Voltage-Gated K^+ Channels

Click Channel Type to Access Validation Data:

- $K_V1.1$
- $K_V1.2$
- $K_V1.3$
- $K_V1.4$
- $K_V1.5$
- $K_V1.6$
- $K_V1.7$
- $K_V1.8$
- $K_V2.1$
- $K_V2.1/K_V9.2$
- $K_V3.1$
- $K_V3.2$
- $K_V3.3$
- $K_V4.2$/KChiP2
- $K_V4.3$/KChiP1
- $K_V4.3$/KChiP2
- hKCNQ1/hminK ($K_V7.1/KCNE_1$)
- $K_V7.2/K_V7.3$
- $K_V7.3/K_V7.5$
- $K_V7.4$
- $K_V7.4/K_V7.5$
- $K_V12.2$
- $K_{ir}2.1$
- $K_{ir}6.2$/SUR 2A

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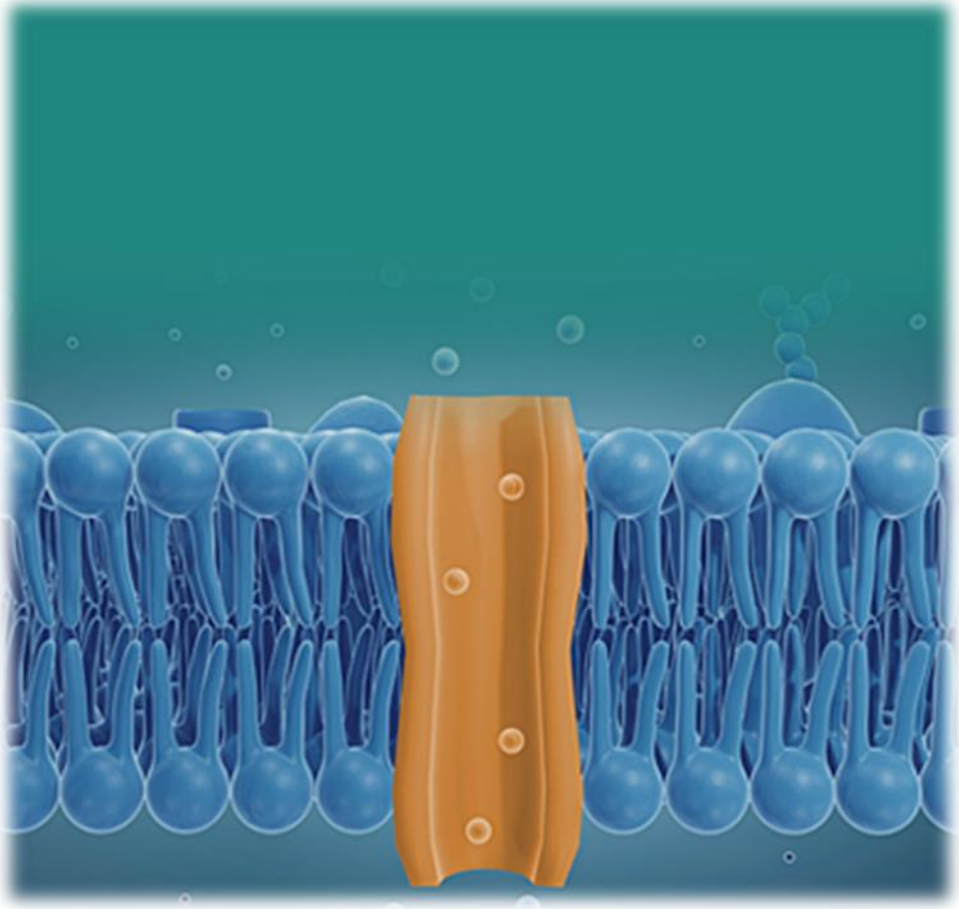

Voltage-Gated Potassium Channels Introduction

BACK

Voltage-gated potassium channels are a large and diverse family. There are 12 subfamilies of human voltage-gated potassium channel genes and approximately 40 pore forming genes. K_V channels are distributed widely in the nervous system, cardiac and other tissues. K_V channels regulate action potential waveforms and firing patterns in excitable cells. K_V channels also have a role in regulating cell proliferation, migration and maintaining cell volume in a wide variety of tissues.

Voltage-gated potassium (K_V) channels are one of the most highly evolutionarily conserved and largest ion channel families [1]. K_V channels contsist of either four identical, or similar pore-forming α-subunits, other auxiliary subunits including β subunits affect the channel function and/or trafficking [2,3]. Individual pore-forming subunits of K_V channels consist of six transmembrane segments (S1-S6), with the voltage sensing properties of the channel contained in the first four transmembrane segments (S1-S4). The ion conducting pore is contained within and flanked by the (S5-S6) transmembrane segments.

There are approximately 80 human potassium channel genes when accessory subunits are included, including the 40 aforementioned pore forming genes [2]. Early studies identified A-type K+ channels that rapidly inactivate, as well as Delayed Rectifier K^+ channels without fast inactivation. Fast inactivation, can alter action potential durations during repetitive firing. Hodgkin and Huxley originally identified the delayed rectifier potassium current in squid, in cardiac tissue they play critical roles in the timing of action potential repolarization.

Voltage-Gated Potassium Channels Introduction (cont.)

BACK

K_V channels are diverse with some of this diversity due to the mixing of K_V channel subunits within individual conducting channels. K_V1, K_V2, K_V3, K_V4 and K_V7 families all have homomeric and heteromeric channels with a wide range of functional properties [2,5]. K_V2 channels can assemble with K_V5, K_V6, K_V8 or K_V9 family members in the nervous system and smooth muscles [6]

Action potential modulation is achieved by functional differences in the voltage dependence and kinetics of KV channels [4]. K_V1, K_V4 and K_V7 channels are activated by relatively low levels of membrane depolarization (low voltage activated). K_V2 and K_V3 channels require greater levels of depolarization for activation(high voltage activated). Low voltage activated K_V channels are known to affect the threshold for action potential firing and the number of action potentials seen during bursts (or excitatory synaptic potentials). The high voltage activated K_V channels are capable of modulating action potential duration and firing pattern [4].

Therapeutic Potential of Potassium Channel Modulators

BACK

The modulation of potassium channel activity has been shown to change action potential firing patterns. This has led to researchers identifying the modulation of K^+ channels as having possible therapeutic value [7]. Various neurological and psychological disorders may involve alterations in normal action potential firing patterns, K_V channel inhibitors or activators can potentially rescue action potential firing patterns as seen in the figure (adapted from [7]). Voltage-gated potassium channels have also been implicated in cell proliferation and migration and thus may be potential treatments for cancer and metastasis [8-14].

In addition to the therapeutic potential mentioned above mutations of K_V channel genes may cause neurological channelopathies such as epilepsy and episodic ataxia, cardiac diseases, and deafness [15-18].

References

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K_V1.1 (CYL3014)

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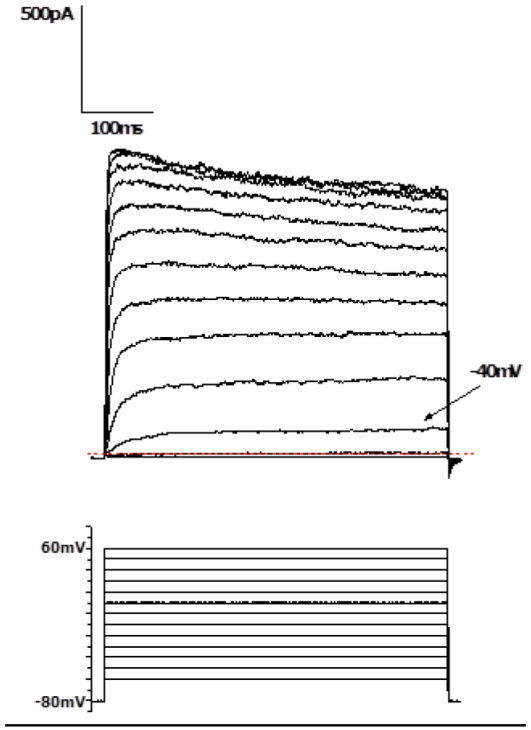

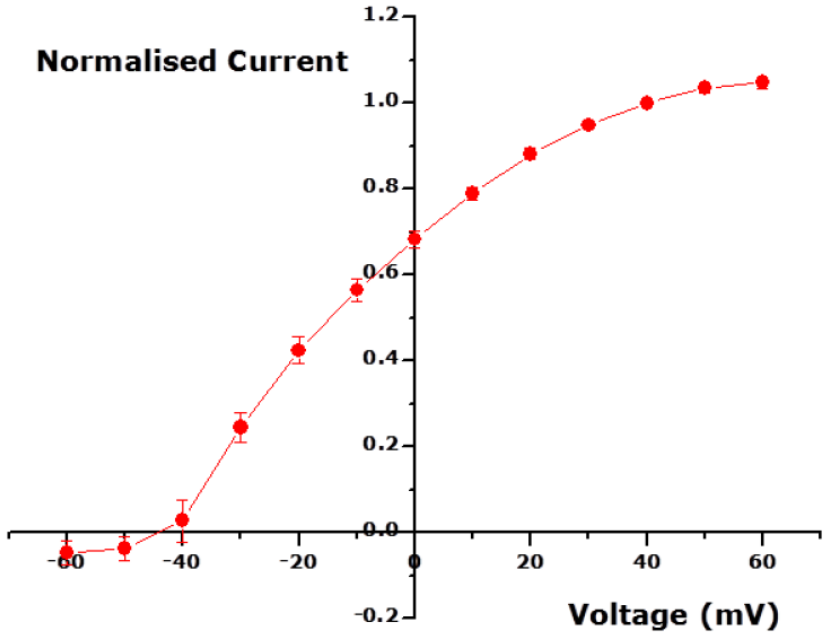

K_V1.1 Raw Data Currents and Current-Voltage (I/V) relationship: Membrane currents **(Upper Left)** elicited by depolarising voltage pulses **(lower left)**, stepped in 10 mV increments from -60 mV to + 60 mV from a holding potential of -80 mV every 15 s. The red dotted line indicates zero current level. **Right:** Current-voltage relationship. Current amplitudes were measured at the end of the 500 ms step, and normalised to the current amplitude obtained at +40 mV. The mean normalised data from four cells is shown. The mean current at +40 mV was 970 ± 220 pA (n=4) (Manual Patch Clamp Data).

$K_V1.1$

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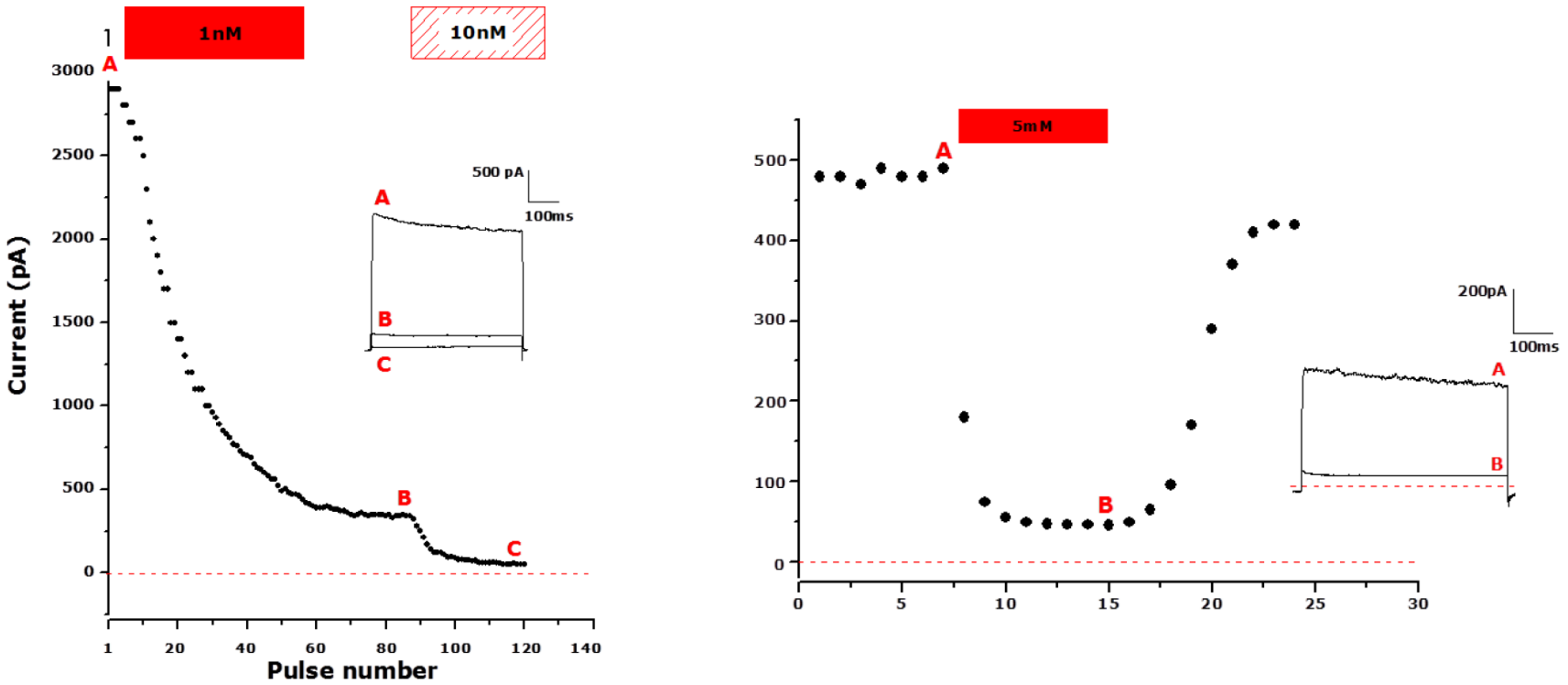

$K_V1.1$ Dendrotoxin K and TEA Pharmacology : Left: The effect of Dendrotoxin K (1-10 nM) on the $hK_V1.1$ current. K_V currents were evoked continuously, every 10s, by stepping the membrane voltage from a holding potential of -80 mV to +60 mV for 500 ms. Peak currents at +60 mV are plotted against pulse number below. The inset shows illustrative examples of current traces taken before (A), immediately after 1 nM DTX-K (B) and during bath addition of 10 nM DTX-K after steady state block had been achieved (C). The red and red-hatched bars depict the presence of 1 and 10 nM DTX-K respectively. **(Right):** The effect of TEA (5 mM) on the $hK_V1.1$ current. K_V currents were evoked every 10 s by stepping the membrane voltage from a holding potential of -80 mV to +60 mV for 500 ms. Peak currents at +60 mV are plotted against pulse number below. The inset shows two illustrative examples of current traces taken before (A) and during TEA addition after steady state block had been achieved (B). The red bar depicts the presence of 5 mM TEA. Red dotted lines **(Left and Right)** indicate zero current level. (Manual Patch Clamp Data).

K_V1.2 (CYL3015)

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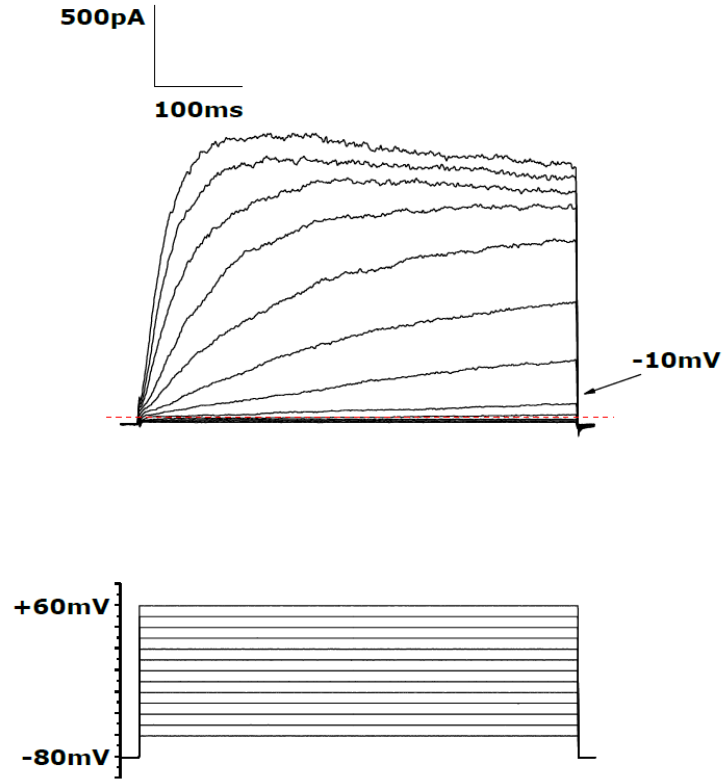

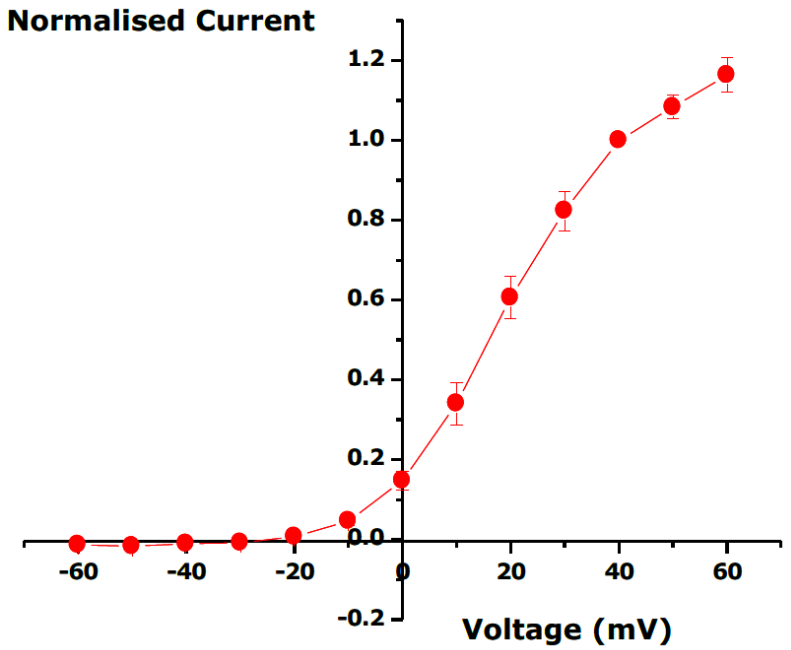

K_V1.2 Raw Data Currents and Current-Voltage (I/V) relationship: Membrane currents **(Upper Left)** elicited by depolarising voltage pulses **(Lower Left)**, stepped in 10 mV increments from –60 mV to + 60 mV from a holding potential of –80 mV every 15 s. The red dotted line indicates zero current level. **Right:** Current/voltage relationship. Current amplitudes were measured at the end of the 500 ms step, and normalised to the current amplitude obtained at +40 mV. The mean normalised data from six cells is shown. The mean current at +40 mV was 2000 ± 570 pA (n=6) (Manual Patch Clamp Data).

$K_V1.2$

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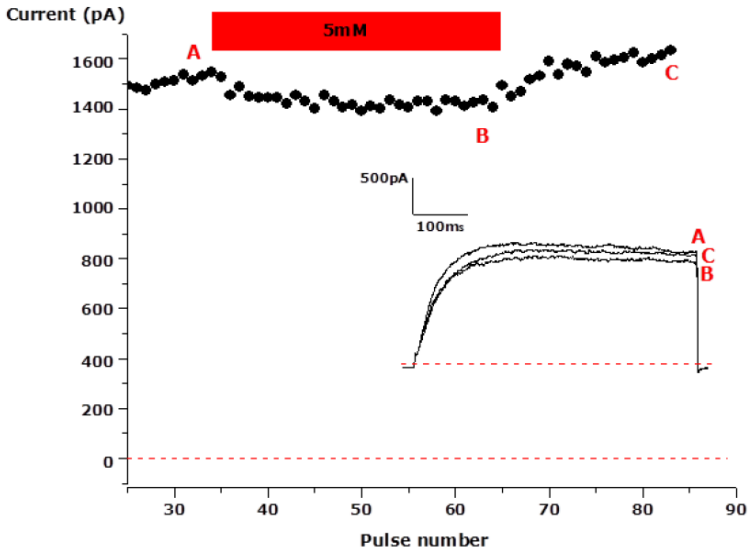

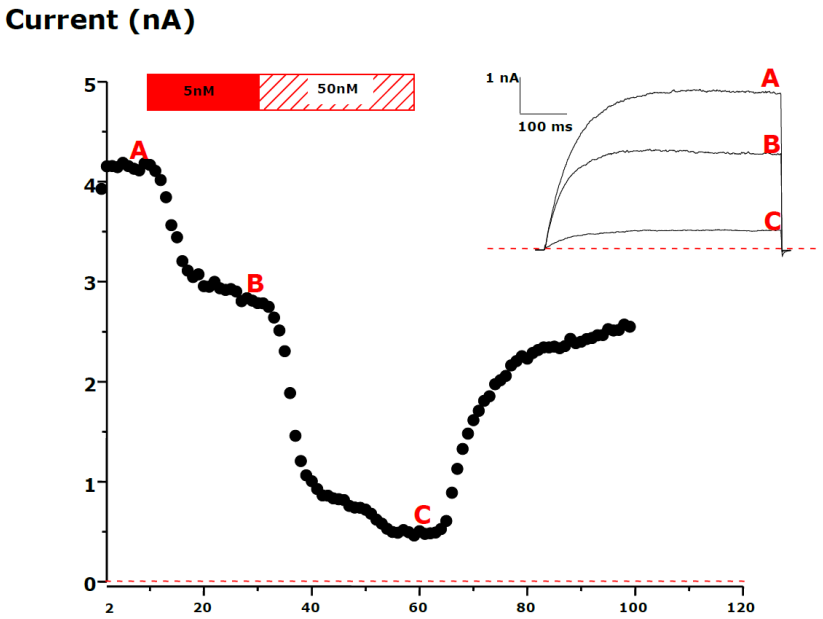

<u>$K_V1.2$ TEA and Tityustoxin Kα Pharmacology :</u> The effect of TEA (5 mM) on the hKv1.2 current. Kv currents were evoked every 10 s by stepping the membrane voltage from a holding potential of –80 mV to +60 mV for 500 ms. Peak currents at +60 mV are plotted against pulse number. The inset shows three illustrative examples of current traces taken before (A), during (B) and after (C) TEA addition. The red bar depicts the presence of 5 mM TEA. **<u>(Right):</u>** The effect of Tityustoxin Kα (5-50 nM) on the hKv1.2 current. hKv1.2 currents were evoked continuously, every 10 s, by stepping the membrane voltage from a holding potential of –80 mV to +60 mV for 500 ms. Peak currents at +60 mV are plotted against pulse number below. The inset shows illustrative examples of current traces taken before (A) and at the end of 5 nM (B) and 50 nM (C) bath application of TsTx-Kα. The red and red-hatched bars depict the presence of 5 and 50 nM TsTx-Kα respectively. Red dotted lines **(Left and Right)** indicate zero current level. <u>(Manual Patch Clamp Data).</u>

$K_V1.2$

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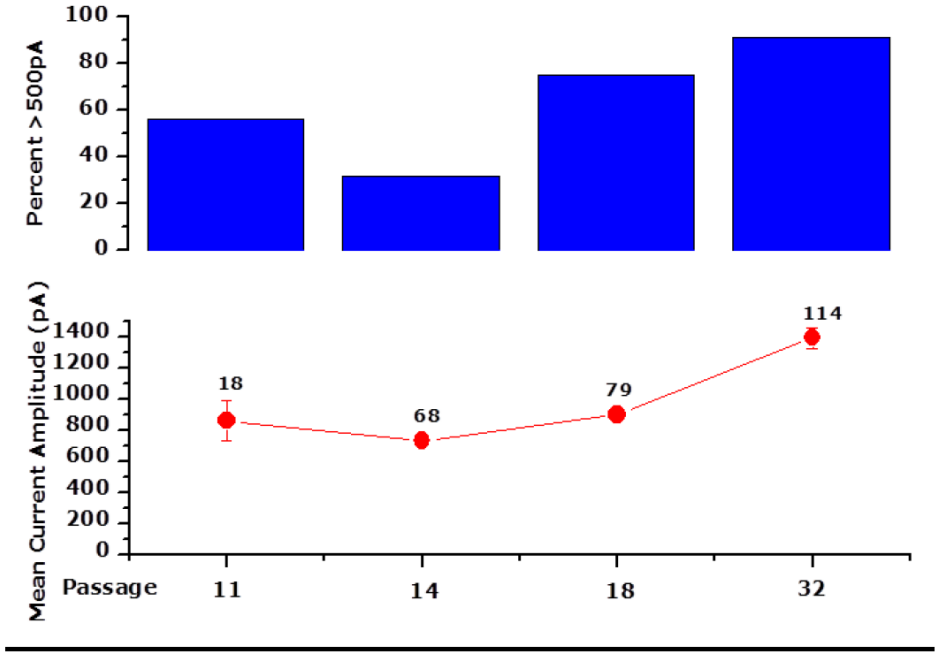

Include? Recollect the Data?

