Using high-throughput automated patch clamp electrophysiology to identify novel TREK-1 modulators in an animal venom library

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Introduction

- Two-pore domain K^+ (K_{2P}) channels are a family of fifteen four-pass transmembrane K^+ channels that dimerise to form a functional K^+ channel complex capable of regulating neuronal membrane potential and excitability through a background K^+ conductance[(1)].
- K_{2P} channels are modulated by a plethora of stimuli, such as pH change, polyunsaturated fatty acids, membrane stretch, certain phospholipids, temperature, small molecules and various general anaesthetics[(1)].
- TREK-1 (Figure 1), a member of the K_{2P} family, is implicated in a range of CNS pathologies, such as depression, epilepsy and chronic pain, due to its role in regulating neuronal excitability. Therefore, TREK-1 is an attractive therapeutic target[(2,3)].

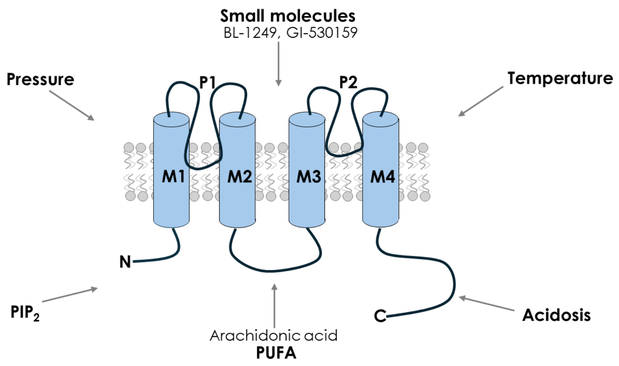

Figure 1. Channel schematic of monomeric TREK-1. TREK-1 consists of four transmembrane domains (M1 – M4) and two pore-forming domains (P1 – P2). TREK-1 dimerises, as homomers or heteromers with other K_{2P} subtypes, to form a functional K^+ channel. TREK-1 can be activated by physical stimuli, e.g. membrane stretch and temperature, as well as chemical stimuli, e.g. intracellular H^+, polyunsaturated fatty acids (PUFAs), small molecules, and phosphatidylinositol 4,5-biphosphate (PIP_2)[(2,3)].

Aims

- Develop a high-throughput, electrophysiological assay of TREK-1 function to identify novel modulators.
- The assay was optimized to identify both activators and inhibitors, providing comprehensive mechanistic data for high value, limited supply screening libraries, such as the venom fraction library used in this study (Targeted Venom Discovery Array, T-VDA, Venomtech, UK).

Materials and methods

Cell culture

- Human TREK-1 (hTREK-1) was stably expressed in a Chinese hamster ovary (CHO) cell line.

Electrophysiology assay

- Automated patch clamp platforms: QPatch 48 and Qube 384 (both Sophion Biosciences).
  - Multi-hole acquisition and 25 °C (unless otherwise stated).
- Voltage protocol: a ramp (800 ms) from -100 mV to +60 mV at 0.1 Hz (holding voltage: – 60 mV). Current amplitude was recorded at +60 mV for QPatch 48 experiments and at +10 mV for Qube 384 experiments.

Venom fraction screening

- 384-well assay plates (3) containing 1 µg of uniquely separated venom fraction per well (320 wells/plate) were provided from Venomtech, UK (Targeted Venom Discovery Array, T-VDA). Fractions were resuspended at 10 ng/µL in extracellular solution for use.
- Taxa of venom fractions screened: Dendroaspis angusticeps (D.ang), Dendroaspis jamesoni (D.jam), Dendroaspis polyepis (D.pol), Dendroaspis viridis (D.vir), Heterometrus silenus (H.sil), Heterometrus spinifer (H.spi), Hotentotta jayakari (H.jay), Chilobrachys huahini (C.hua), Cyriopagopus albostriatum (H.lbo), Grammaostola porteri (G.por), Oxyuranus scutellatus (O.scu).

Results

1. Validation of TREK-1 currents

TREK-1 current expression was verified following robust activation with the TREK-selective agonists, BL-1249 (10 µM, Figure 2A, C) and GI-530159 (30 µM, Figure 2B, C) on the QPatch 48 platform.

