

Supplementary Materials for

TWIK-1 Two-Pore Domain Potassium Channels Change Ion Selectivity and Conduct Inward Leak Sodium Currents in Hypokalemia

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Text

Fig. S1. TWIK-1 WT K⁺ channels show altered ion selectivity and conduct inward leak Na⁺ currents in subphysiological [K⁺]_o.

Fig. S2. Effects of removing 5 mM [K⁺]_o on TWIK-1 WT K⁺ channels and five other types of K2P channels.

Fig. S3. Effects of removing 5 mM [K⁺]_o on THIK-1 WT and THIK-1•I112T mutant channels.

Table S1. Reversal potentials and whole-cell currents of TWIK-1 WT and TWIK-1•K274E channels in 5 and 0 mM [K⁺]_o.

References

SUPPLEMENTARY MATERIALS

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Fig. S1. TWIK-1 WT K^+ channels show altered ion selectivity and conduct inward leak Na^+ currents in subphysiological $[K^+]_o$.

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TWIK-1 WT K^+ channels show altered ion selectivity and conduct inward leak Na^+ currents in subphysiological $[K^+]_o$. TWIK-1 WT K^+ channels produced no detectable macroscopic currents in an asymmetric physiological K^+ gradient in most transfected CHO cells and very small currents in the remainder. Detectable currents through human TWIK-1 WT K^+ channels were recorded in about 1 out of 18 transfected CHO cells; whole-cell currents at +80 mV were between 250 pA to 480 pA in 5 mM $[K^+]_o$. These currents clearly differed from the endogenous K^+ currents in CHO cells, which at +80 mV are ~40 pA (n=20 cells) in 5 mM $[K^+]_o$ (1). We observed that TWIK-1

WT K^+ channels showed altered ion selectivity in subphysiological $[K^+]_o$ (fig. S1), as observed for TWIK-1•K274E mutant K^+ channels (Fig. 1, main text). These results were not seen in either non-transfected CHO cells (n=20 cells) or those transfected with the GFP reporter gene alone (n=20 cells).

Five other K2P channels maintain their K^+ -selectivity in the absence of extracellular K^+ . TWIK-1 K^+ channels have the highest relative permeability of Na^+ to K^+ (~0.52) in 0 mM $[K^+]_o$ (Fig. 1E; figs. S1D-E and S2A). Replacing 5 mM $[K^+]_o$ with 0 mM $[K^+]_o$ shifted the reversal potentials of TREK-1, TASK-1, TASK-3, THIK-1, and TRESK-2 channels in the hyperpolarizing direction; no detectable inward Na^+ current was observed in 0 mM $[K^+]_o$ (fig. S2B-F). Thus, these five K2P channels maintained their K^+ -selectivity in the absence of extracellular K^+ . TASK-1 channels did not yield detectable whole-cells currents in most transfected CHO cells, so we used a TASK-1• Δ i20 mutant with a 20 amino acid deletion within the C-terminal, which shows increased abundance at the plasma membrane relative to wild-type TASK-1 (2). Removing 5 mM $[K^+]_o$ reduced the amplitude of TASK-1• Δ i20 whole-cell currents, so that whole-cell currents at +80 mV were decreased to 41%, suggesting that subphysiological $[K^+]_o$ may decrease the channel activity. Previous studies indicate that lowering $[K^+]_o$ to subphysiological levels decreases the activities of Kv1.4 and hERG voltage-gated K^+ channels (3-4).