K_V1.3 (CYL3016)

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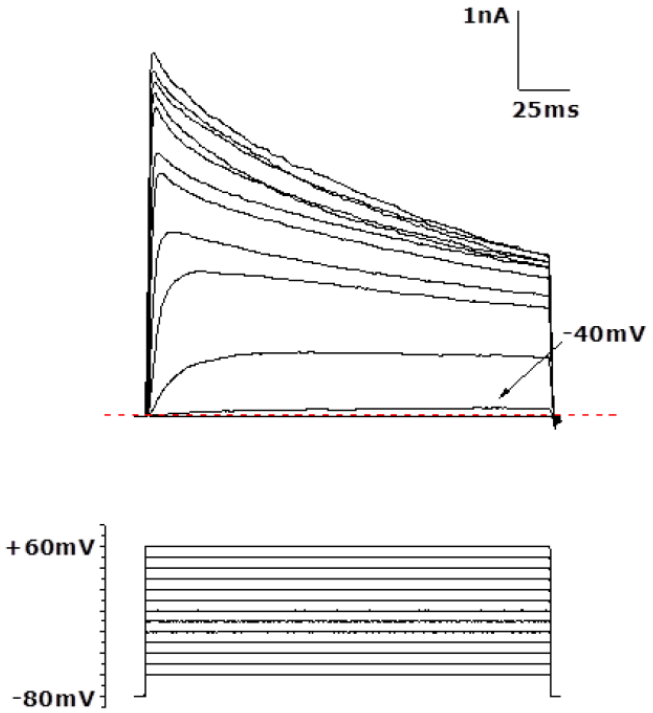
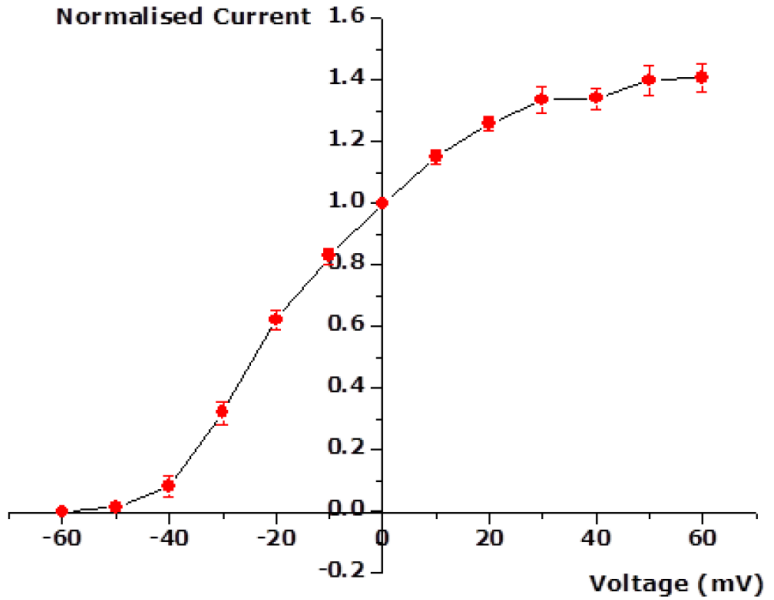
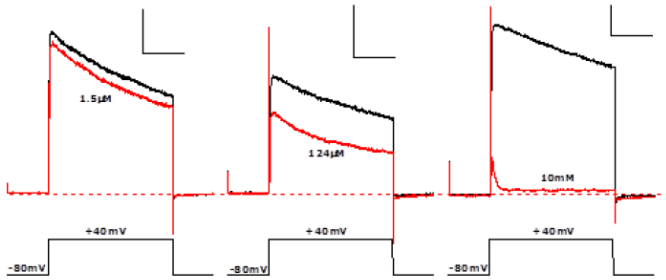
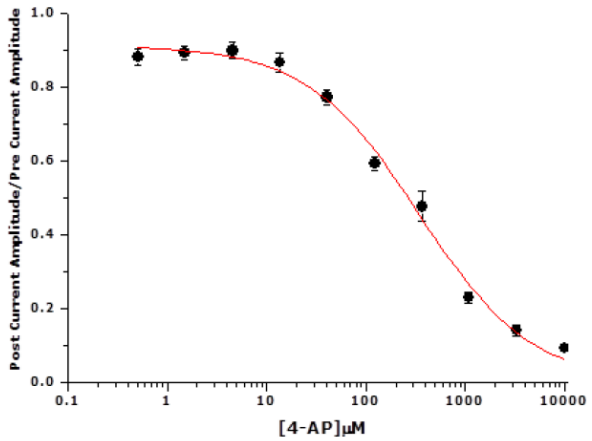


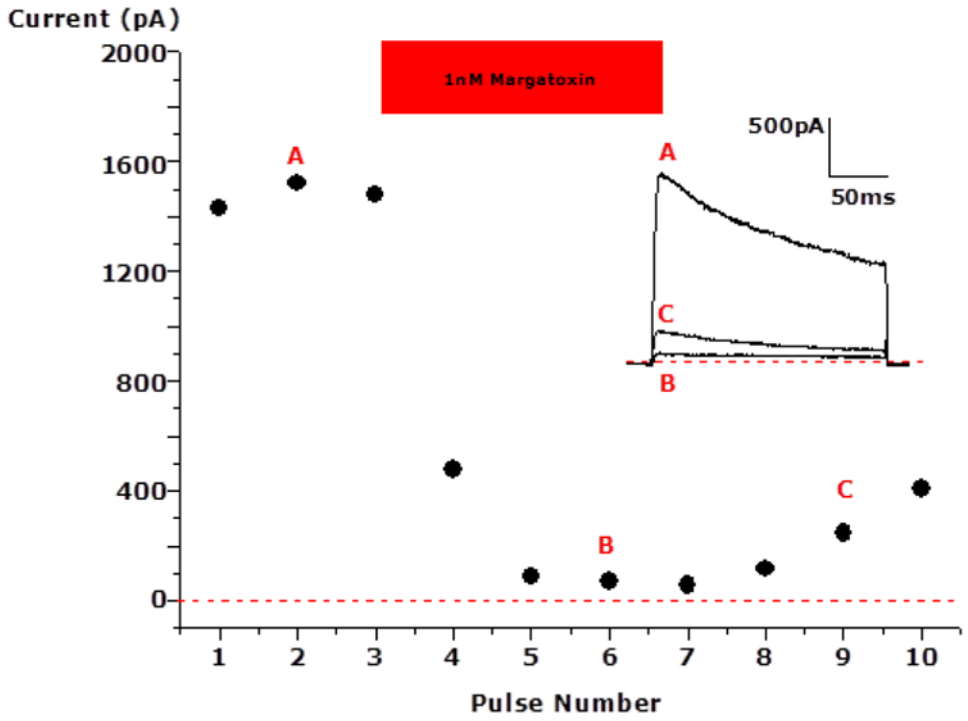


K_V1.3 Raw Data Currents and Current Voltage (I/V) relationship: Membrane currents **(Left)** elicited by 200 ms depolarising voltage pulses, stepped in 10 mV increments from –60 mV to + 60 mV from a holding potential of –80 mV every 60 s. The red dotted line indicates zero current level. Current-voltage relationship **(Right)**, peak current amplitudes were measured during the 200 ms step, and normalized to the current amplitude obtained at 0 mV. The mean current at 0 mV was 4000 ± 1000 pA (n=5). (Manual Patch Clamp Data).

K_V1.3







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<u>K_V1.3 Pharmacology: Left:</u> The effect of 4-AP on hKv1.3 current **(top)**. Kv1.3 currents were evoked by stepping from a holding potential of –80 mV to +40 mV for 300 ms prior to (black traces in A) and 5 min after 4-AP addition (red traces in A). Typical effects of 1.5 µM, 124 µM and 10 mM respectively are shown. The vertical and horizontal calibration bars are 1 nA and 100 ms respectively for all traces. A 10 point dose-response curve of the data is shown **(bottom)** and could be described by a Hill equation giving an estimated IC_{50} value of 344 µM. Each data point represents the mean of between 15-16 cells. **Right:** The effect of Margatoxin (1 nM) on the hKv1.3 current. Kv1.3 currents were evoked every 60 s by stepping the membrane voltage from a holding potential of –80 mV to –10 mV for 200 ms. Peak currents at -10 mV are plotted against pulse number. The inset shows three illustrative examples of current traces taken before (A), during (B) and after (C) Margatoxin addition. The red bar depicts the presence of 1 nM Margatoxin. **(Left and Right)** The red dotted lines indicate zero current levels. <u>(IonWorks HT Data).</u>

$K_V1.3$

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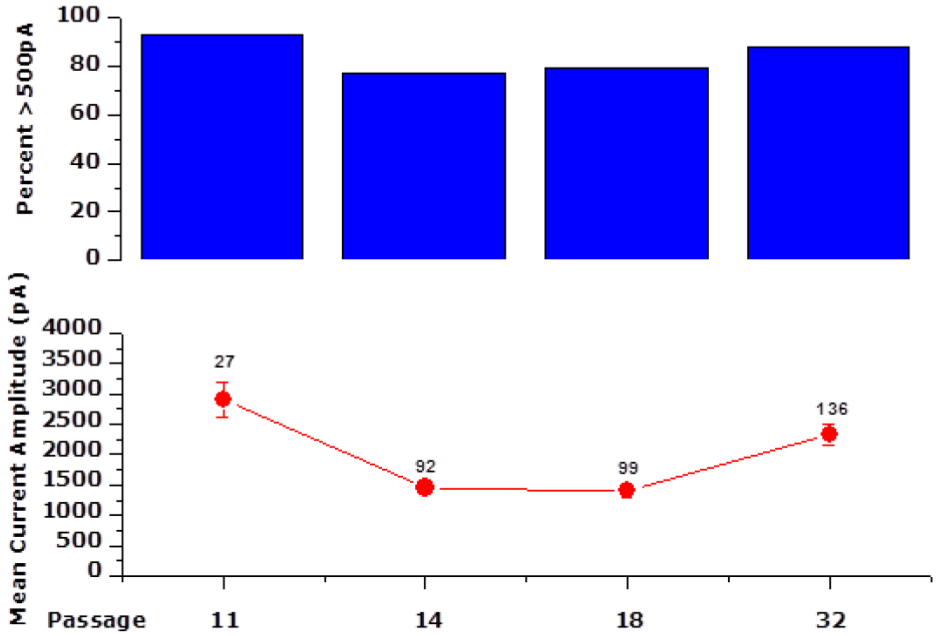

$K_V1.3$ Stability and Expression: Stability of expression over passage. The upper panel shows the percentage of cells expressing a mean peak outward current >500 pA for cell passages 11, 14, 18 and 32. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles). (IonWorks HT Data).

K_V1.4 (CYL3017)

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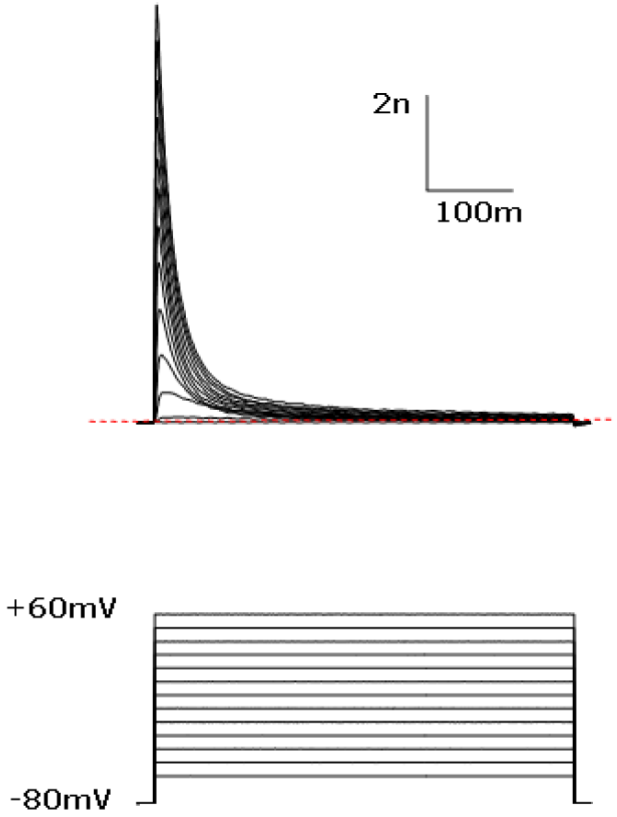

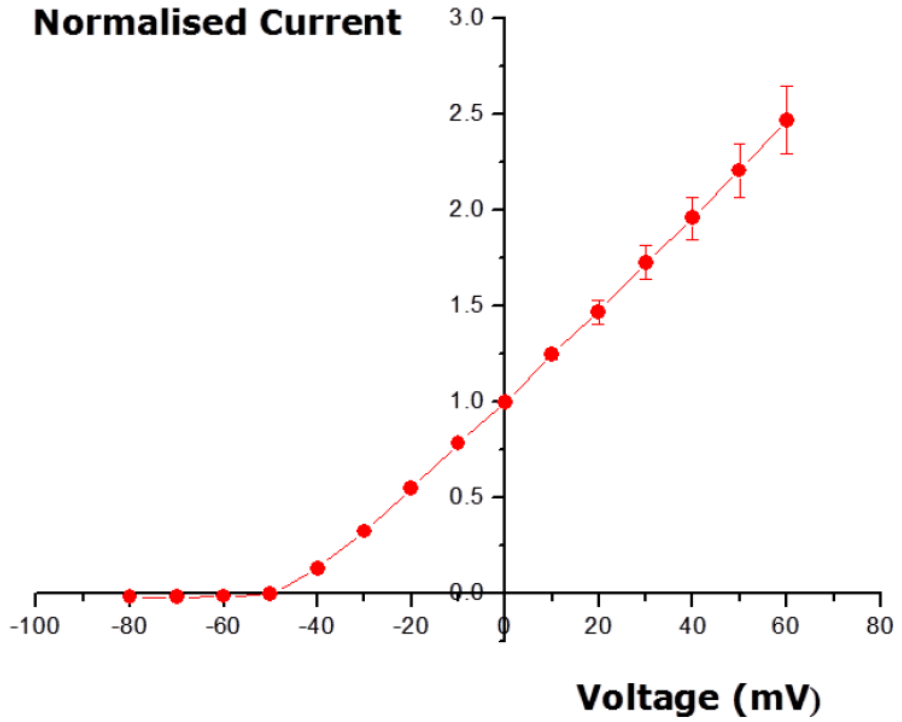

K_V1.4 Raw Data Currents and Current-Voltage (I/V) relationship: Membrane currents **(Left)** elicited by depolarising voltage pulses stepped in 10 mV increments from –60 mV to + 60 mV from a holding potential of –80 mV every 5 s. The red dotted line in the upper panel indicates zero current level. Current-voltage relationship **(Right)**. Peak current amplitudes were measured and normalised to the current amplitude obtained at 0 mV. The mean current at 0 mV was 3600 ± 2700 pA (n=3). (Manual Patch Clamp Data).

$K_V1.4$

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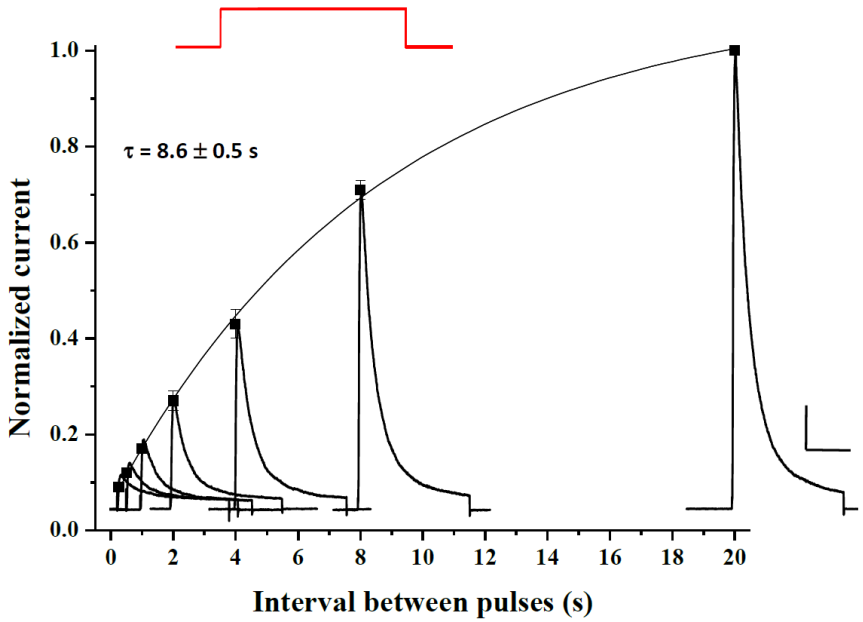

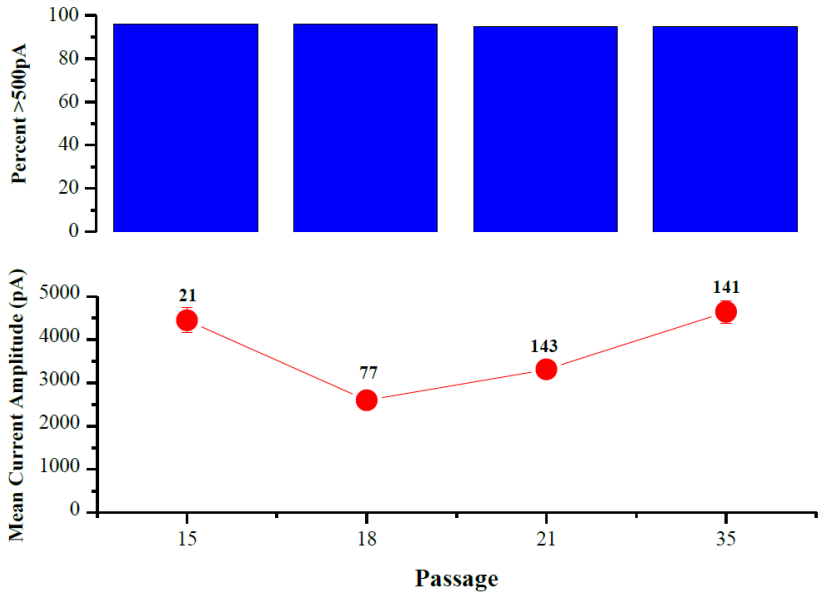

$K_V1.4$ Recovery from Inactivation Using a Two-Pulse Protocol; Stability of Expression over Passage: Using a gapped two pulse voltage protocol with variable interval between pulses a single exponential curve **(Left)** was fitted to the mean peak current of the second pulse. Currents evoked by a voltage step from –80 mV to -10 mV for 250 ms. Currents normalized to the peak current of the first pulse (control). Scale bars represent 100 ms and 500 pA (n = 3) (Manual Patch Clamp Data). **Right:** The upper panel shows the percentage of cells expressing a mean peak outward current of >500 pA for cell passages 15, 18, 21, and 35. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles) (IonWorks HT Data).

