarz, Function of K2P channels in the mammalian node of ranvier, *J. Physiol.* 599 (2021) 4427–4439.
- [11] H. Kanda, J. Ling, S. Tonomura, et al., TREK-1 and TRAAK are principal K⁺ channels at the nodes of ranvier for rapid action potential conduction on mammalian myelinated afferent nerves, *Neuron* 104 (2019) 960–971.
- [12] J. Noël, K. Zimmerman, J. Busserolles, et al., The mechano-activated K⁺ channels TRAAK and TREK-1 control both warm and cold perception, *EMBO J.* 28 (2009) 1308–1318.
- [13] P. Blanc, E. Génin, B. Jesson, et al., Genetics and postsurgical neuropathic pain an ancillary study of a multicentre survey, *Eur. J. Anaesthesiol.* 36 (2019) 342–350.
- [14] Y. Qiu, L. Huang, J. Fu, et al., TREK channel family activator with well-defined structure-activity relationship for pain and neurogenic inflammation, *J. Med. Chem.* 63 (2020) 3665–3677.
- [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] M. Takahira, M. Sakurai, N. Sakurada, et al., Fenamates and diltiazem modulate lipid-sensitive mechano-gated 2P domain K(+) channels, *Pflug. Arch. Eur. J. Physiol.* 451 (2005) 474–478.
- [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] J. Lv, Y. Liang, S. Zhang, et al., DCPIB, an inhibitor of volume-regulated anion channels, distinctly modulates K2P channels, *ACS Chem. Neurosci.* 10 (2019) 2786–2793.
- [19] L. Beltran, M. Beltran, A. Aguado, et al., 2-Aminoethoxydiphenyl borate activates the mechanically gated human KCNK channels KCNK 2 (TREK-1), KCNK 4 (TRAAK) and KCNK 10 (TREK-2), *Front. Pharmacol.* 4 (2013) 63.
- [20] S. Schrecke, Y. Zhu, J.W. McCabe, et al., Selective regulation of human TRAAK channels by biologically active phospholipids, *Nat. Chem. Biol.* 17 (2021) 89–95.
- [21] K. Gada, L.D. Plant, Two-pore domain potassium channels: emerging targets for novel analgesic drugs, *Br. J. Pharmacol.* 176 (2019) 256–266.
- [22] D. McCoull, E. Ococks, J.M. Large, et al., A “target class” screen to identify activators of two-pore domain potassium (K2P) channels, *SLAS Discovery* 26 (2021) 428–438.
- [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] 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.
- [25] P.D. Wright, D. McCoull, Y. Walsh, et al., Pranlukast is a novel small molecule activator of the two-pore domain potassium channel TREK2, *Biochem. Biophys. Res. Commun.* 520 (2019) 35–40.
- [26] A. Mathie, E.L. Veale, R.G. Holder, Heterologous expression of ion channels in mammalian cell lines, *Methods Mol. Biol.* 2188 (2021) 51–65.
- [27] D.P. Flaherty, D.S. Simpson, M. Miller, et al., Potent and selective inhibitors of the TASK-1 potassium channel through chemical optimisation of a bis-amide scaffold, *Bioorg. Med. Chem. Lett* 24 (2014) 3968–3973.
- [28] S.G. Brohawn, Z. Su, R. MacKinnon, Mechanosensitivity is mediated directly by the lipid membrane in TRAAK and TREK1 K⁺ channels, *Proc. Natl. Acad. Sci. Unit. States Am.* 111 (2014) 3614–3619.
- [29] D. Regoli, A. Boudon, J.L. Fauchère, Receptors and antagonists for substance P and related peptides, *Pharmacol. Rev.* 46 (1994) 551–599.
- [30] J.J. Hale, S.G. Mills, M. MacCoss, et al., Structural optimisation affording 2-(R)-(1-(R)-3,5-bis(trifluoromethyl)phenoxy)-3-(S)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methylmorpholine, a potent, orally active, long-acting morpholine acetal human NK-1 receptor antagonist, *J. Med. Chem.* 41 (1998) 4607–4614.
- [31] A.J.C. Loucif, P.-P. Saintot, J. Liu, et al., GI-530159, a novel, selective, mechanosensitive two-pore-domain potassium (K_{2p}) channel opener, reduces rat dorsal root ganglion neuron excitability, *Br. J. Pharmacol.* 175 (2018) 2272–2283.
- [32] K.M.J. Brands, J.F. Payack, J.D. Rosen, et al., Efficient synthesis of NK1 receptor antagonist Aprepitant using a crystallization-induced diastereoselective transformation, *J. Am. Chem. Soc.* 125 (2003) 2129–2135.
- [33] A. Ozaita, E. Vega-Saenz de Miera, Cloning of two transcripts, HKT4.1a and HKT4.1b, from the human two-pore K⁺ channel gene KCNK4. Chromosomal localization, tissue distribution and functional expression, *Brain Res. Mol. Brain Res.* 102 (2002) 18–27.