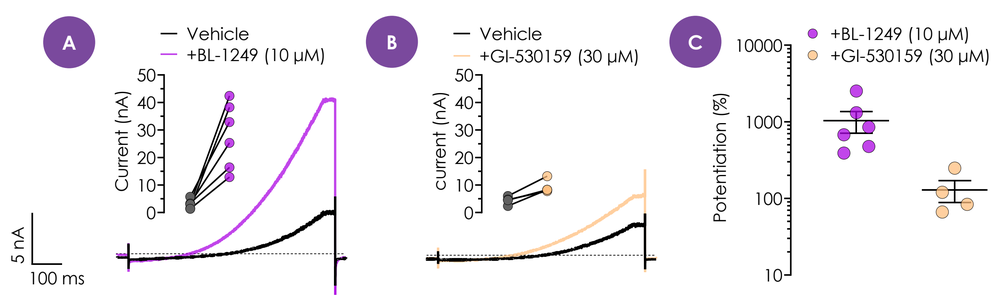

Figure 2. Validation of TREK-1 currents using a QPatch 48. (**A**, **B**) Representative TREK-1 currents elicited by an 800 ms ramp from -100 mV to +60 mV in the presence and absence of BL-1249 (**A**, 10 µM) and GI-530159 (**B**, 30 µM), inserts represent potentiation in individual cells. The quantified % increase in TREK-1 current amplitude for both compounds at +60 mV (**C**). Recordings performed in multi-hole acquisition using a QPatch48 platform at 25°C. Data displayed as Mean ± SEM.

2. Optimisation of TREK-1 current amplitude

To develop an assay for dual detection of potentiation and inhibition of hTREK-1, an assay window for both potentiators and inhibitors is required. A baseline current amplitude of >1 nA is ideal for screening inhibitors and a potentiation window of >1000 % by BL-1249 is ideal for screening potentiators. Temperatures ≥ 32.5 °C significantly increased baseline current amplitude, but reduced BL-1249 potentiation (% increase relative to vehicle) to <1000 %, meaning temperature is not a viable approach (Figure 3A,C). Incubating CHO-hTREK-1 cells with the histone deacetylase inhibitor, sodium butyrate (NaB, 3 mM), for 48 h, increases baseline current amplitude above 1 nA while still maintaining a >1000 % BL-1249 potentiation window (Figure 3B,D). Therefore, enhancing channel expression via NaB treatment is the optimal approach for establishing assay conditions.