$K_V1.4$

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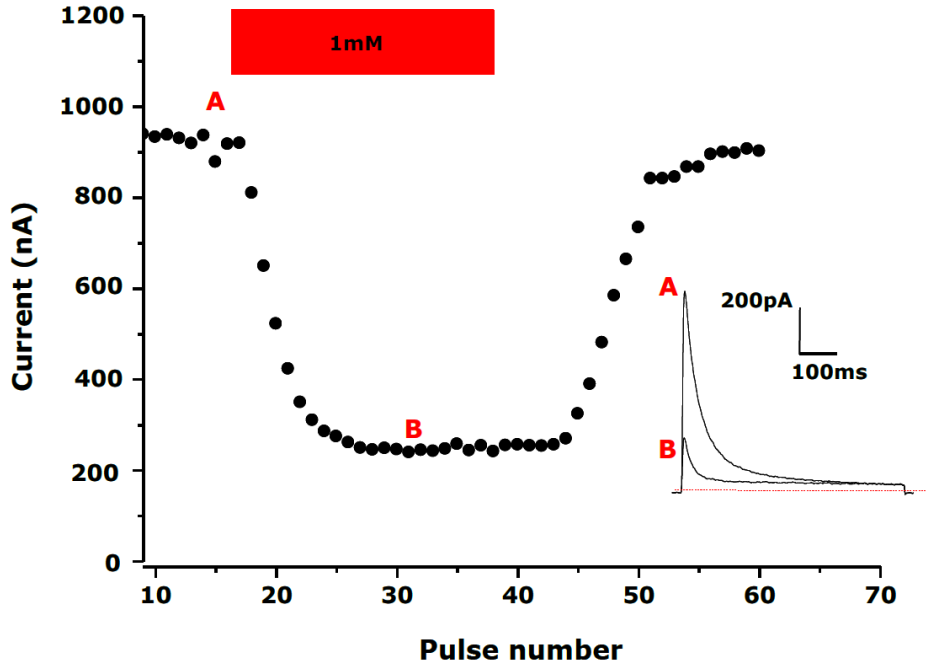

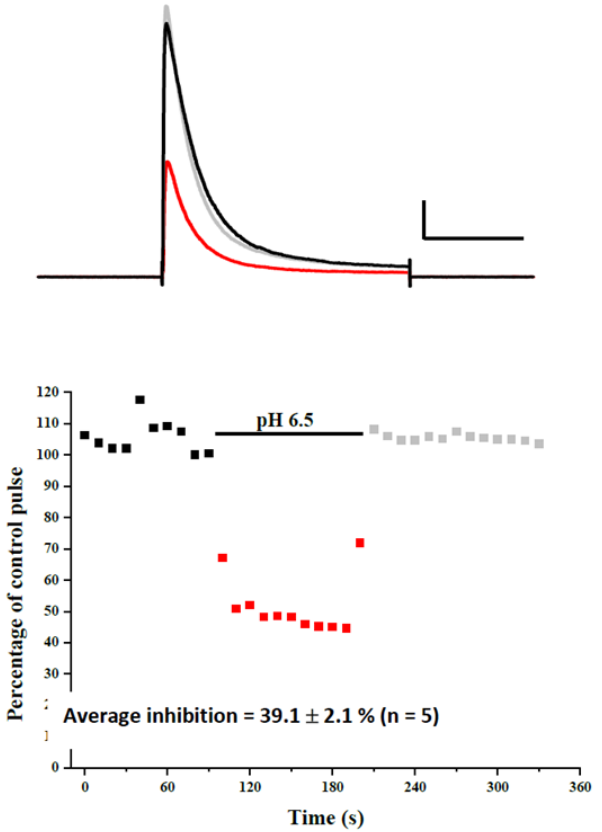

$K_V1.4$ Pharmacology of 4-AP and the Effect of Acidosis: The effect of 4-AP (1 mM) on the hKv1.4 current. Kv currents were evoked continuously, every 10 s, by stepping the membrane voltage from a holding potential of –80 mV to –10 mV for 500 ms. Peak currents at –10 mV are plotted against pulse number below. The inset shows an illustrative example of a current traces taken before (A) and during addition of 1 mM 4-AP, when steady state block had been achieved. The red bar depicts the presence of 1 mM 4-AP, red dotted line on the inset indicates zero current level. **Right (top):** Kv currents were evoked every 10 s, by stepping from a holding potential of –80 mV to –10 mV for 500 ms. A, a typical current evoked in external solution at pH 7.3 (Black trace), after lowering the pH to 6.5 (red trace) and following the return to pH 7.3 external solution (Grey trace). Scale bar represents 200 ms and 1 nA. **Right (bottom):** The rapid and reversible effect of acidosis on the peak currents of the Kv1.4 (Manual Patch Clamp Data).

K_V1.5 (CYL3018)

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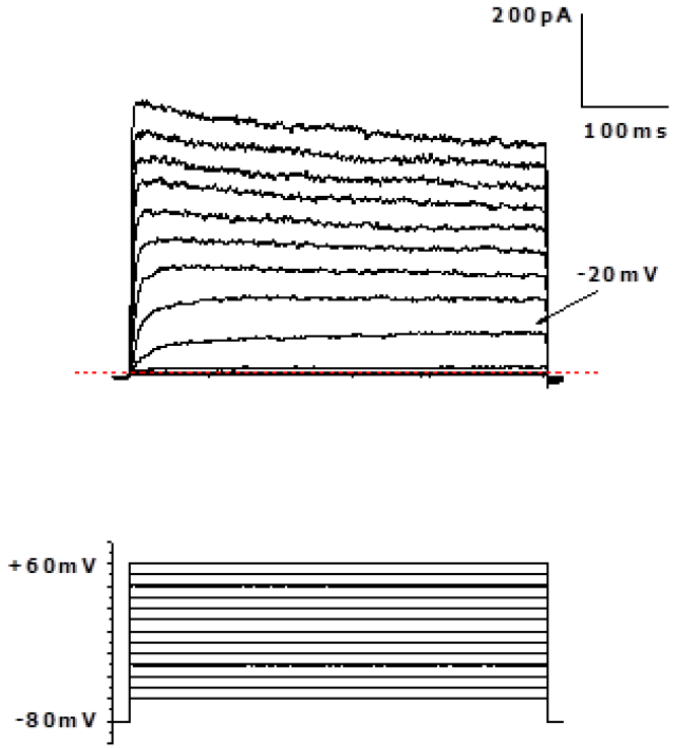
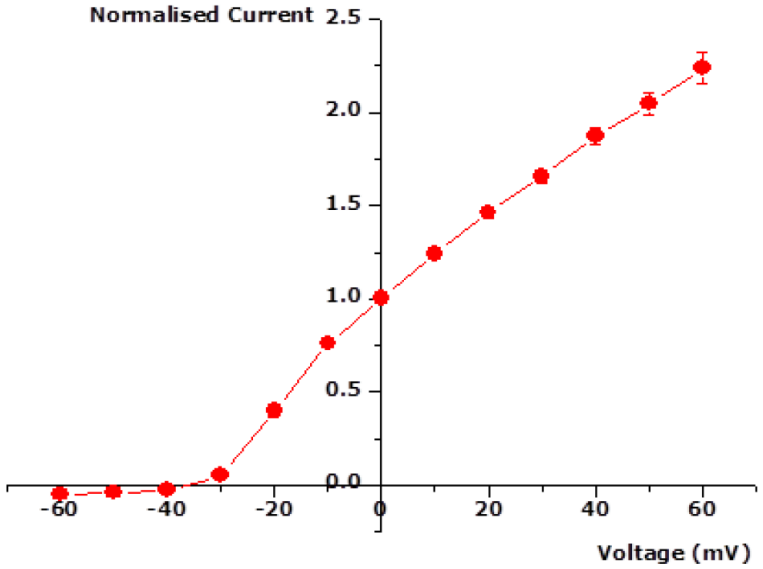
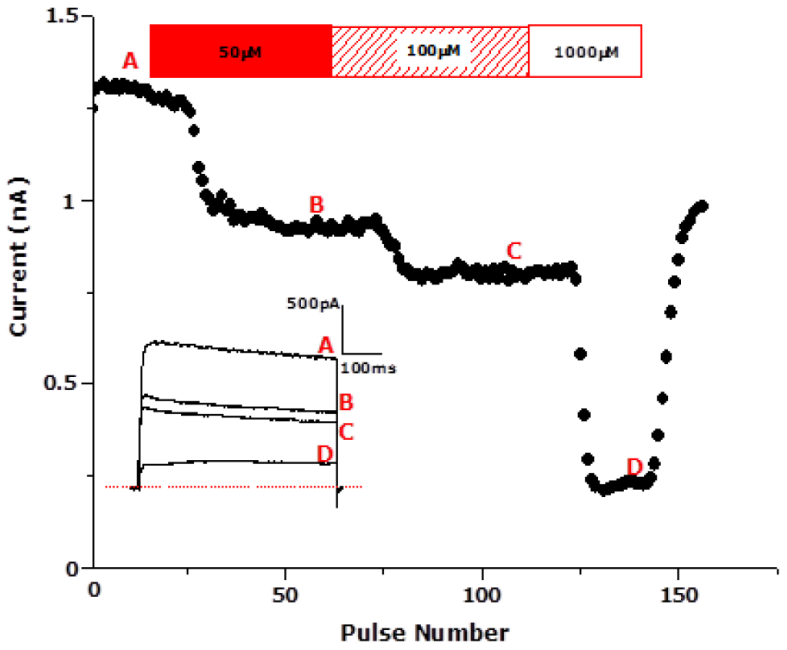
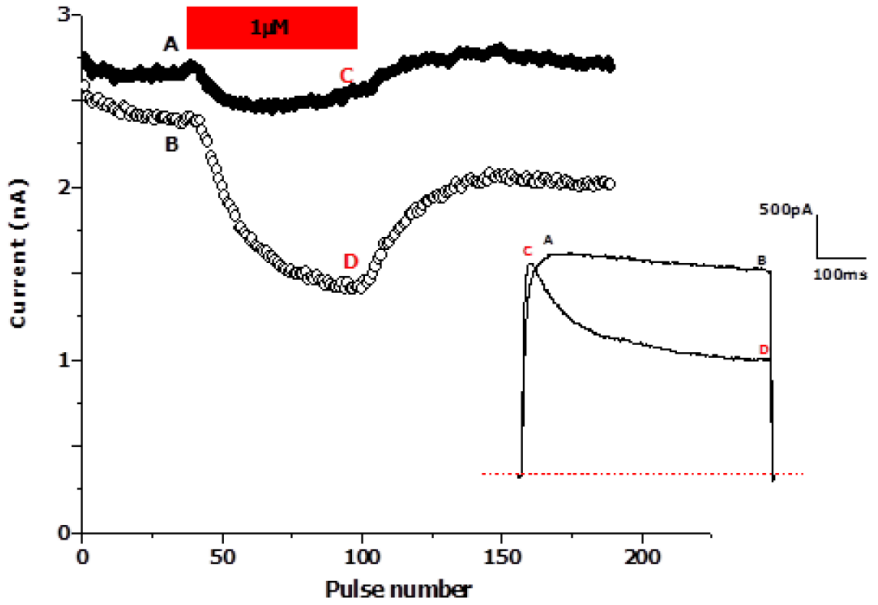




K_V1.5 Raw Data Currents and Current-Voltage (I/V) relationship: Membrane currents **(Left)** elicited by depolarising voltage pulses stepped in 10 mV increments from –60 mV to + 60 mV from a holding potential of –80 mV every 5 s. The red dotted line in the upper panel indicates zero current level. Current-voltage relationship **(Right)**. Current amplitudes were measured at the end of the 500 ms step, and normalised to the current amplitude obtained at 0 mV. The mean normalised data is shown (n =4). The mean current at +40 mV was 2340 ± 1000 pA (Manual Patch Clamp Data).

$K_V1.5$



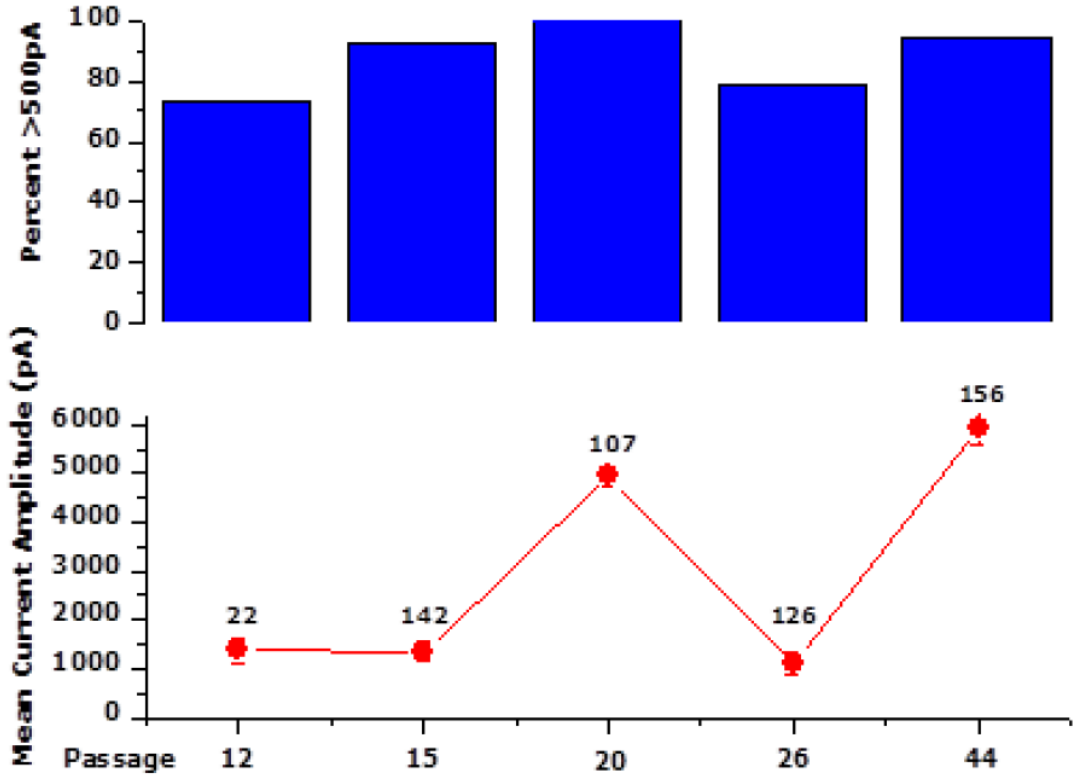


BACK

$K_V1.5$ Recovery from Inactivation Using a Two-Pulse Protocol; Stability of Expression over Passage: Left: The effect of 4-AP (50-1000 µM) on the hKv1.5 current. Kv currents were evoked continuously, every 10 s, by stepping the membrane voltage from a holding potential of –80 mV to +0 mV for 500 ms. Peak currents at 0 mV are plotted against pulse number below. The inset shows illustrative examples of current traces taken before (A) and at the end of 50 µM (B), 100 µM (C) & 1000 µM bath application of 4-AP. The red, red-hatched and open bars depict the presence of 50 µM, 100 µM and 1000 µM 4-AP respectively. **Right:** The effect of Loratadine (1 µM) on the hKv1.5 current. Kv currents were evoked continuously, every 5 s, by stepping the membrane voltage from a holding potential of –80 mV to +0 mV for 500 ms. Peak current (solid circles) and current at the end of the depolarising step (open circles) are plotted against pulse number below. The inset shows two traces each showing the points at which peak and end measurements were taken. Peak (A) and end (B) prior to addition of Loratadine and peak (C) and end (D) after addition of Loratadine. The amplitudes at these points are also indicated on the main graph. The red bar depicts the presence of 1 µM Loratadine. **Left and Right:** The red dotted line on the inset indicates zero current level. (Manual Patch Clamp Data).

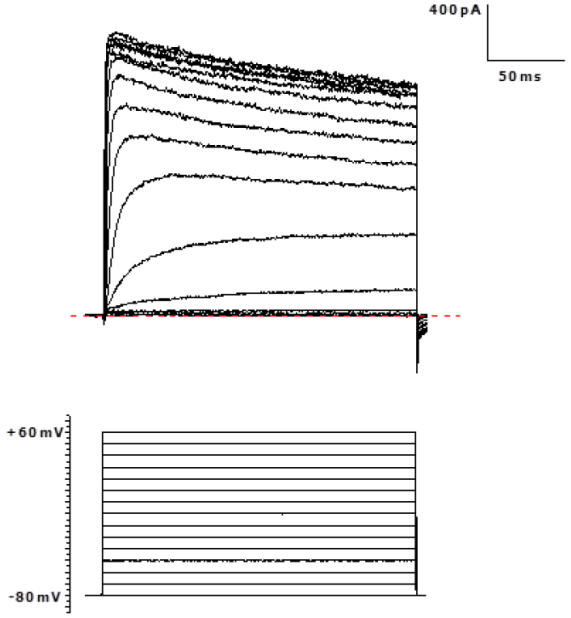
$K_V1.5$

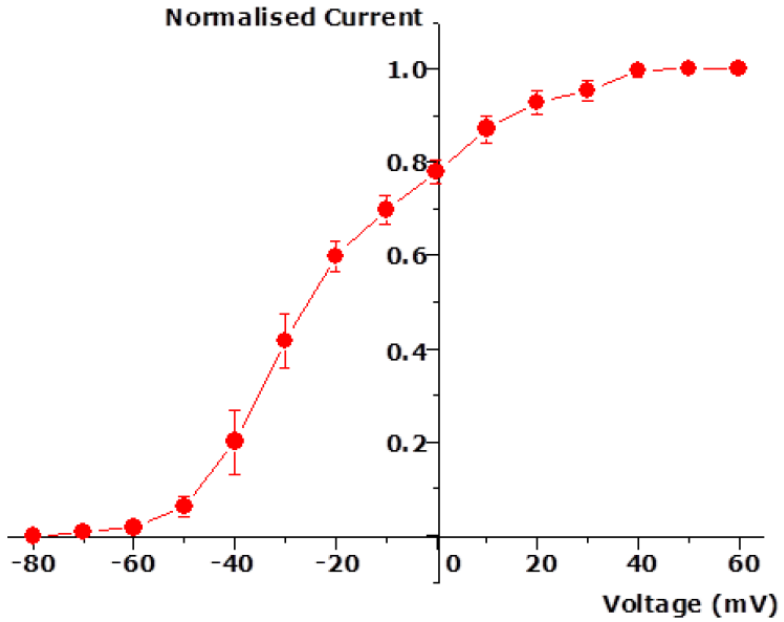
BACK



$K_V1.5$ Stability and Expression over Passage: The upper panel shows the percentage of cells expressing a mean peak outward current of >500 pA for cell passages 12, 15, 20, 26 and 44. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles). (IonWorks HT Data).

K_V1.6 (CYL3019)



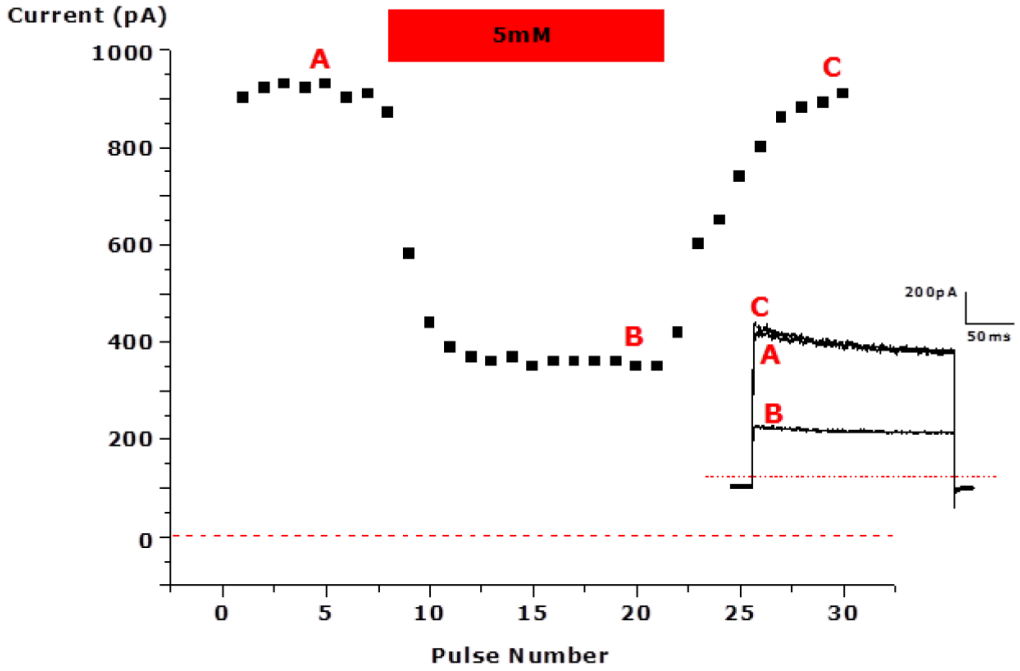


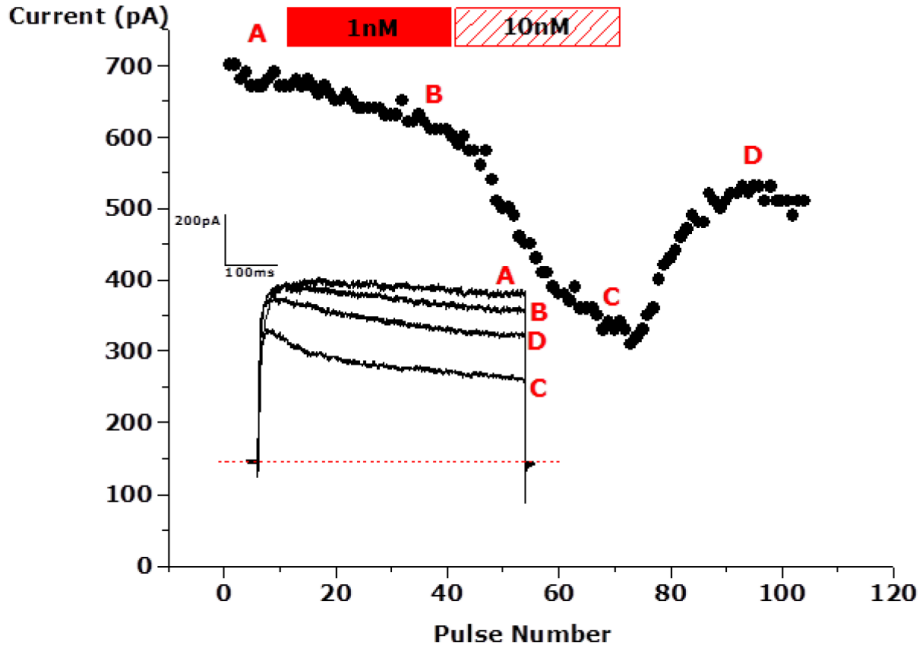
BACK

KV1.6 Raw Data Currents and Current-Voltage (I/V) relationship: Left: Membrane currents **(upper panel)** elicited by 200 ms depolarising voltage pulses **(lower panel)**, stepped in 10 mV increments from -80 mV to + 60 mV from a holding potential of -80 mV every 10 s. The red dotted line in the upper panel indicates zero current level. Right: Current/voltage relationship. Current amplitudes were measured at the end of the 200 ms step, and normalized to the current amplitude obtained at +60 mV. The mean normalized data from five cells is shown. The mean current at +60 mV was 1740 ± 314 pA (n=5). (Manual Patch Clamp Data).