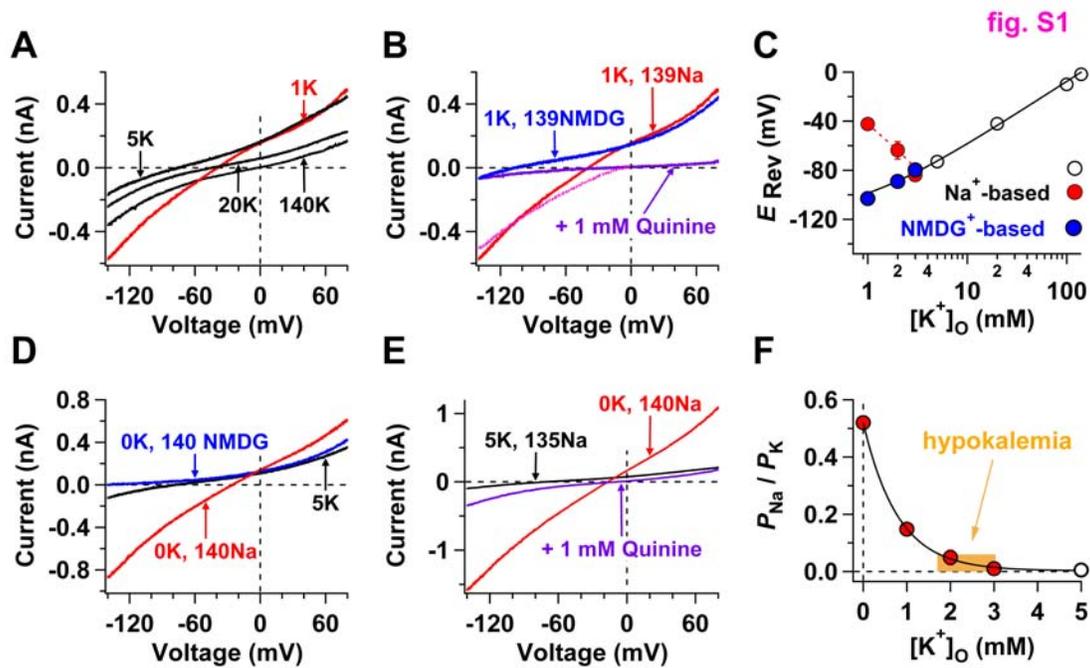


Fig. S1. TWIK-1 WT K^+ channels show altered ion selectivity and conduct inward leak Na^+ currents in subphysiological $[K^+]_o$. (A) Whole-cell currents of TWIK-1 WT channels are shown from four different transfected CHO cells in Na^+ -based bath solutions containing the indicated $[K^+]_o$. (B) Whole-cell currents of TWIK-1 WT channels are shown from two different transfected CHO cells in Na^+ -based (red line) or $NMDG^+$ -based (blue line) bath solutions with 1 mM $[K^+]_o$. Dash pink line represents net inward Na^+ currents calculated by comparing both currents. (C) Reversal potentials (E_{rev}) of TWIK-1 WT channels were plotted as a function of $[K^+]_o$. E_{rev} values were measured in Na^+ -based (open and red filled circles, $n=3-9$ cells for each data point) or $NMDG^+$ -based (blue filled circles, $n=3-5$ cells for each data point) bath solutions with various $[K^+]_o$. The continuous curve is a fit for open circles with the GHK equation, as described in Fig. 1, yielding a Na^+ permeability relative to that of K^+ of 0.005. (D-E) Whole-cell currents are shown for TWIK-1 WT channels before and after Na^+ -based bath solutions were changed from 5 mM $[K^+]_o$ (black lines) to 0 mM (red lines) $[K^+]_o$. Whole-cell currents in 0 mM $[K^+]_o$ represent those obtained at equilibrium. Quinine blockade confirmed TWIK-1 currents in B and E (purple lines). (F) The calculated values for relative permeability of Na^+ to K^+ (P_{Na}/P_K) were plotted as a function of $[K^+]_o$. The superimposed single-exponential fit yielded a slope factor of $[K^+]_o$ -dependence at 0.80 mM per e-fold increase in P_{Na}/P_K . The $[K^+]_o$ range consistent with hypokalemia are marked with an orange box.