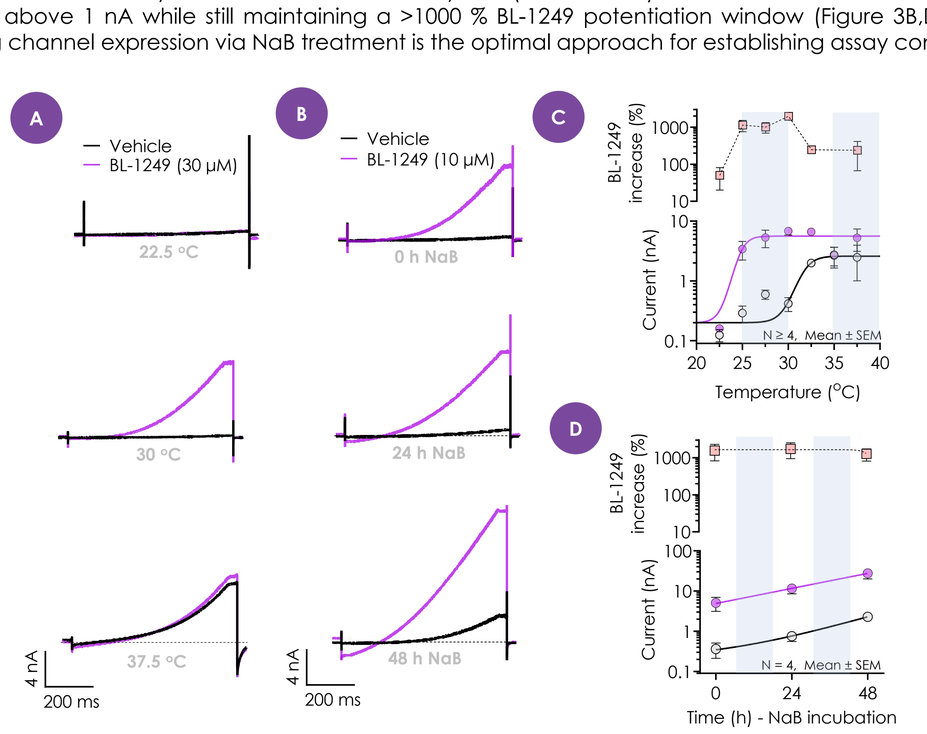

Figure 3. Optimisation of hTREK-1 parameters for dual screening of agonist and antagonist activity using a QPatch 48. (**A**,**C**) The effect of increasing recording temperature on hTREK-1. (**A**) Representative traces depicting the current response at baseline (vehicle) and maximal activation (BL-1249, 30 µM). (**C**) Current amplitude at +60 mV, in vehicle (grey) and BL-1249 (purple), and the extent of BL-1249 potentiation (%), was quantified at each temperature interval. Note, potentiation at 35°C was <10 %. (**B**,**D**) The effect of sodium butyrate (NaB) treatment at 30 °C on CHO-hTREK-1 cells. (**B**) Representative traces depicting the current response at baseline (vehicle) and following maximal activation (BL-1249, 10 µM) at 25 °C. (**D**) Current amplitude at +60 mV, in vehicle (grey) and BL-1249 (purple), and the extent of BL-1249 potentiation (%), was quantified at each NaB treatment timepoint. Recordings performed in multi-hole acquisition using a QPatch 48 platform. Data displayed as Mean ± SEM.

3. TREK-1 assay development using a Qube 384 platform

hTREK-1 was initially examined on the Qube 384 to ensure compatibility with QPatch 48 results. An outward current was observed in non-transfected cells at +60 mV, unlike with the QPatch48 (Figure 4A,B). Therefore, current amplitude measurements were made at +10 mV for Qube experiments. BL-1249 demonstrated comparable potentiation of hTREK-1 current amplitude as the QPatch, validating the use of the Qube for high-throughput investigation of hTREK-1 (Figure 4C,D).

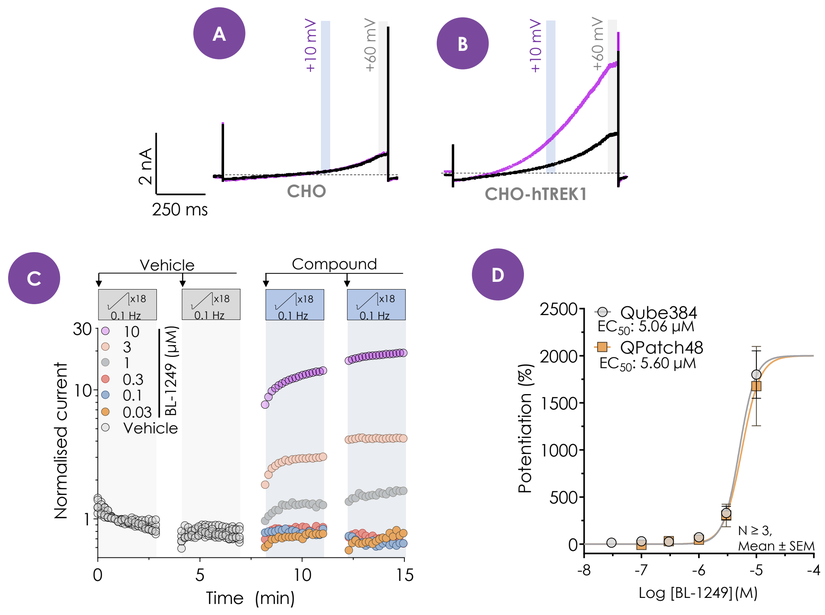

Figure 4. Validation of hTREK-1 currents using a Qube 384. (**A**,**B**) Representative current response in the presence and absence of BL-1249 of non-transfected CHO (**A**) and stably expressing CHO-hTREK-1 cells (**B**). Current response at +10 mV and +60 mV highlight differences in current amplitudes between CHO and CHO-hTREK-1 cells. (**C**) Assay paradigm of Qube 384 displayed on top, current-time plots demonstrating BL-1249 potentiation of hTREK-1 below. (**D**) Potentiation comparable between Qube 384 and QPatch 48.