$K_V1.6$

BACK





$K_V1.6$ Pharmacology: Left: The effect of TEA (5 mM) on the hKv1.6 current. Kv1.6 currents were evoked every 10 s by stepping the membrane voltage from a holding potential of -80 mV to +60 mV for 200 ms. Currents measured at the end of the step to +60 mV are plotted against pulse number below. The inset shows three illustrative examples of current traces taken before (A), during (B) and after (C) TEA addition. The red bar depicts the presence of 5 mM TEA and the red dotted line indicates the zero current level. **Right:** The effect of Margatoxin (1-10 nM) on the hKv1.6 current. Kv currents were evoked continuously, every 10 s, by stepping the membrane voltage from a holding potential of -80 mV to 0 mV for 500 ms. Currents at the end of the step to 0 mV are plotted against pulse number below. The inset shows illustrative examples of current traces taken before the addition of Margatoxin (A), at the end of 1 nM and 10 nM Margatoxin (B and C respectively) and after wash out of Margatoxin (D). The red and red-hatched bars depict the presence of 1 and 10 nM Margatoxin respectively and the red dotted line indicates zero current level.

$K_V1.6$

BACK

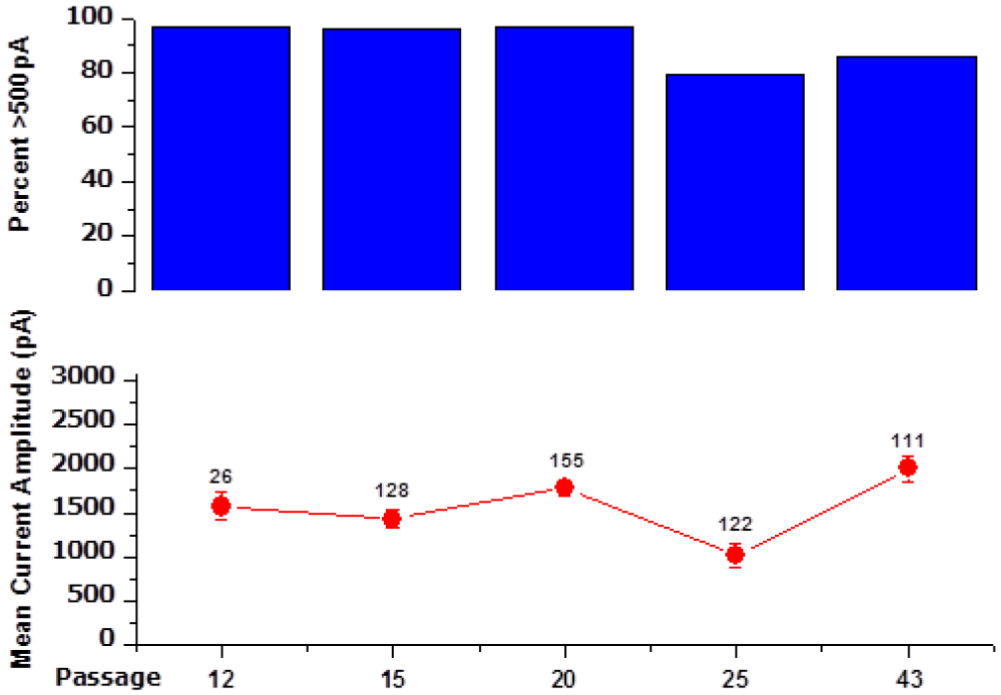
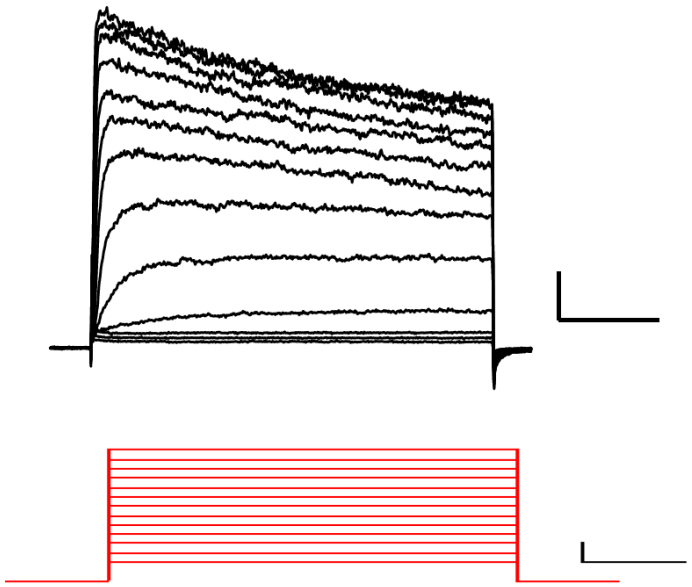
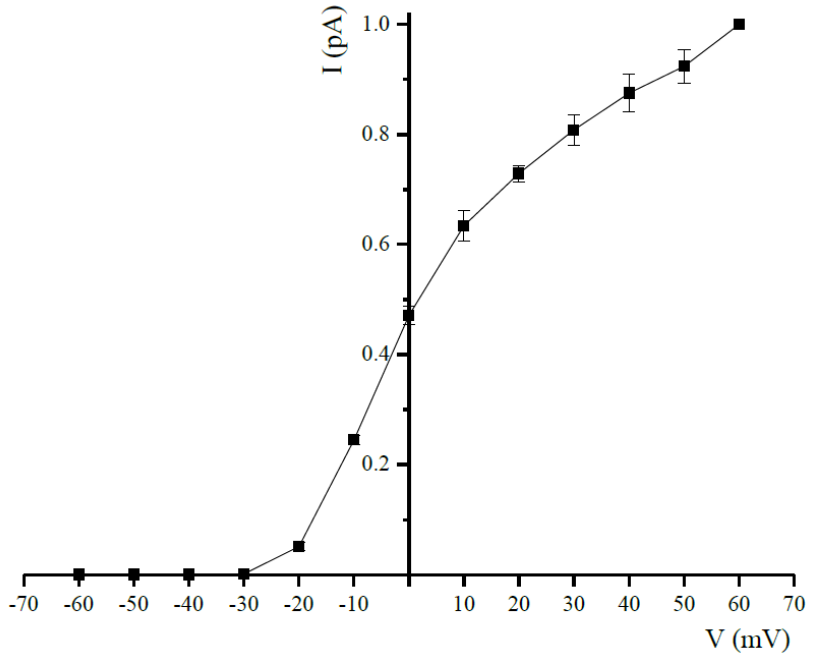


Figure 5. Stability of Expression over Passage: The upper panel shows the percentage of cells expressing a mean peak outward current of >500 pA for cell passages 12, 15, 20, 25 and 43. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles).

K_V1.7 (CYL3020)

BACK

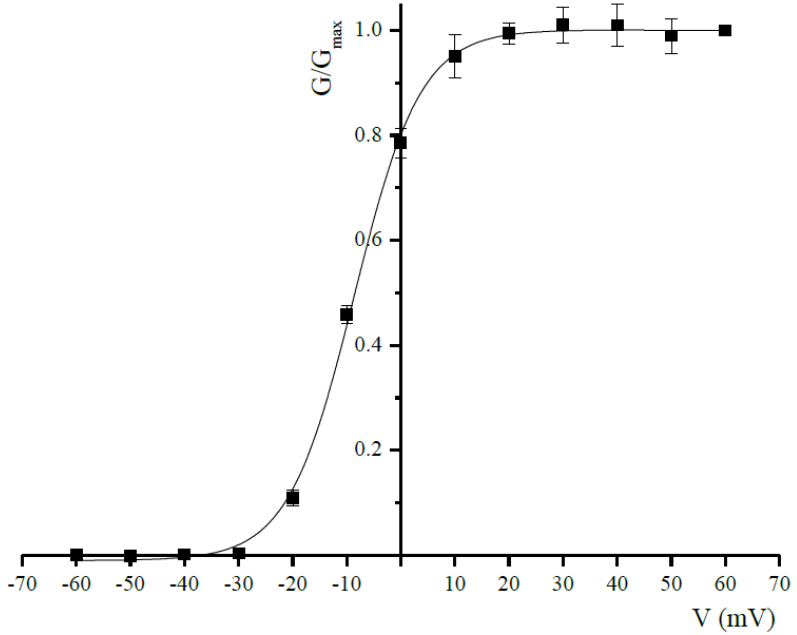




K_V1.7 Raw Data Currents and Current-Voltage (I/V) Relationship: **Left:** Typical hKv1.7 currents (upper panel) elicited by depolarising voltage pulses (lower panel), stepped from -60 mV to + 60 mV in 10 mV increments every 30 s from a holding potential of -80 mV. Scale bars represent 50 ms (x-axis) and 200 pA (y-axis) in the upper panel and 50 ms (x-axis) and 20 mV (y-axis) in the lower panel respectively. **Right:** Current amplitudes were measured at the end of the 200 ms step and normalized to the current amplitude obtained at +60 mV. The mean current at +60 mV was 2.69 ± 0.8 nA (n=4) (Manual Patch Clamp Data)

$K_V1.7$

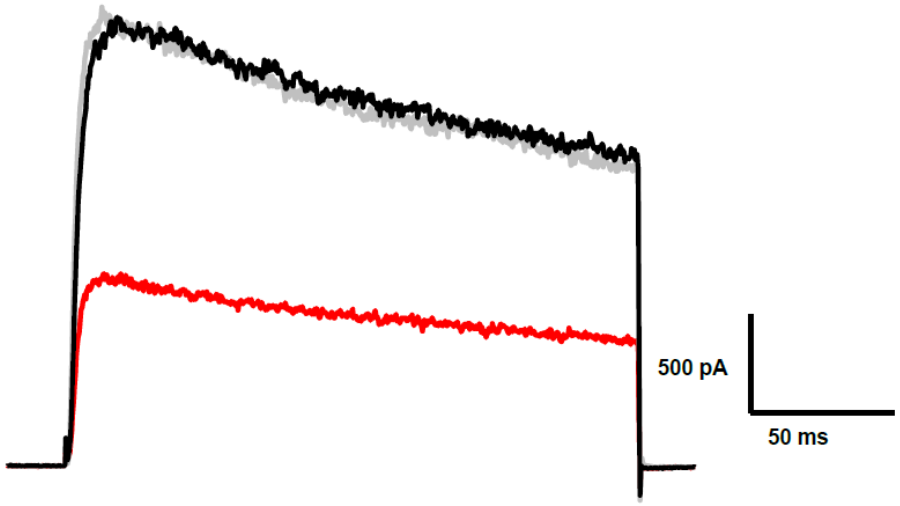
BACK

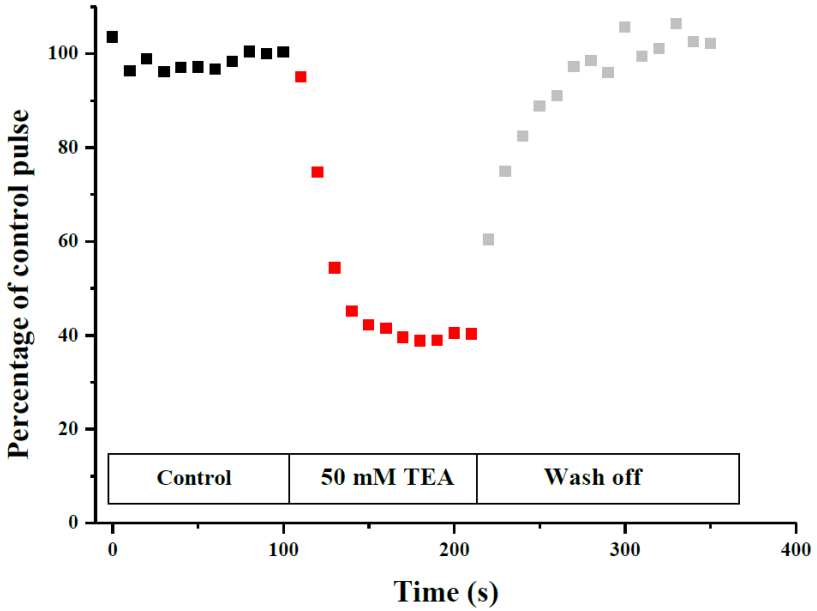


K_V1.7 Conductance-Voltage (G/V) Curve: The current (I) was converted into conductance (G) by use of the following equation: G = I/(V-EK), where the Nernst value (EK) was calculated as -89.82 mV. Data was fitted with a single Boltzmann equation. V½ = -8.6 ± 0.3 mV and slope (k) = 6.1 ± 0.3 mV. Values represent mean ± SEM (n = 4).

$K_V1.7$

BACK

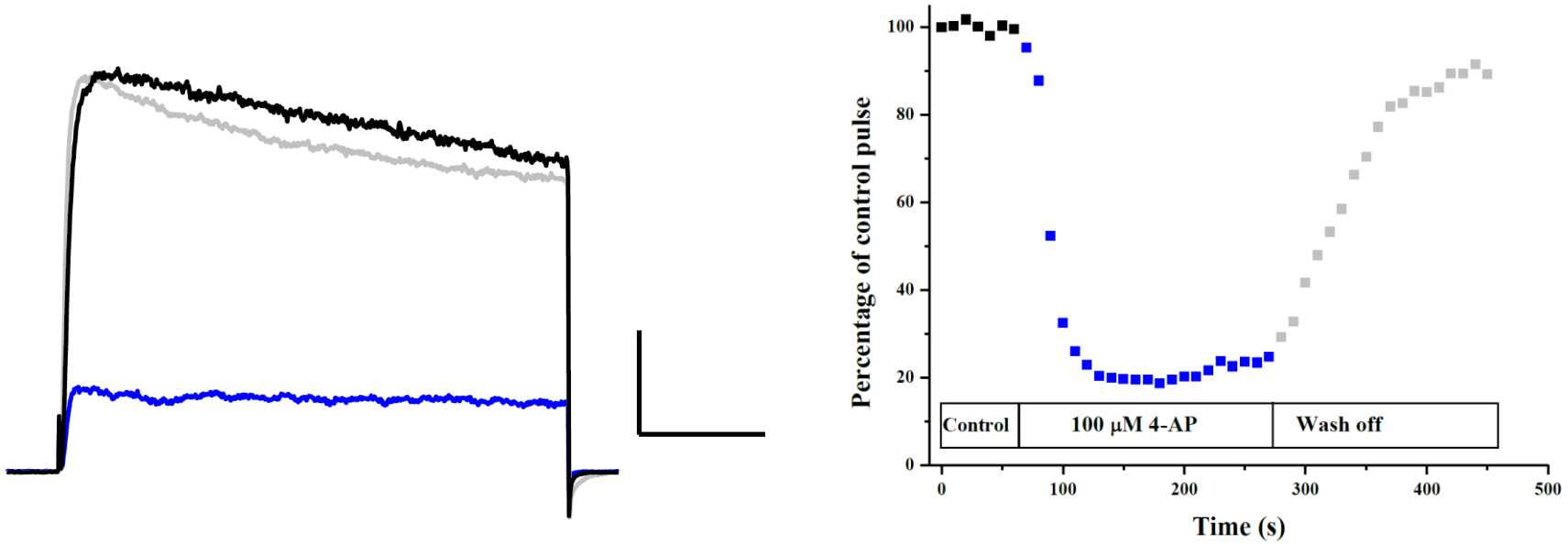




Blockade of $K_V1.7$ currents by Tetraethylammonium (TEA): **Left:** hKv1.7 current traces evoked by stepping the membrane voltage to 0 mV from a holding potential of –80 mV in the presence of TEA; before the addition of TEA (control - black), after addition of 50 mM TEA (red) and after wash-off of TEA (grey). Scale bars represent 50 ms (x-axis) and 500 pA (y-axis). Right: Typical hKv1.7 currents evoked by stepping to 0 mV from –80 mV in the presence of 50 mM TEA. Current amplitudes at the end of the step are plotted against time. Currents normalized to control current. Inhibition = 56-61%. hKv1.7 channels were stepped for 200 ms every 10 s.

$K_V1.7$

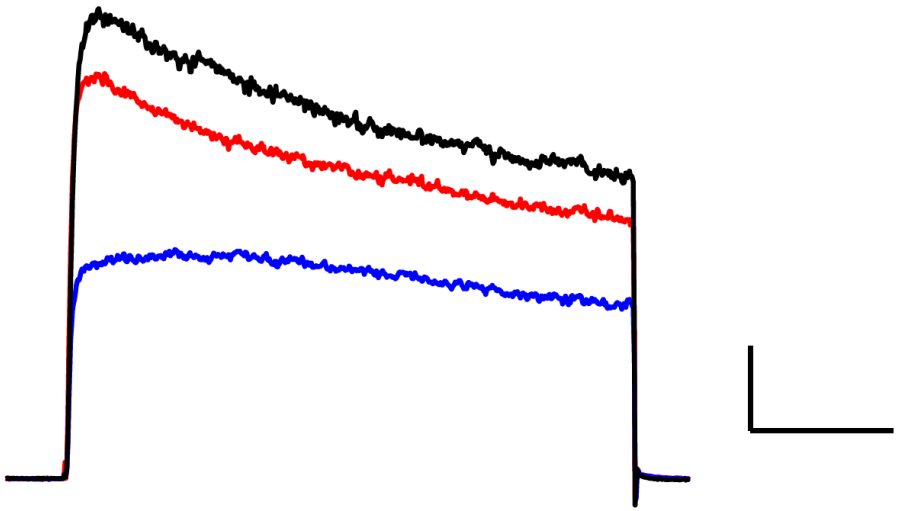
BACK

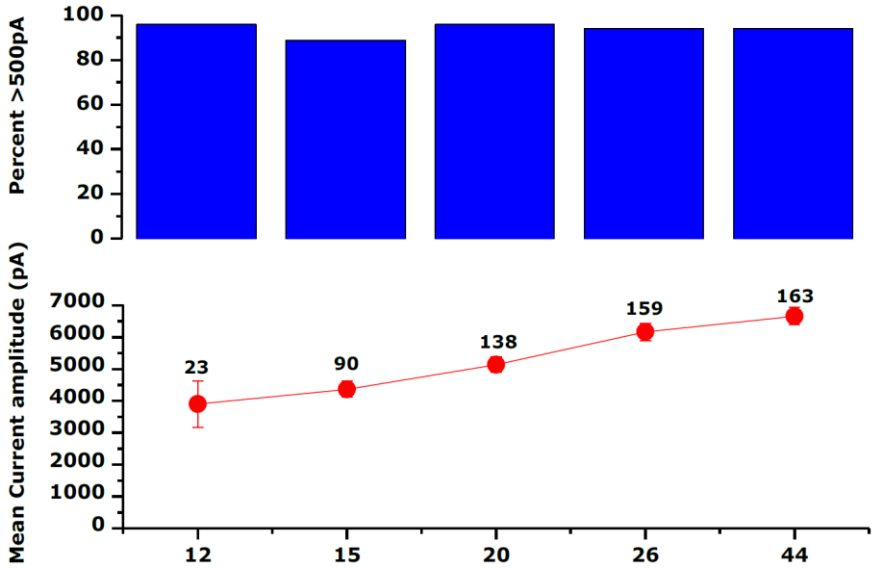


Blockade of $K_V1.7$ currents by 4-Aminopyridine (4-AP): **Left:** hKv1.7 currents traces evoked by stepping the membrane voltage to 0 mV from a holding potential of –80 mV in the presence of 4-AP; before the addition of 4-AP (control - black), after addition of 100 µM 4-AP (blue) and after wash off of 4-AP (grey). Scale bars represent 50 ms (x-axis) and 1 nA (y-axis). **Right:** Typical hKv1.7 currents evoked by stepping to 0 mV from –80 mV in the presence of 4-AP. Current amplitudes at the end of the step are plotted against time below. Currents normalized to control current. Inhibition = 82.6 ± 2.6 % (n = 3). hKv1.7 channels were stepped for 200 ms every 10 s.