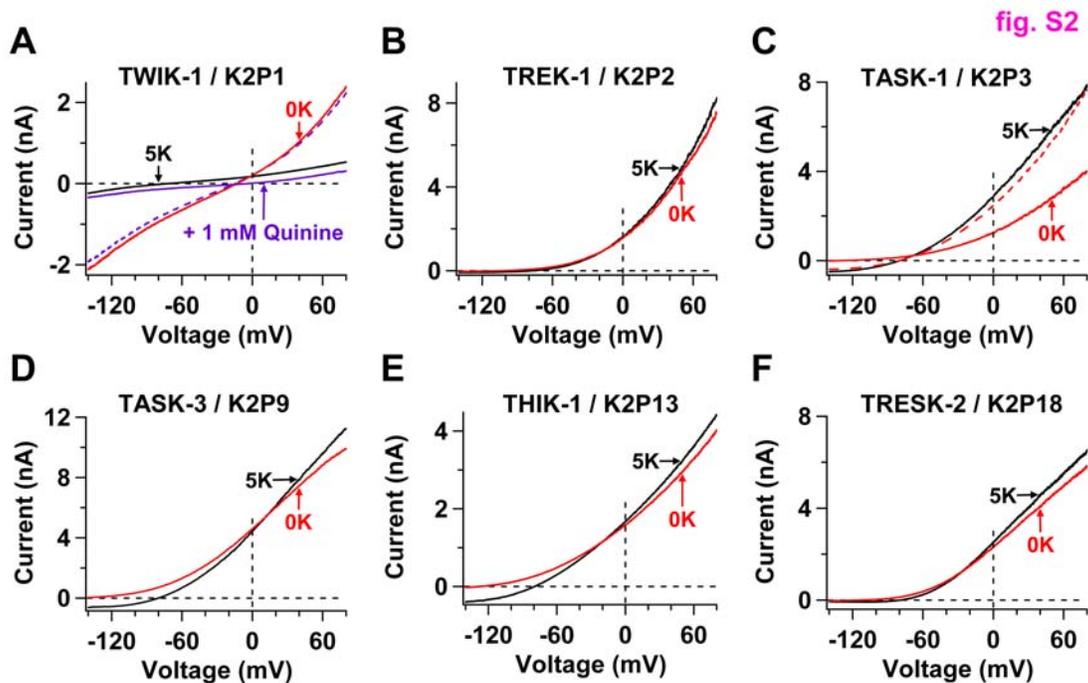


Fig. S2. Effects of removing 5 mM $[K^+]_o$ on TWIK-1 WT K^+ channels and five other types of K2P channels. (A-F) Whole-cell currents are shown for indicated K2P (A, human TWIK-1 WT; B, rat TREK-1; C, human TASK-1• Δ i20; D, human TASK-3; E, mouse THIK-1; F, mouse TRESK-2) channels heterologously expressed in CHO cells before and after Na^+ -based bath solutions were changed from 5 mM $[K^+]_o$ (black lines) to 0 mM (red lines) $[K^+]_o$ (n=6-12 cells). Whole-cell currents in 0 mM $[K^+]_o$ were obtained at equilibrium. Purple lines in A show reversible block of TWIK-1 currents by quinine (solid line, block; dash line, wash out). Dash red line in C represents whole-cell currents following restoration of 5 mM $[K^+]_o$ in Na^+ -based bath solutions.