4. Validation of pharmacological assessment of TREK-1 activity using a Qube 384 platform

Various reference compounds, including potentiators and inhibitors, were tested against hTREK-1 for further validation of the assay (Figure 5A-C). Potency values were similar to published data (Figure 5D,E).

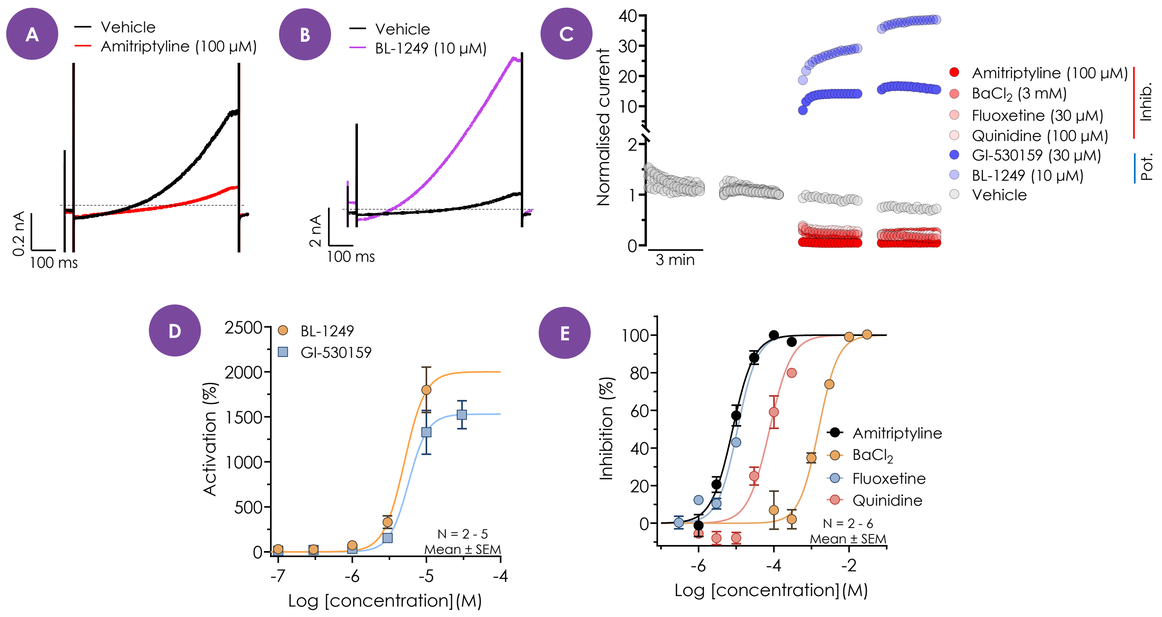

Figure 5. Validation of pharmacological assessment of hTREK-1 activity using known reference compounds on a Qube 384 platform. (**A**,**B**) Representative traces of hTREK-1 current response when exposed to amitriptyline (**A**, 100 µM) or BL-1249 (**B**, 10 µM). (**C**) Representative current-time plots of cells exposed to vehicle (0.3 % v/v DMSO) or inhibitor ("Inhib.") or potentiator ("Pot."). (**D**,**E**) Concentration-response curves for potentiators (**D**) or inhibitors (**E**). IC_{50}s (µM): amitriptyline – 8.08, $BaCl_2$ – 1552, fluoxetine – 10.8, quinidine – 77.6. EC_{50}s (µM): BL-1249 – 5.06, GI-530159 – 5.73. Inhibitors within 3-fold of literature values[4-6]. potentiators within 5-fold[7,8].

5. Venom fraction library screening of TREK-1

A total of 591 venom fractions, likely consisting of a single peptide per fraction, were successfully screened against hTREK-1 using the Qube 384 platform (Figure 6A). A variety of peptides produced a significant response (Figure 6B,C). Further work will be performed to validate hits and assess potency and selectivity.