$K_V1.7$

BACK

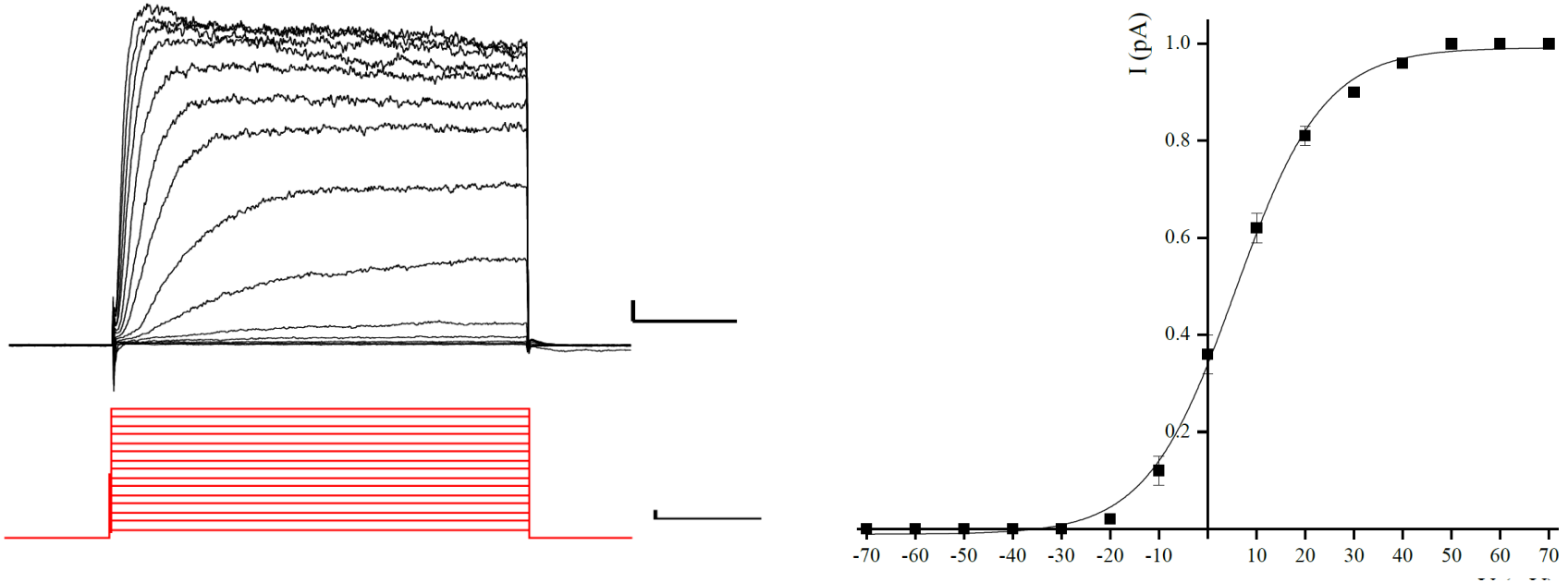




Blockade of $K_V1.7$ Currents by Stichodactyla Toxin (ShK) and Stability of expression over passage: **Left:** hKv1.7 current traces evoked by stepping the membrane voltage to 0 mV from a holding potential of –80 mV in thepresence of ShK (10 nM and 50 nM); before the addition of ShK (control - black), after the addition of 10 nM ShK (red), 50 nM ShK (blue). Scale bars represent 50 ms (x-axis) and 500 pA (y-axis). hKv1.7 channels were stepped for 200 ms every 10 s. (Manual Patch Clamp Data) Right: upper panel shows the percentage of cells expressing a mean peak outward current >500pA for cell passages 12, 15, 20 and 44. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles) (IonWorks HT Data).

K_V1.8 (CYL3021)

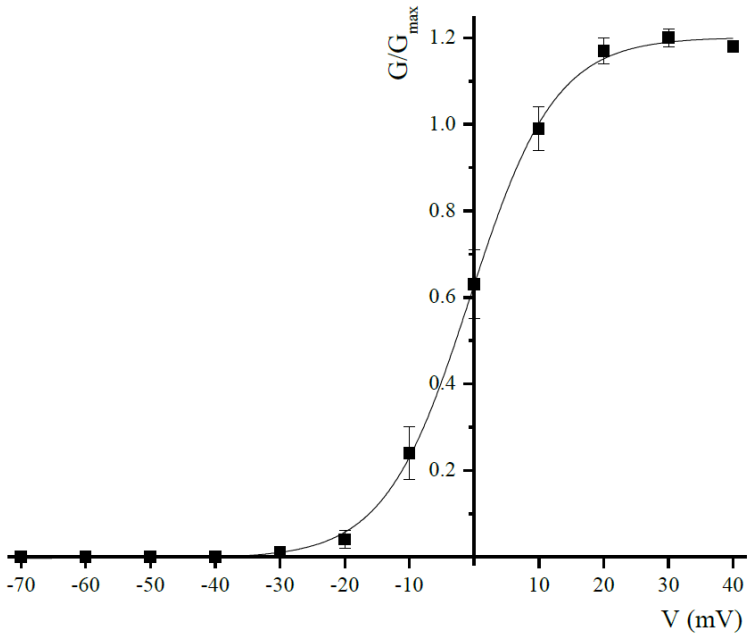
BACK



K_V1.8 Raw Data Currents and Current-Voltage (I/V) relationship: **Left:** Typical hKv1.8 currents (upper panel) elicited by depolarising voltage pulses (lower panel) from –70 mV to +70 mV in 10 mV increments every 10 s from a holding potential of –80 mV. Scale bars represent 50 ms (x-axis) and 100 pA (y-axis) in the upper panel and 50 ms (x-axis) and 10 mV (y-axis) in the lower panel respectively. Right: Current amplitudes were measured at the end of the 200 ms step and normalized to the current amplitude obtained at +70 mV. The mean current at +70 mV was 606.1 ± 136.2 pA (n=10). (Manual Patch Clamp Data)

$K_V1.8$

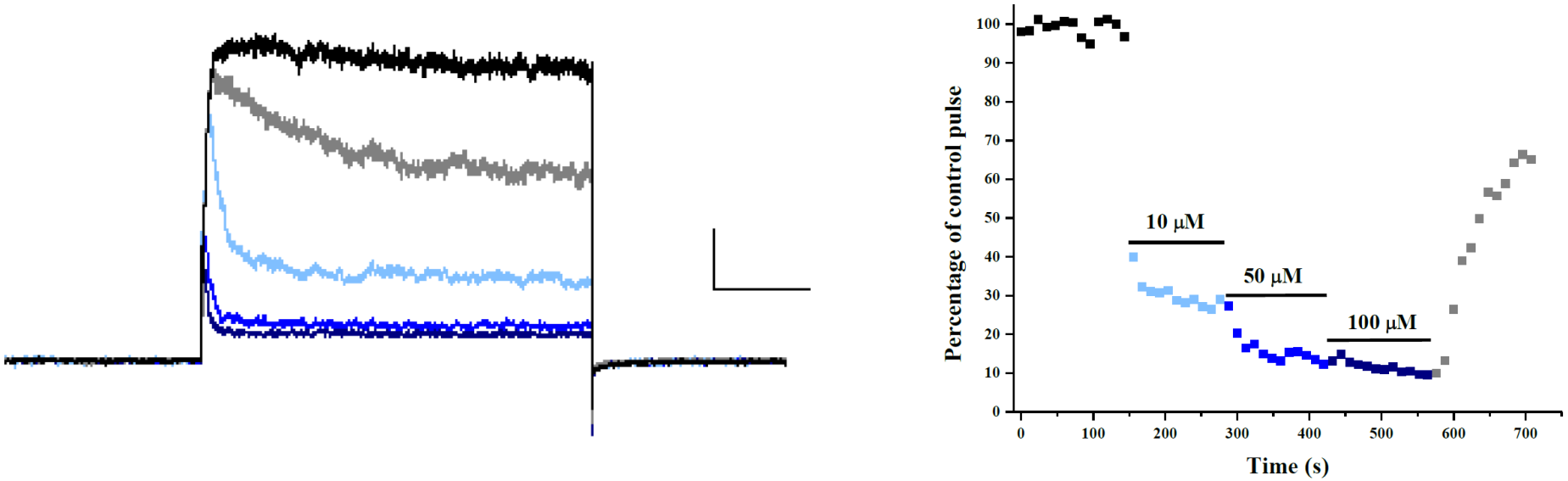
BACK



Conductance-Voltage (G/V) Plot, Stability of Currents over Passage: **Left:** he current (I) was converted into conductance (G) by use of the following equation: G = I/(V-EK), where the Nernst value (EK) was calculated as -89.82 mV. Data was fitted with a single Boltzmann equation. V½ = -0.7 ± 0.3 mV and slope (k) = 6.6 ± 0.2 mV. Values represent means ± SEM (n = 10). (Manual Patch Clamp Data)

$K_V1.8$

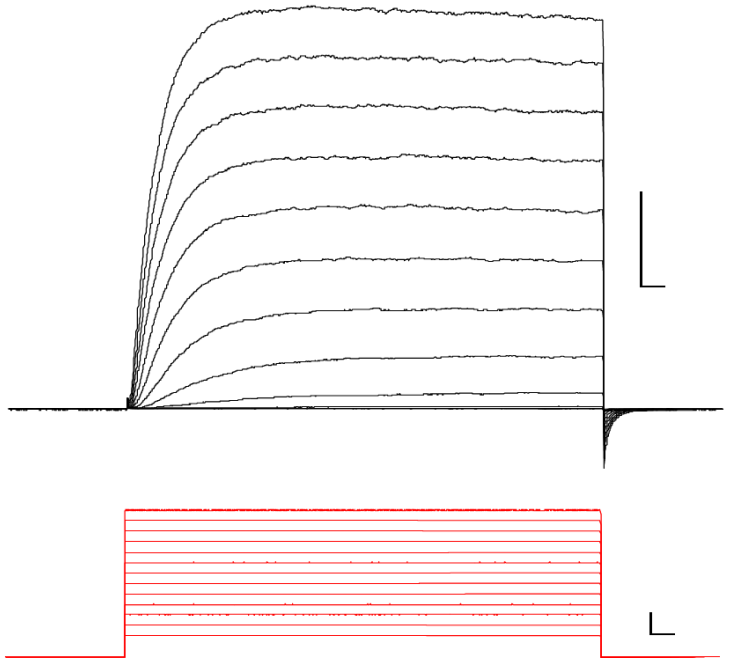
BACK

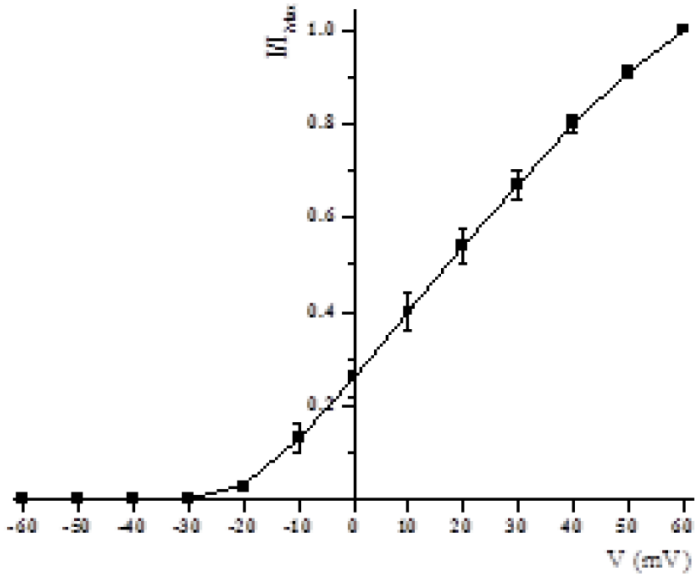


Blockade of $K_V1.8$ Currents by Verapamil: **Left:** hKv1.8 current traces evoked by stepping to +40 mV from a holding potential of –80 mV in the presence of various concentrations of verapamil; before the addition of verapamil (control - black trace), after addition of 10 µM (light blue), 50 µM (blue), 100 µM (dark blue) and after wash off of verapamil (grey). Scale bars represent 250 ms (x-axis) and 100 pA (y-axis). **Right:** Typical hKv1.8 currents evoked by stepping to +40 mV from –80 mV in the presence of increasing concentrations of verapamil. Current amplitudes at the end of the step are plotted against time. Currents normalized to control current. hKv1.8 channels were stepped for 1 s every 12 s. (Manual Patch Clamp Data)

K_V2.1 (CYL3022)

BACK

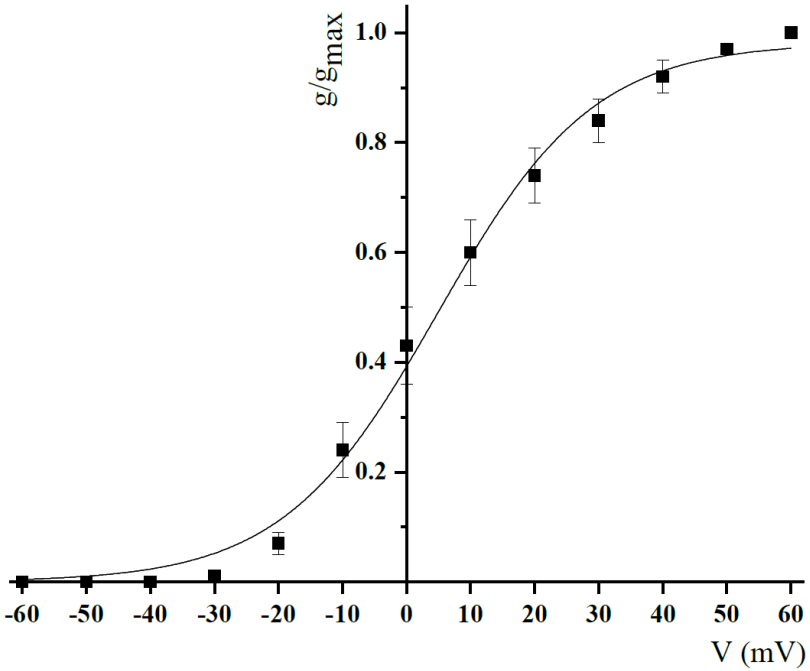


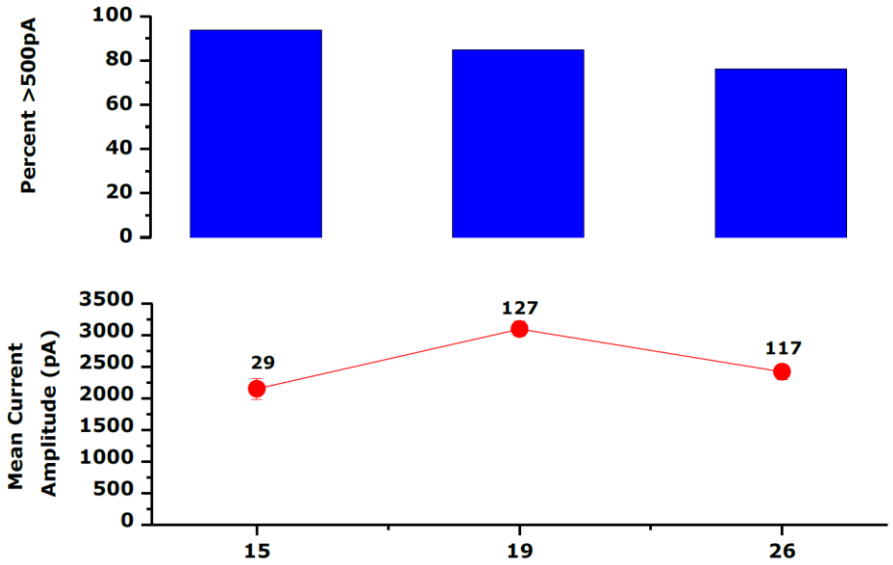


K_V2.1 Raw Data Currents and Current-Voltage (I/V) Relationship: **Left:** Typical hKv2.1 current traces (upper panel) elicited by depolarizing voltage pulses from –60 mV to +60 mV in 10 mV increments (lower panel) from a holding potential of –80 mV. Scale bars represent 10 ms and 2 nA (upper panel) and 10 ms and 20 mV (lower panel) respectively. **Right:** Current amplitudes measured at the end of the 200 ms step. Currents normalized to the current amplitude obtained at +60 mV. The mean current at 60 mV was 5.63 ± 0.86 nA (n=11). (Manual Patch Clamp Data)

$K_V2.1$

BACK

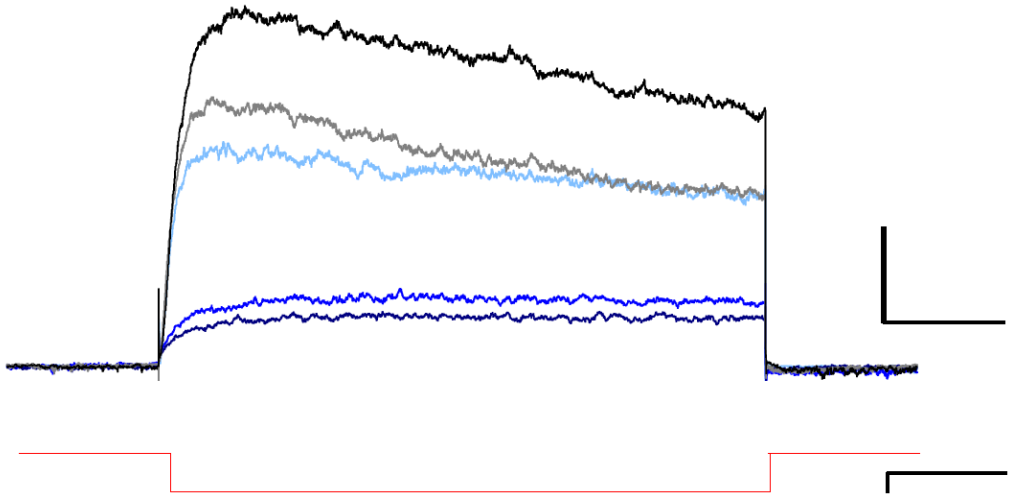


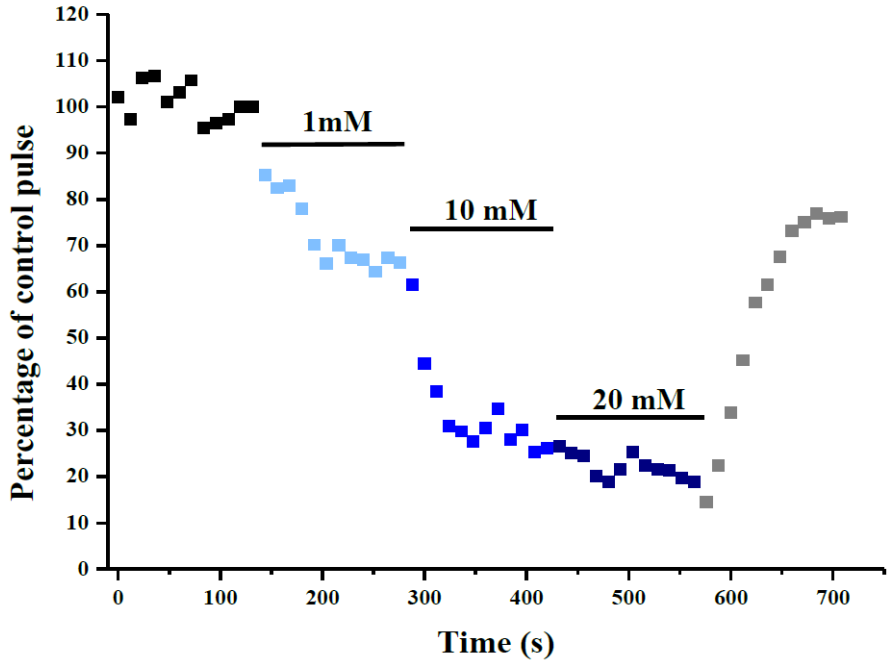


$K_V2.1$ Conductance-Voltage (G/V) Relationship, Stability and Expression over Passage: **Left:** Current (I) converted into conductance (G) by use of the following equation: G = I/(V-EK), where the Nernst EK was calculated as -89.82 mV. Data was fitted with a single Boltzmann equation. V½ = 4.9 ▯ 1.1 mV and slope (k) = 12.1 ▯ 0.9 mV. Values represent means ▯ SEM n = 11. (Manual Patch Clamp Data) **Right**: The upper panel shows the percentage of cells expressing a mean peak outward current >500 pA for cell passages 15, 19 and 26. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers adjacent red circles). (IonWorks HT Data)

$K_V2.1$

BACK

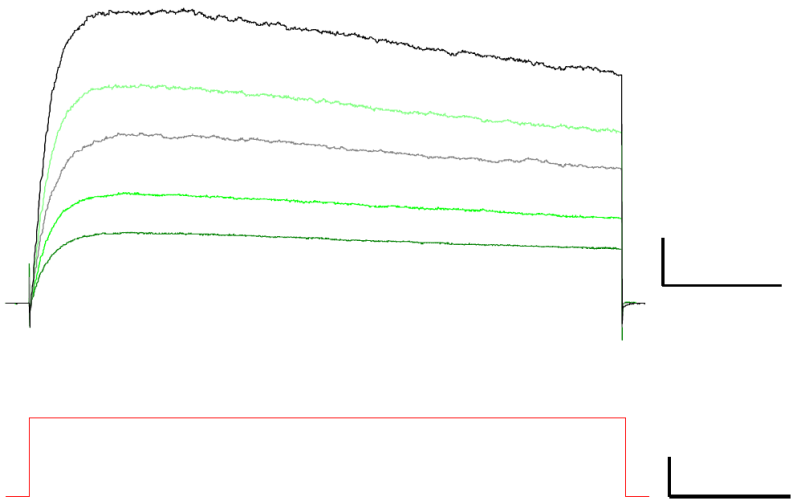


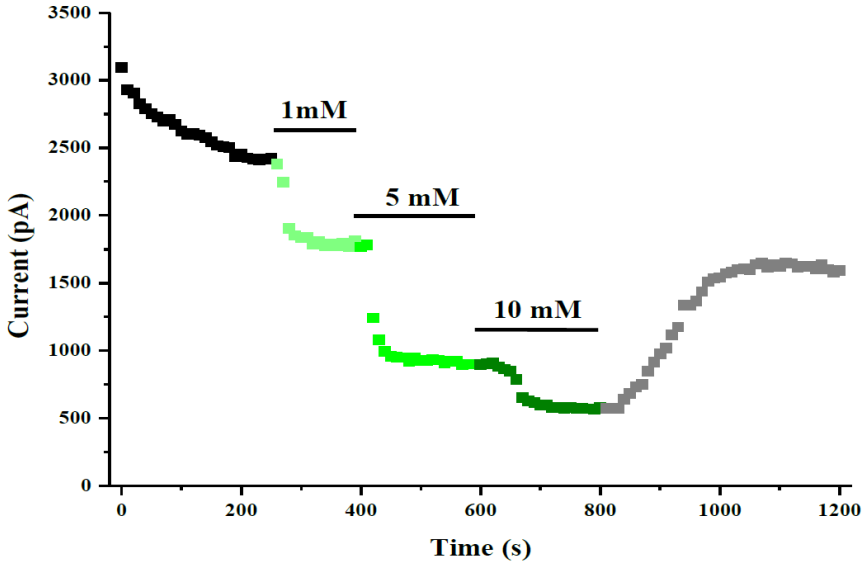


Blockade of $K_V2.1$ Currents by 4-Aminopyridine (4-AP): **Left:** hKv2.1 current traces evoked by stepping to –20 mV from a holding potential of –80 mV in the presence of various concentrations of 4-AP. Upper panel; before the addition of 4-AP (Control - black trace), after addition of 1 mM 4-AP (light blue), 10 mM 4-AP (blue), 20 mM 4-AP (dark blue) and after wash off of 4-AP (grey). Scale bars represent 200 ms and 200 pA. Lower panel; voltage-step protocol. Scale bars represent 200 ms and 30 mV. **Right:** Mean hKv2.1 currents evoked by stepping to –20 mV from –80 mV. Currents normalized to control current. hKv2.1 channels were stepped for 1 s every 12 s. (Manual Patch Clamp Data)