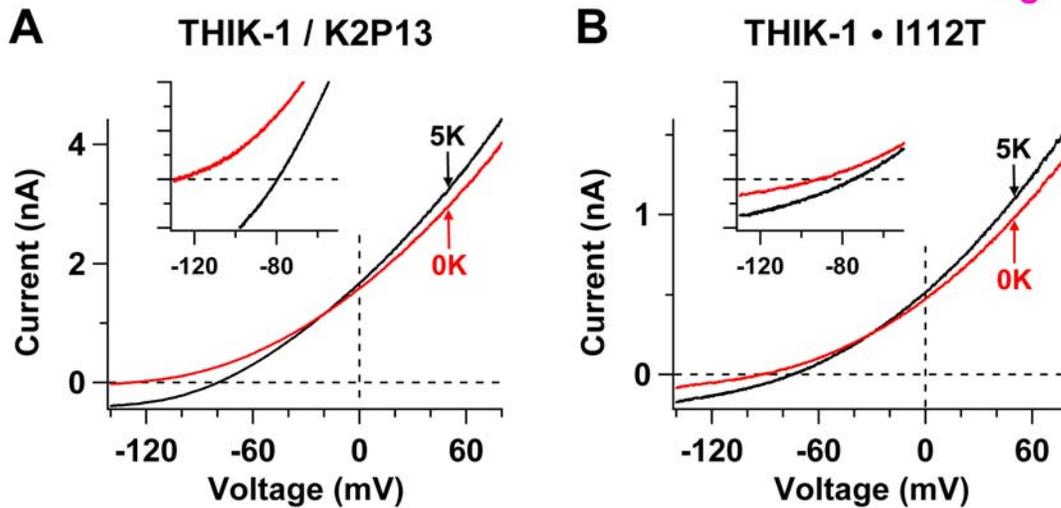


Fig. S3. Effects of removing 5 mM [K⁺]_o on THIK-1 WT and THIK-1•I112T mutant channels. (A-B) Whole-cell currents are shown for mouse THIK-1 WT (A) or THIK-1•I112T mutant (B) channels heterologously expressed in CHO cells before and after Na⁺-based bath solutions were changed from 5 mM [K⁺]_o (black lines) to 0 mM [K⁺]_o (red lines) (n=5-7 cells). Whole-cell currents in 0 mM [K⁺]_o were obtained at equilibrium. Inserts: current traces are shown in shorter voltage ranges (-130 mV to -50 mV) and narrower current amplitudes (-200 pA to +400 pA) so that reversal potentials are clearly visible. Reversal potentials of THIK-1•I112T mutant channels shifted from -72.3 ± 0.8 mV to -90.5 ± 1.1 mV (n=7 cells), reflecting values for permeability of Na⁺ relative to that of K⁺ of ~ 0.005 and ~ 0.019 , respectively. Thus, THIK-1•I112T mutant channels are still highly K⁺-selective in the absence of extracellular K⁺.

Table S1. Reversal potentials and whole-cell currents of TWIK-1 WT and TWIK-1•K274E channels in 5 and 0 mM $[K^+]_o$. Reversal potentials (E_{rev}) and whole-cell currents at +80 mV ($I_{+80\text{ mV}}$) and -140 mV ($I_{-140\text{ mV}}$) of TWIK-1 and TWIK-1•K274E channels before and after Na^+ -based bath solutions were changed from 5 mM $[K^+]_o$ to 0 mM $[K^+]_o$. Parameters in 0 mM $[K^+]_o$ represent those obtained equilibrium. N represents number of measured cells.

Channel	5 mM $[K^+]_o$			0 mM $[K^+]_o$			N
	E_{rev} (mV)	$I_{+80\text{ mV}}$ (pA)	$I_{-140\text{ mV}}$ (pA)	E_{rev} (mV)	$I_{+80\text{ mV}}$ (pA)	$I_{-140\text{ mV}}$ (pA)	
Human TWIK-1	-72.9 ± 1.0	348 ± 40	-140 ± 20	-17.0 ± 1.2	1332 ± 199	-1333 ± 177	9
Human TWIK-1•K274E	-73.0 ± 1.0	617 ± 80	-294 ± 40	-16.8 ± 0.9	2243 ± 400	-2354 ± 450	8
Rat TWIK-1	-74.3 ± 1.2	274 ± 35	-137 ± 25	-18.6 ± 0.9	679 ± 124	-931 ± 172	5
Rat TWIK-1•K274E	-72.2 ± 0.7	706 ± 87	-392 ± 63	-17.3 ± 0.8	2116 ± 370	-2397 ± 368	12

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