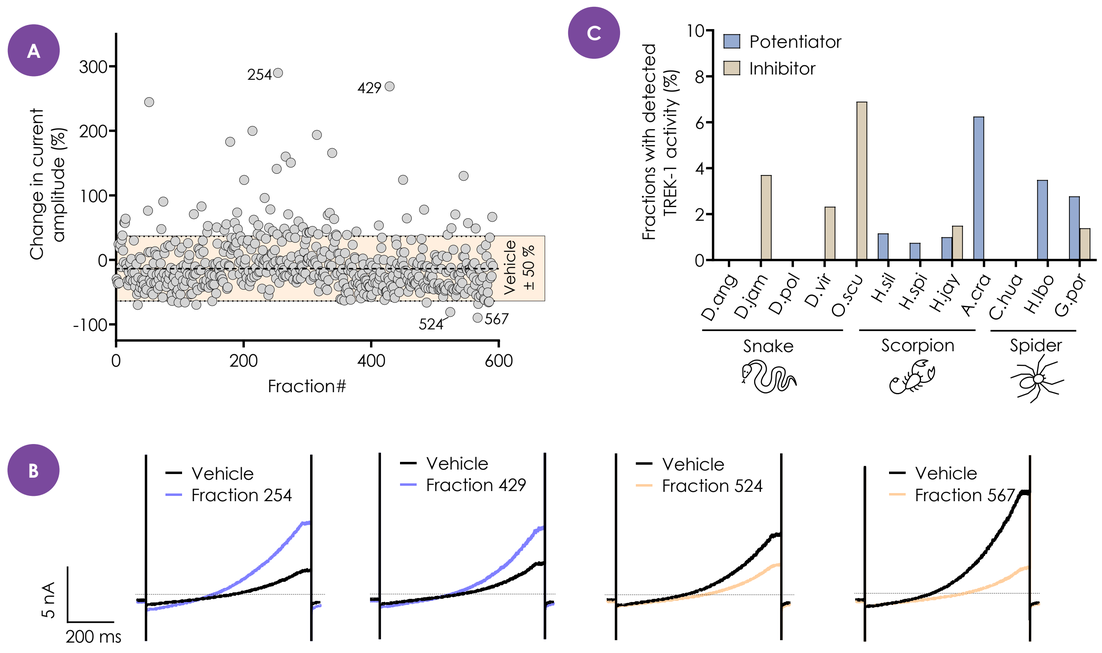

Figure 6. Venom fraction screen of hTREK-1 activity on a Qube384 platform. (**A**) hTREK-1 activity was assessed using a venom fraction library and changes in current amplitude at +10 mV measured. A peptide was considered active if it induced a response greater than 50 % from the mean of the vehicle response (-13.7 %). (**B**) Representative traces of the two most active openers and inhibitors displayed. (**C**) A qualitative assessment of current responses in the presence of venom fractions was combined with the quantitative assessment in (**A**) to identify the high-confidence hits.

Conclusions

- Metrion has developed a robust hTREK-1 screening assay on the high throughput automated patch clamp Qube 384 platform capable of identifying both activators and inhibitors of the hTREK-1 channel.
- The optimized screening assay was employed in the screening of a venom library (Targeted Venom Discovery Array, Venomtech, UK), detecting peptides with inhibitory and potentiating modalities.
- Further work will confirm hits and elucidate potency and selectivity of the most promising fractions.

References

1) Decher N *et al.* (2021). *Cell Physiol Biochem* 2021;55(S3):87-107.
2) Honoré E *et al.* (2007). *Nat Rev Neurosci*. 2007 Apr;8(4):251-61.
3) Djillani *et al*. (2019). *Front Pharmacol* 2019; 10: 379
4) Park H *et al*. (2016*). Korean J Physiol Pharmacol* 2016;20(4):379-385.
5) Ma X-Y *et al*. (2011*). J Biol Chem*. 2011 Nov 18;286(46):39813-22.
6) Bae Y *et al*. (2020). *Int J Mol Sci.* 2020 Dec 17;21(24):9639.
7) Pope L *et al.* (2018). *ACS Chem Neurosci* 2018 Dec 19;9(12):3153-3165.
8) Loucif AJC *et al.* (2018). *Br J Pharmacol* 2018 Jun;175(12):2272-2283.

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