$K_V2.1$

BACK

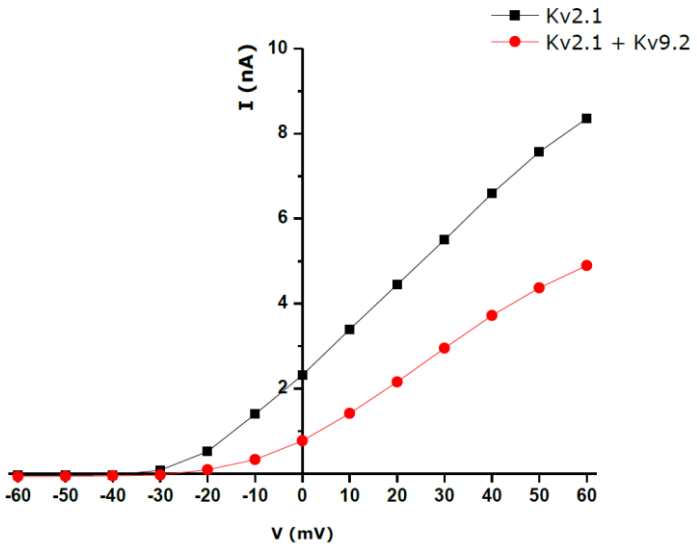


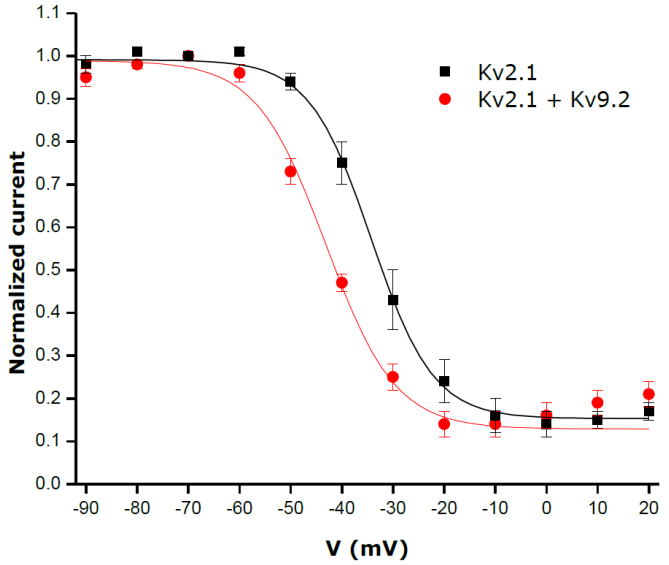


Blockade of $K_V2.1$ Currents by Tetraethylammonium (TEA): **Left:** hKv2.1 current traces evoked by stepping to 0 mV from a holding potential of –80 mV in the presence of various concentrations of TEA. Upper panel: before the addition of TEA (Control - black trace), after addition of 1 mM TEA (light green), 5 mM TEA (green), 10 mM TEA (dark green) and after wash off of TEA (grey). Scale bars represent 200 ms and 200 pA. Lower panel: Voltage-step protocol. Scale bars represent 200 ms and 30 mV. **Right:** Typical hKv2.1 currents produced by depolarizing pulse in the presence of various concentrations of TEA. hKv2.1 channels were stepped from a holding potential of –80 mV to 0 mV for 500 ms every 10 s. (Manual patch Clamp Data)

$K_V2.1/K_V9.2$ (CYL3067)

BACK

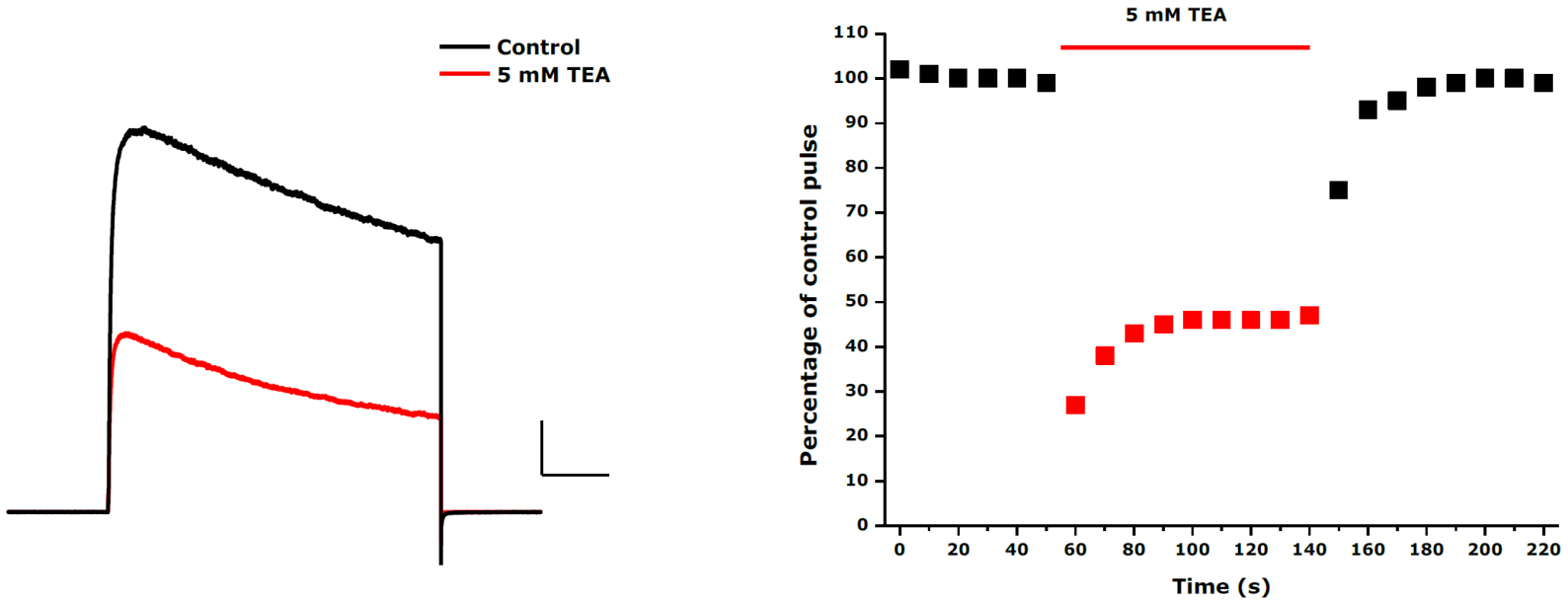




Current-Voltage (I/V) Relationships, Inactivation of hKv2.1 and hKv2.1/hKv9.2: **Left:** Current-voltage (I/V) relationship of hKv2.1 and hKv2.1/hKv9.2: The I/V relationship for both cell lines is shown in Figure 1. The biophysical properties of the hKv2.1 and hKv2.1/hKv9.2 channels were studied by stepping from the holding potential of the cell (-80 mV) to voltages of –60 mV to +60 mV in 10 mV steps every 10 s. The duration of each step was 200 ms.$K_V2.1/9.2$ Current-Voltage (I/V) relationship and Biophysics: **Right:** Inactivation of the hKv2.1/hKv9.2 current. Cells were stepped from a holding potential of –80 mV to a test pulse of 30 mV for 200 ms following pre-pulse voltages ranging from -90 to +20 mV for 10 s in 10 mV steps. Sweeps every 20 s. Currents normalized to current at pre-pulse of –70 mV. n = 3 for hKv2.1 and n = 5 for hKv2.1/hKv9.2. (Manual Patch Clamp Data)

$K_V2.1/K_V9.2$

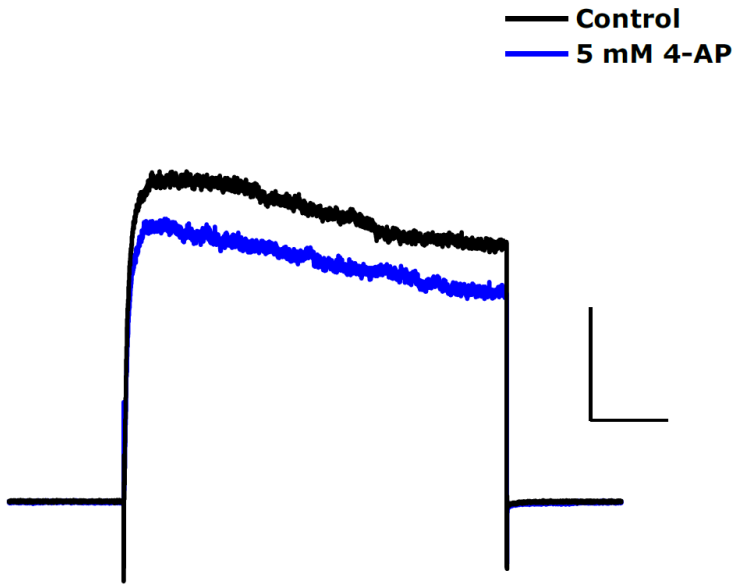
BACK

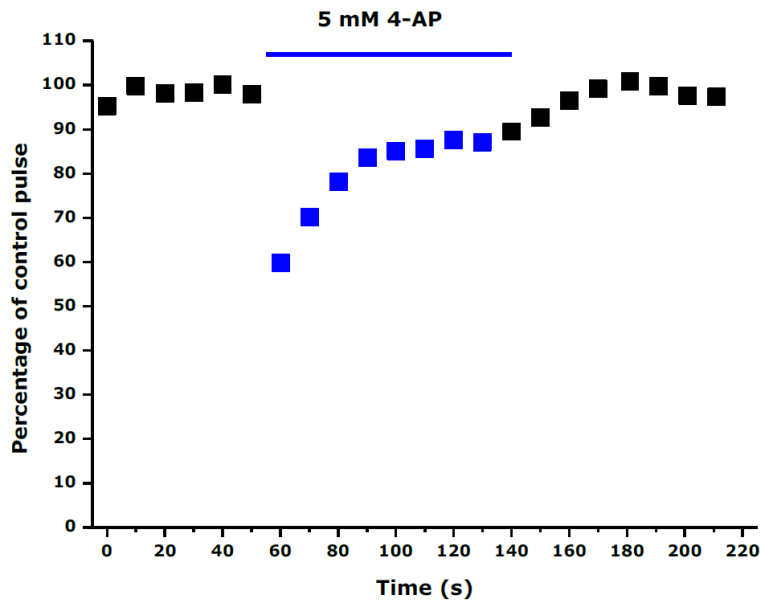


Blockade of $K_V2.1/9.2$ Currents by Tetraethylammonium (TEA): **Left:** Current traces evoked by stepping to +40 mV for 1 s from a holding potential of –80 mV, before (black trace) and after application of (red) 5 mM TEA. Traces show the level of steady state block that is achieved after an approximate 80 s incubation with 5 mM TEA. Scale bars represent 200 ms and 1 nA. **Right:** Typical time course of inhibition of hKv2.1/hKv9.2 currents by 5 mM TEA. Currents were evoked by stepping to +40 mV for 1 s from -80 mV. Sweeps every 10 s. Currents normalized to current before the addition of TEA. (Manual Patch Clamp Data)

$K_V2.1/K_V9.2$

BACK

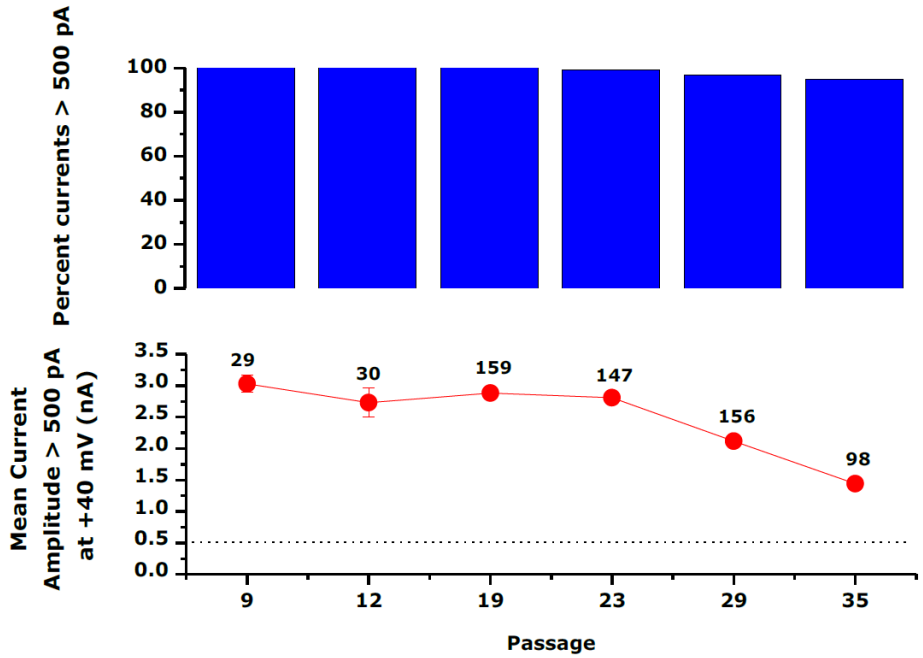




Effect of $K_V2.1/9.2$ currents by 4-Aminopyridine: **Left:** Current traces evoked by stepping to +40 mV for 1 s from a holding potential of –80 mV, before (black trace) and after application of (red) 5 mM TEA. Traces show the level of steady state block that is achieved after an approximate 80 s incubation with 5 mM TEA. Scale bars represent 200 ms and 1 nA. **Right:** Typical time course of inhibition of hKv2.1/hKv9.2 currents by 5 mM TEA. Currents were evoked by stepping to +40 mV for 1 s from -80 mV. Sweeps every 10 s. Currents normalized to current before the addition of TEA (Manual Patch Clamp Data).

$K_V2.1/K_V9.2$

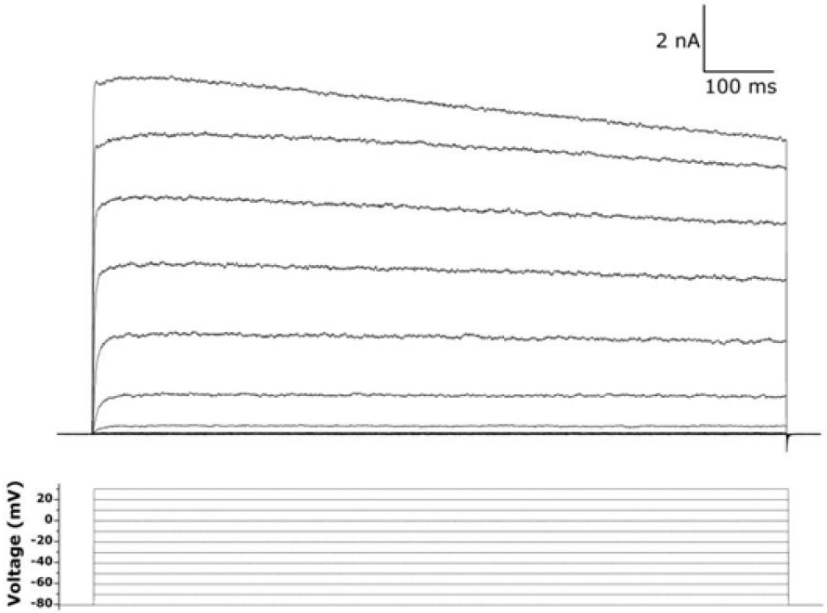
BACK

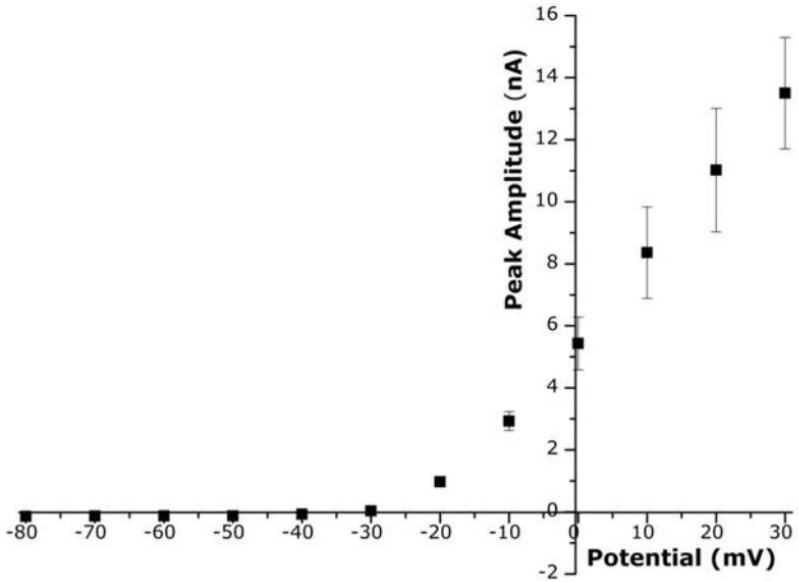


$K_V2.1/9.2$ Stability of Expression over Passage Number: The upper panel shows the percentage of cells expressing a mean peak current >500 pA at 40 mV at cell passages 9, 12, 19, 23, 29 and 35. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of cells (numbers above red circles - out of 32 cells for passages 9 & 12 and out of 160 cells for all other passages) (Manual Patch Clamp Data)

K_V3.1 (CYL3043)

BACK

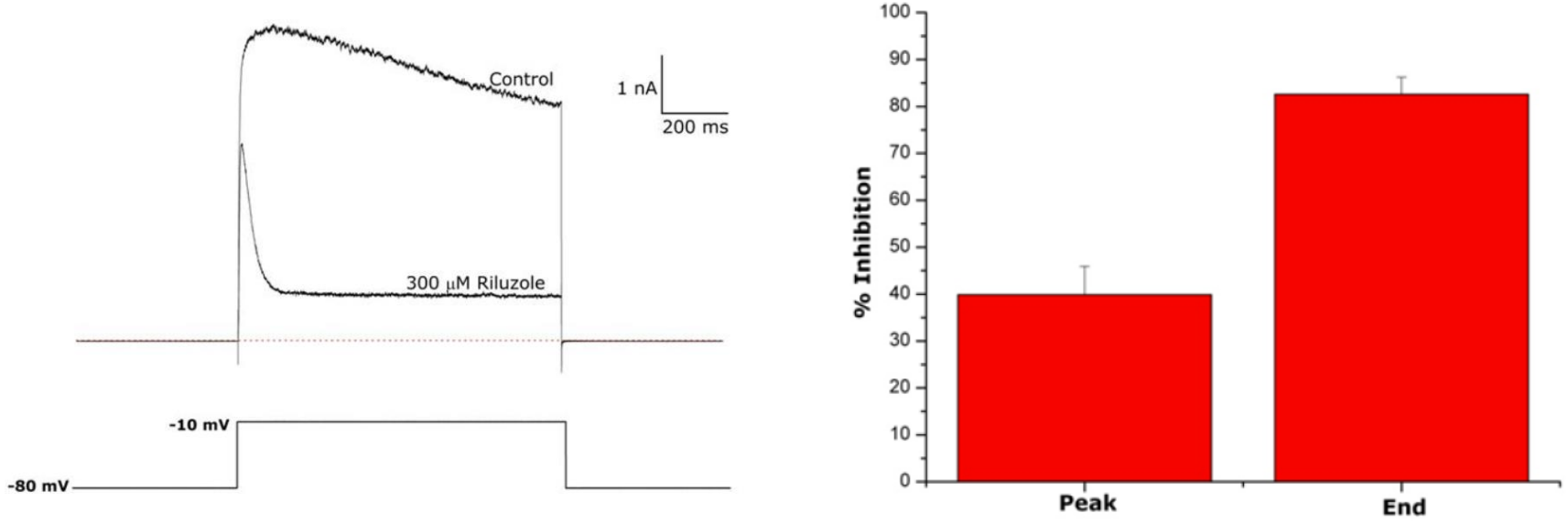




K_V3.1 Raw Data Currents, Current-Voltage (I/V) relationship: **Left:** Sample current traces from a cell held at –80 mV and with currents evoked by consecutive 1 s steps in 10 mV increments from –80 mV to +30 mV. The voltage protocol used is below the current traces. **Right:** Cells were held at –80 mV and currents evoked by 1 s steps in 10 mV increments from –80 mV to +30 mV (as example in Figure 1). Mean peak current amplitudes are plotted against voltage to outline the I/V characteristics (n=3) (Manual Patch Clamp Data).

$K_V3.1$

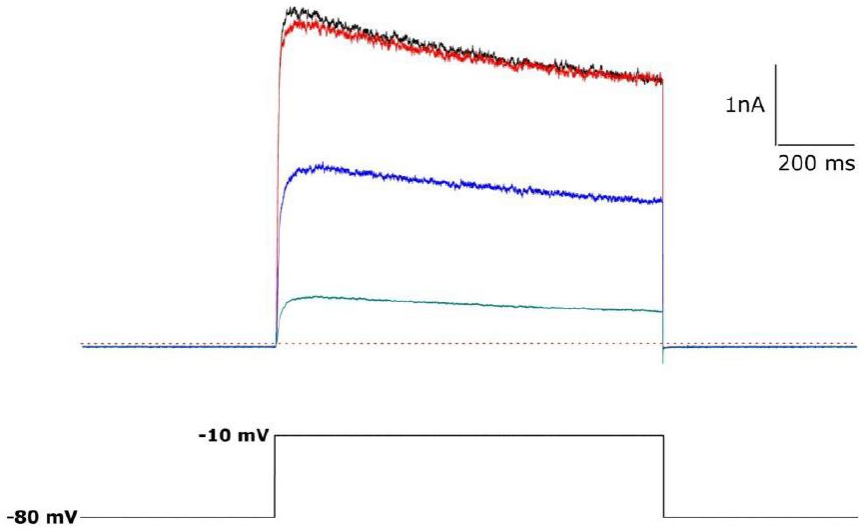
BACK

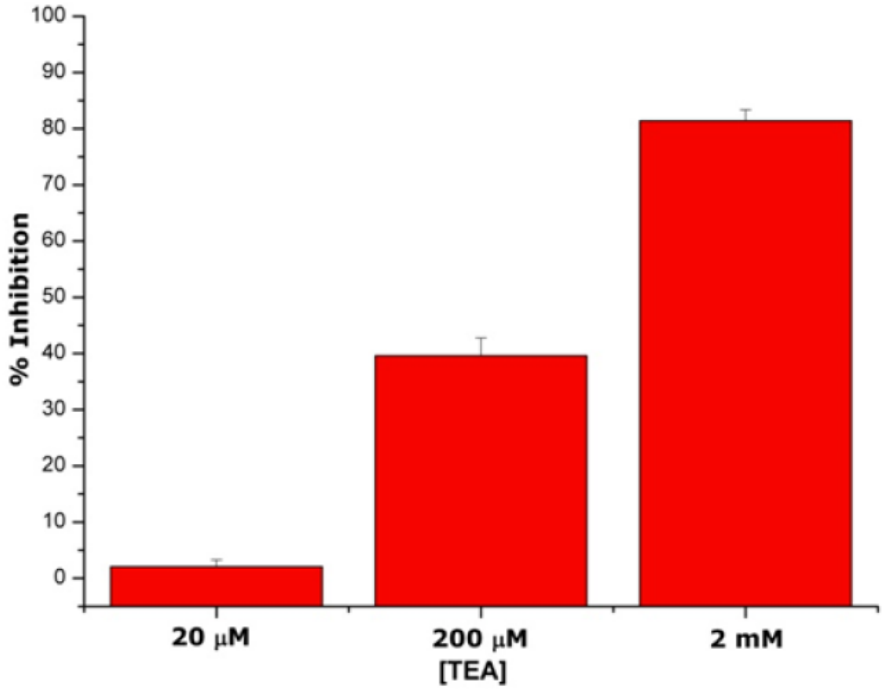


Blockade of $K_V3.1$ Currents by Riluzole: **Left:** Sample current trace for a cell held at –80 mV and current evoked by 1 s steps to –10 mV in control buffer conditions and in the presence of 300 µM Riluzole. The voltage protocol used is below the current traces. **Right:** Effect of riluzole on peak and end hKv3.1 currents. The mean percent inhibition of current by 300 µM riluzole at the peak and at end of the depolarizing pulse (see Figure 3), n=5.

$K_V3.1$

BACK

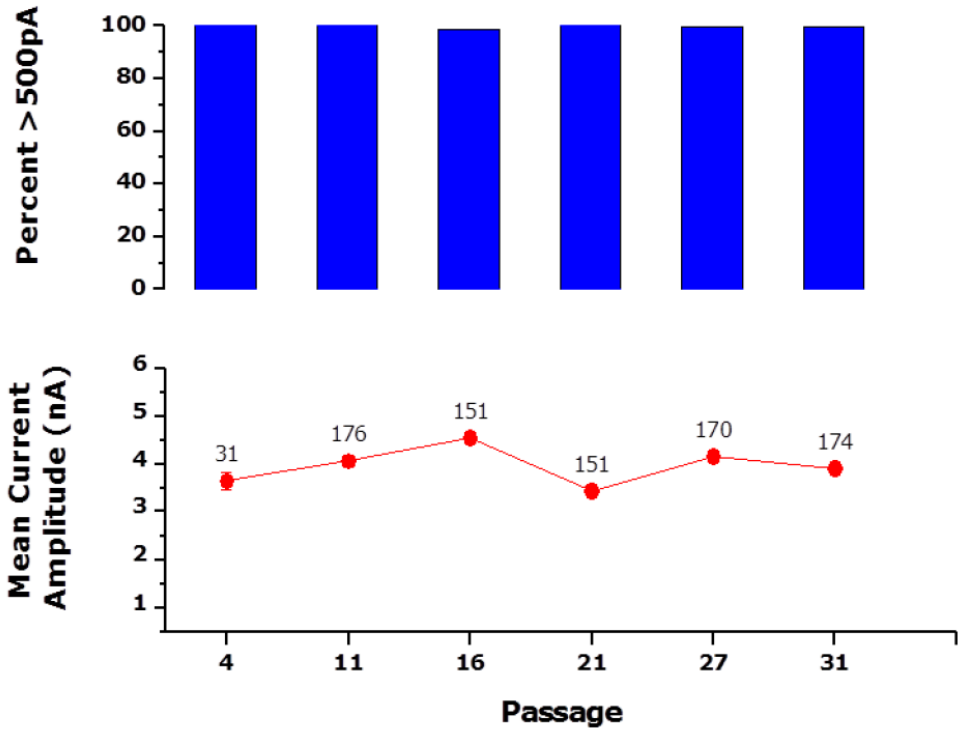




Effect of TEA on $K_V3.1$ Currents: Left: Sample current traces for a cell held at –80 mV with currents evoked by 1 s steps to –10 mV in control buffer (black trace) or perfused with 20 µM (red), 200 µM (blue) and 2mM TEA (cyan). The red dotted line represents zero current level. The voltage protocol used is below the current traces. **Right:** Effect of TEA on hKv3.1 currents. Mean percent inhibition of peak current by 20 µM, 200 µM and 2 mM TEA (n=5). (Manual Patch Clamp Data)

$K_V3.1$

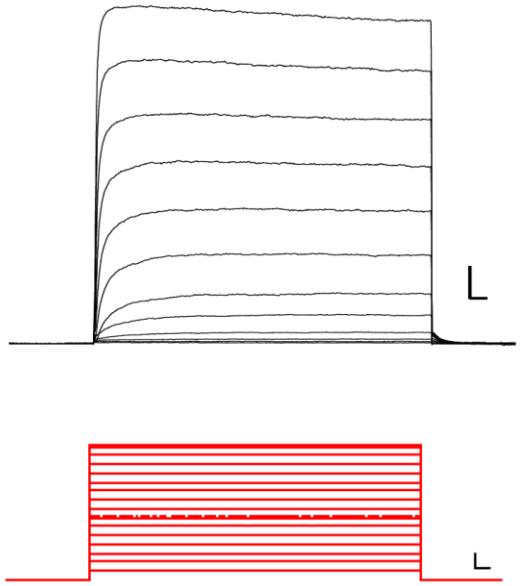
BACK

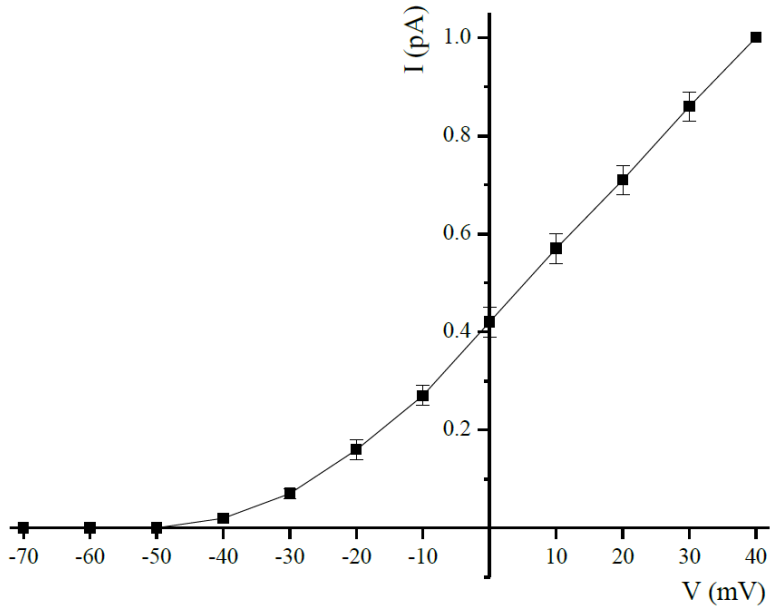


$K_V3.1$ Stability over Passage Number: The upper panel shows the percentage of cells expressing a mean peak current ≥500 pA (+30 mV) at cell passages 4, 11, 16, 21, 27 and 31. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles – out of 32 cells at passage 4 and out of 192 cells for all other passages).

K_V3.2 (CYL3044)

BACK

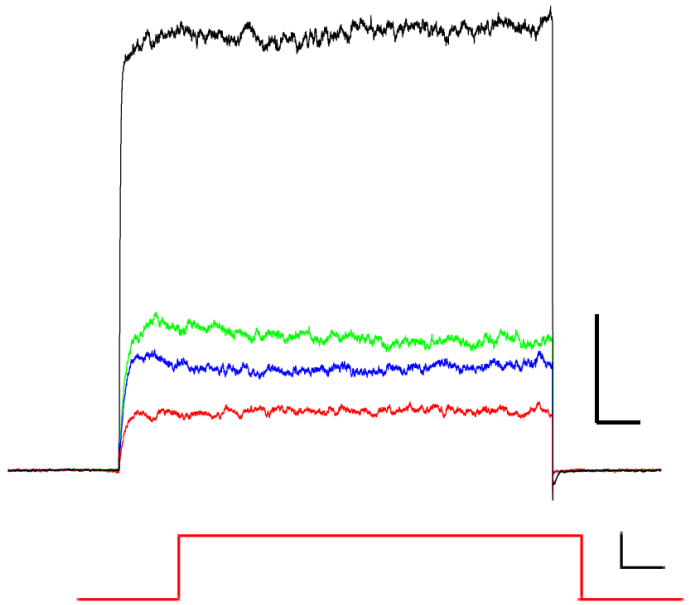


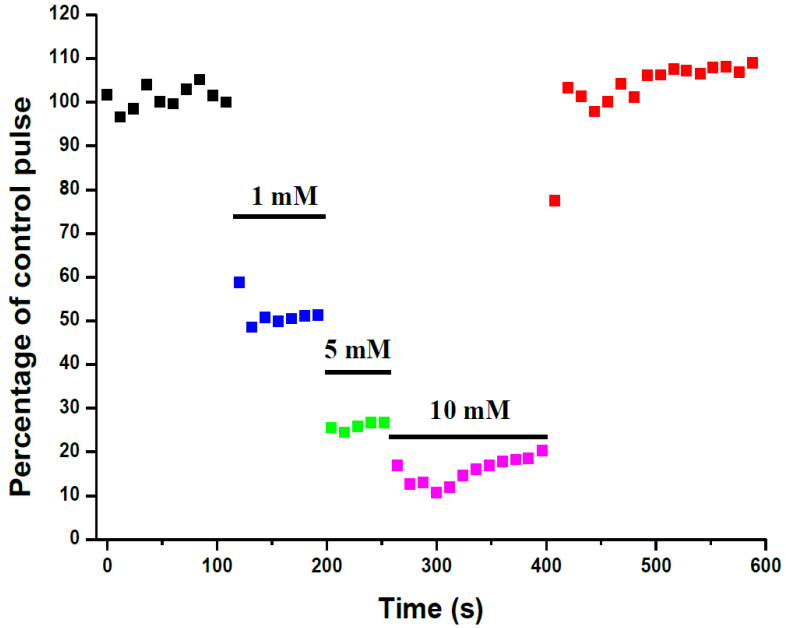


K_V3.2 Raw Current Traces, Current-Voltage (I/V) Relationship: **Left:** Typical current traces (upper panel) elicited by 200 ms depolarising voltage pulses from –70 mV to +40 mV in 10 mV increments (lower panel) from a holding potential of –80 mV. Scale bars represent 10 ms and 1 nA (upper panel) and 10 ms and 20 mV (lower panel) respectively. **Right:** Mean I/V relationship. Current amplitudes were measured at the end of the 200 ms step and plotted against the test voltage. Currents were normalized to the current amplitude obtained at +40 mV. The mean current at +40 mV was 9.55 ± 4.10 nA (n=7). (Manual Patch Clamp Data)

$K_V3.2$

BACK

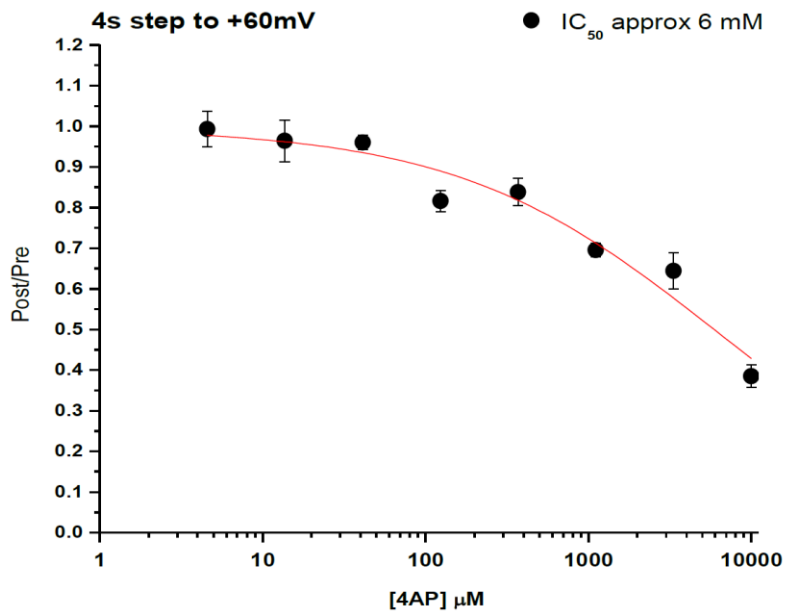


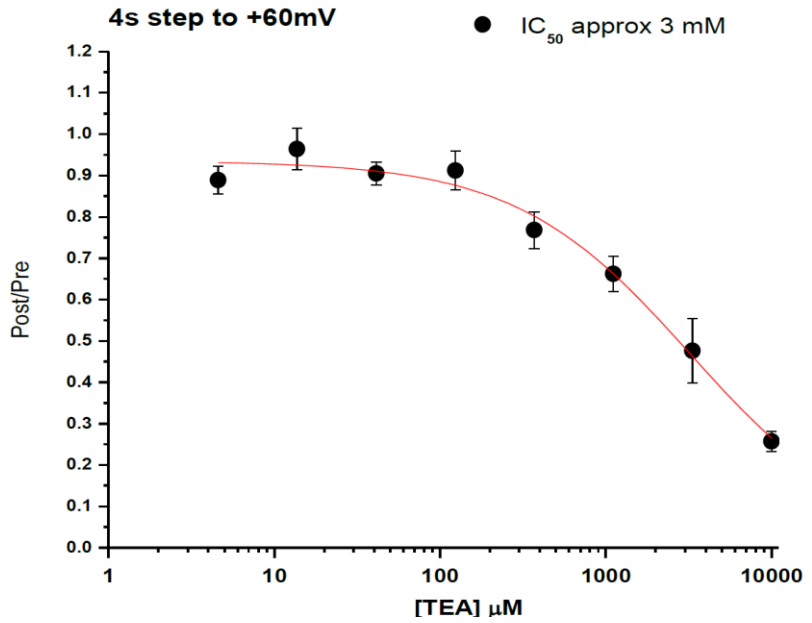


Effect of 4-Aminopyridine on $K_V3.2$ Currents: **Left:** Current traces evoked by stepping to –10 mV from a holding potential of –80 mV in the presence of various concentrations of 4-AP. Upper panel: before the addition of 4-AP (control - black trace), after addition of 0.5 mM 4-AP (green), 1 mM 4-AP (blue) and 5 mM 4-AP (red) respectively. Scale bars represent 100 ms (x-axis) and 500 pA (yaxis). Lower panel: voltage-step protocol. Scale bars represent 100 ms (x-axis) and 35 mV (y-axis). **Right:** Mean currents evoked by stepping to –10 mV for 1 s from –80 mV. Currents normalized to control current. hKv3.2 channels were stepped for 1 s every 12 s. (Manual Patch Clamp Data)

$K_V3.2$

BACK

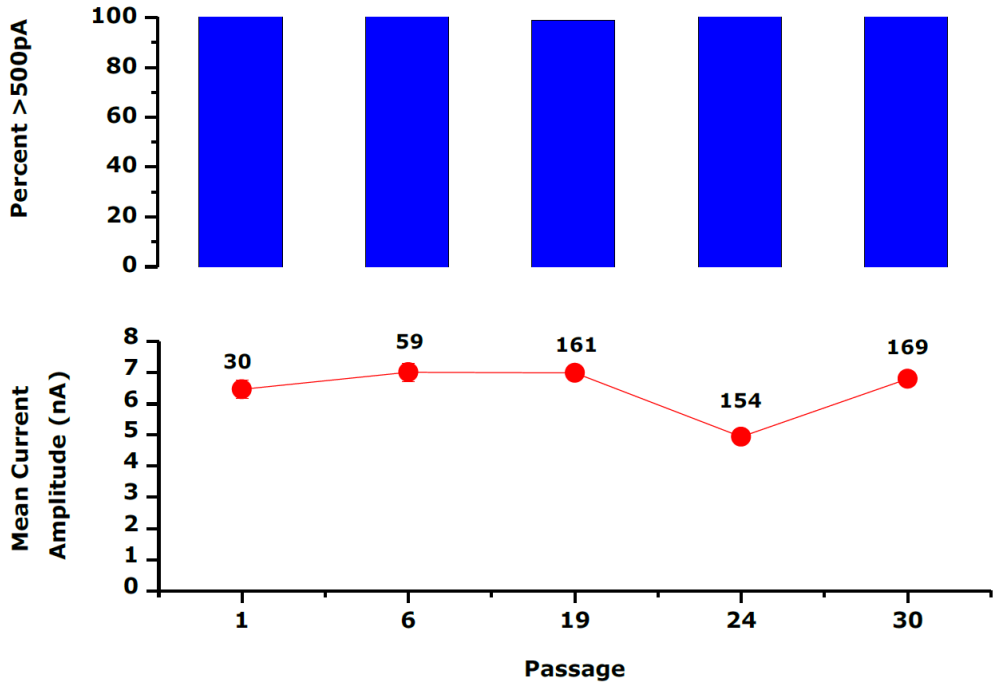




Pharmacology of 4-AP and TEA on $K_V3.2$ Currents: The effect of 4-AP **(Left)** and TEA **(Right)** was tested using a 4 s voltage test step to +60 mV. The hKv3.2-CHO K1 cell line was sensitive to low mM concentrations of both 4-AP (IC50 values were 6 mM and 3 mM for 4-AP and TEA respectively). The Kv3.2 channel is reported to be highly sensitive to both 4-AP and TEA with concentrations of less than 1 mM reducing the current produced by approximately 50% (IonWorks HT Data).

$K_V3.2$

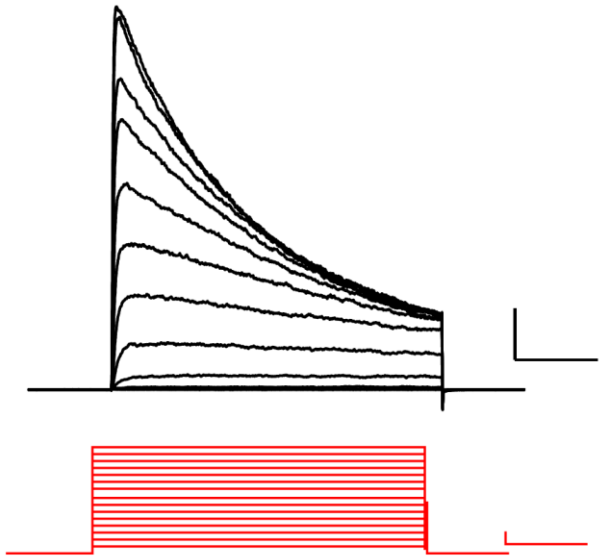
BACK

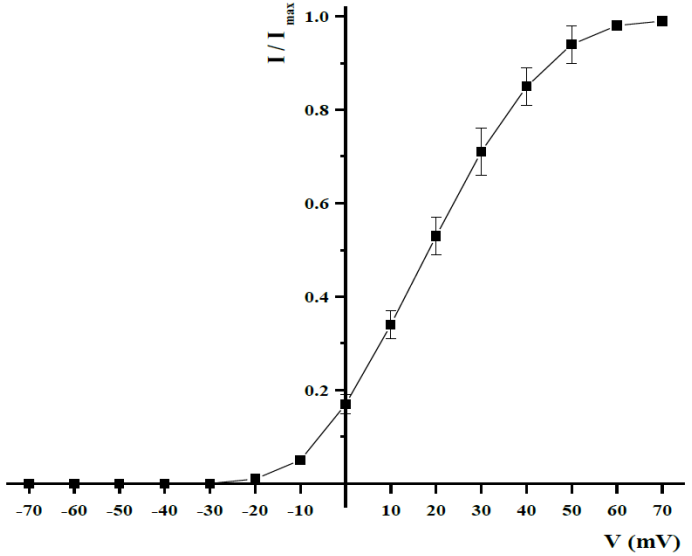


$K_V3.2$ Stability of Expression over Passage Number: The upper panel shows the percentage of cells expressing a mean peak tail current >500 pA at cell passages 1, 6, 19, 24, and 30. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles) (IonWorks HT Data).

K_V3.3 (CYL3045)

BACK

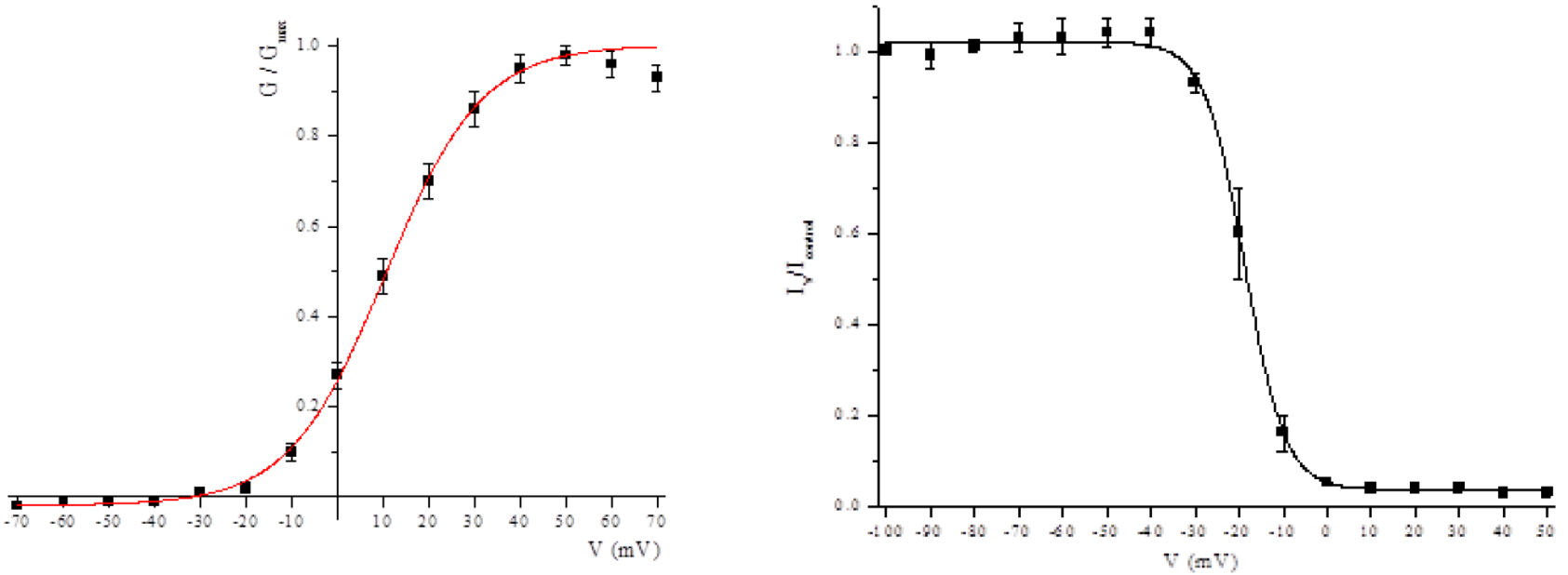




K_V3.3 Raw Data Currents and Current-Voltage (I/V) relationship. Left: Typical current traces (upper panel) elicited by 200 ms depolarising voltage pulses from –70 mV to +70 mV in 10 mV increments (lower panel) from a holding potential of –80 mV. Scale bars represent 50 ms and 2 nA in the upper panel and 50 ms and 20 mV in the lower panel. **Right:** Mean I/V relationship: Peak current amplitudes were measured during the 200 ms step and plotted against the test voltage. Peak currents at a given voltage were normalized to the peak current amplitude obtained at +60 mV. The mean current at +40 mV was 11.69 ± 1.06 nA (n = 5) (Manual Patch Clamp Data).

$K_V3.3$

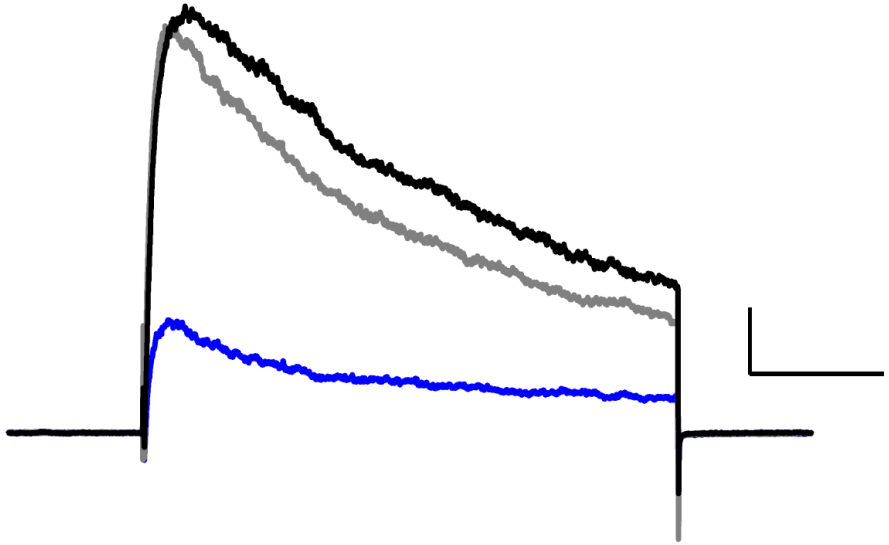
BACK

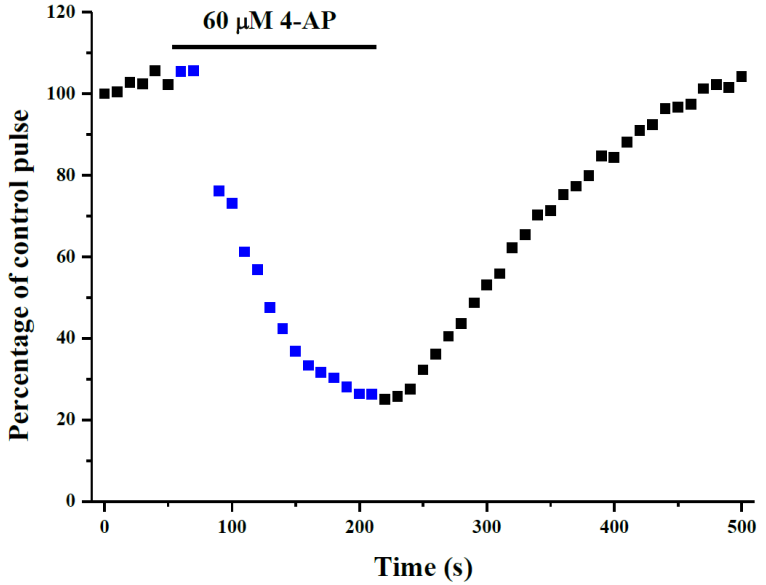


hK$_V$3.3 Current Activation and Inactivation: **Left:** V½ was 10.7 ± 2.0 mV and the slope of the conductance-voltage relationship (k) was 10.4 ± 0.9. Conductance was normalized to the peak conductance and could be described by a Boltzmann equation. V½ = 10.7 ± 2.0 mV; k = 10.4 ± 0.9. **Right:** Cells were stepped from –100 mV to +50 mV for 2 s from the holding potential (-80 mV) in 10 mV increments (conditioning voltage) and then stepped to a test voltage of +50 mV for 25 ms to measure channel availability after each conditioning voltage step. Sweeps every 15 s. The current amplitudes, measured at the end of the test voltage step to +50 mV, were normalized to current obtained after the 2 s step to -100 mV. This is plotted against the conditioning voltage and could be described by a Boltzmann equation. Inactivation V½ =-18.5 ± 1.9 mV and slope (k) = 4.3 ± 0.2 (n = 3) (Manual Patch Clamp Data)

$K_V3.3$

BACK

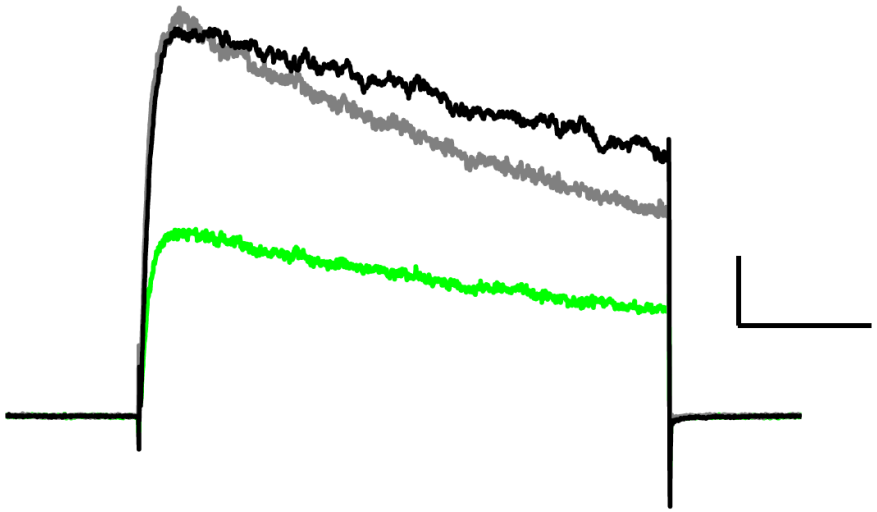


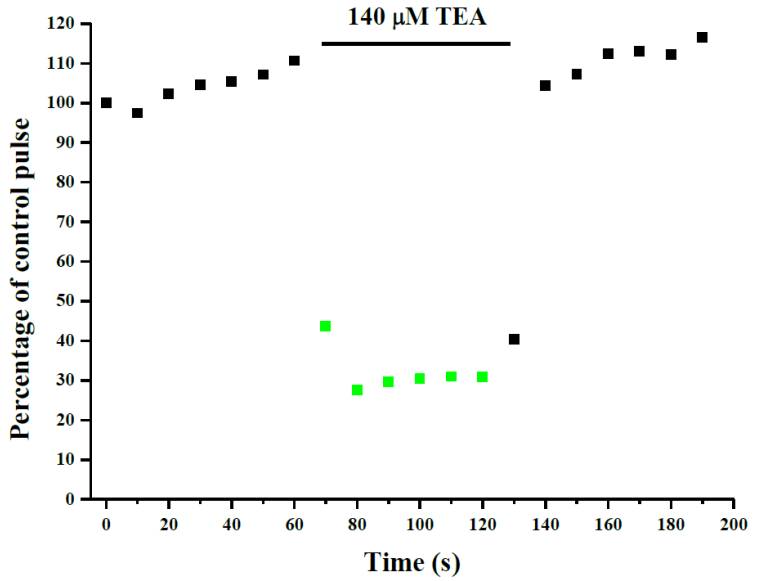


<u>hK$_V$3.3 Blockade by 4-Aminopyridine:</u> Left: Currents were inhibited by micromolar concentrations 4-Aminopyridine (4-AP). The application of 4-AP (60 µM) was found to inhibit the hKv3.3 current by >70 % (Table 1). 4-AP has a reported IC50 of <100 µM on Kv3.3 currents when expressed in mammalian cells (Rashid et al., 2001). Current traces evoked by stepping to 0 mV for 200 ms from a holding potential of –80 mV, before (black trace), in the presence of (blue), and after wash off (grey) of 60 µM 4-AP. Scale bars represent 50 ms and 500 pA. **Right:** Typical time course of inhibition of hKv3.3 currents by 60 µM 4-AP. Currents were evoked by stepping to 0 mV for 200 ms from -80 mV. Sweeps every 10 s. Currents normalized to current before the addition of 4-AP (Manual Patch Clamp Data).

$K_V3.3$

BACK

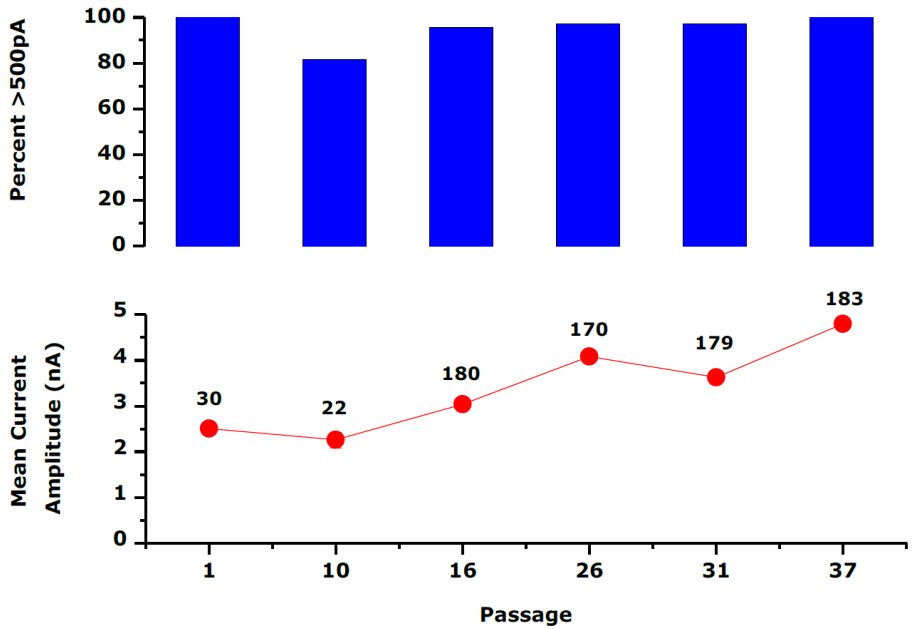




$K_V3.3$ Blockade by Tetraethylammonium: **Left:** 140 µM Tetraethylammonium chloride (TEA) inhibited hKv3.3 currents by approximately 50%. This is in agreement with the published IC_{50} of 140 µM (Vega-Saenz de Miera et al., 1992). Current traces evoked by stepping to 0 mV from a holding potential of –80 mV before (black trace), in the presence (green trace) and after wash off (grey trace) of 140 µM TEA. Scale bars represent 50 ms and 500 pA. **Right:** Typical time course of inhibition of hKv3.3 currents by 140 µM TEA. Currents were evoked by stepping to 0 mV for 200 ms from -80 mV. Sweeps every 10 s. Currents normalized to current before the addition of TEA (Manual Patch Clamp Data).

$K_V3.3$

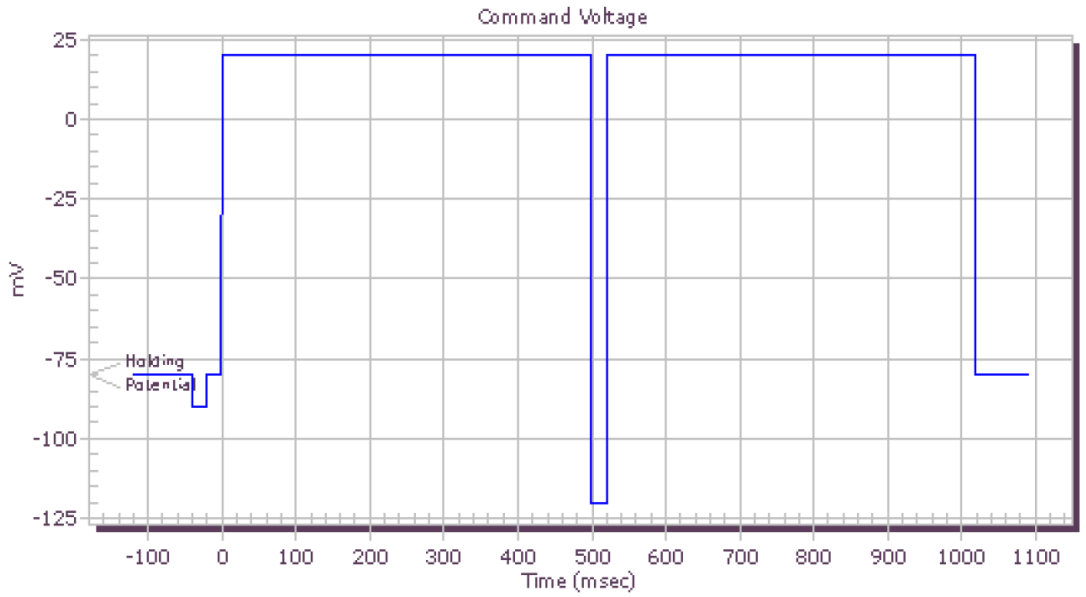
BACK

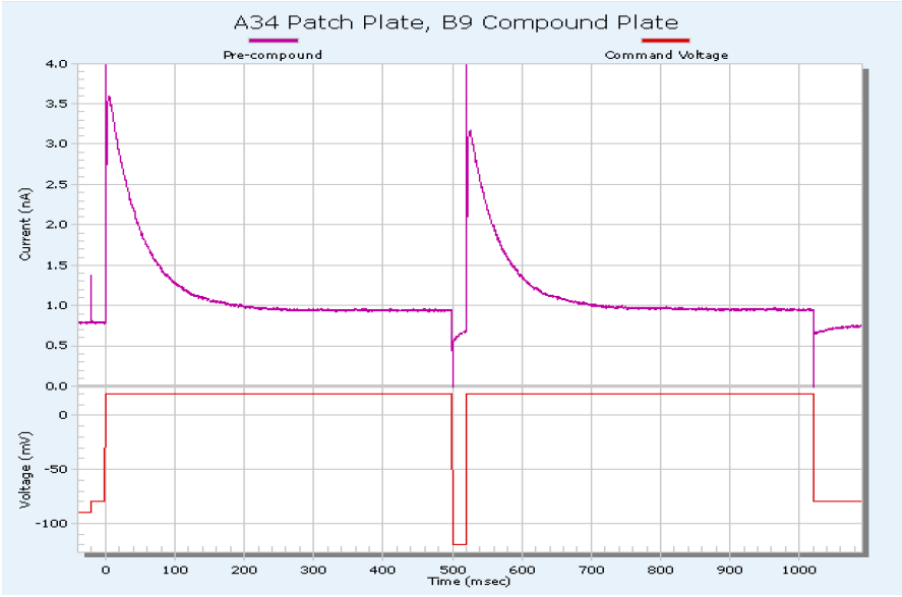


$K_V3.3$ Stability of Expression Over Passage: The upper panel shows the percentage of cells expressing a mean peak tail current >500 pA at cell passages 1, 10, 16, 26, 31 and 37. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles) (IonWorks HT Data)

K_V4.2/KChiP2 (CYL3026)

BACK

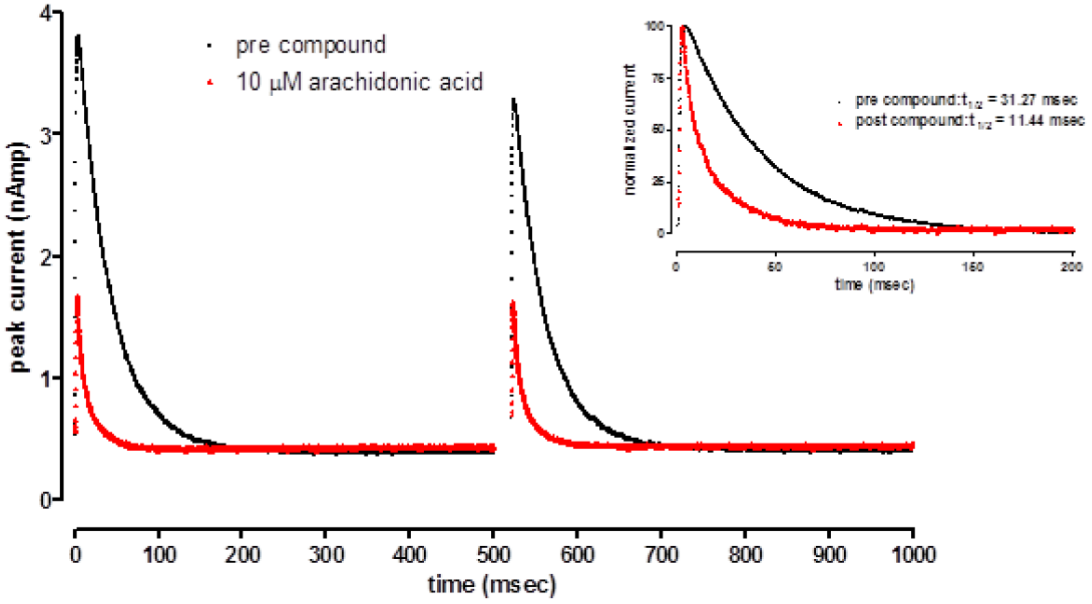


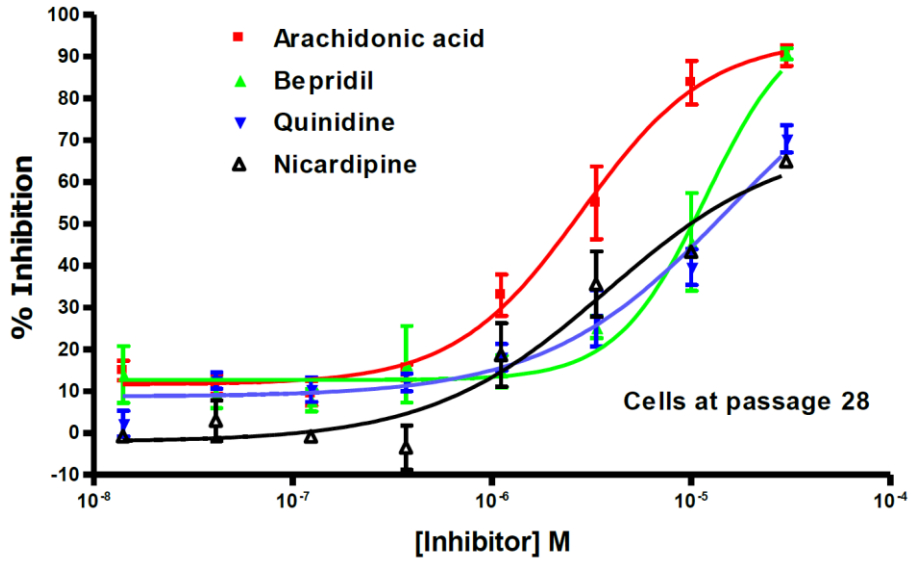


K_V4.2/KChIP2 Voltage Protocol and Raw Current Traces: **Left:** Command voltage protocol for hKv4.2/hKChIP2 clone evaluation using the IonWorks™ HT. Voltage was stepped from a holding potential of -80 mV to +20 mV. All hKv4.2/hKChIP2 channels will have opened and inactivated very quickly at these potentials. Voltage is clamped at +20 for 500 ms followed by a step to -120 mV to ensure closure of all channels. After a delay of 20 ms, the voltage is again stepped to +20 mV to ensure opening/inactivation of all channels. Based on the literature, only channels composed of both hKv4.2 and hKChIP2 subunits should have recovered from inactivation and responded to the second voltage step. Channels composed of hKv4.2 alone should be refractory to the second voltage step. **Right:** Representative trace of hKv4.2/hKChIP2 currents using the indicated command voltage protocol demonstrating the rapid activation and inactivation kinetics characteristic of the channel. The presence of both subunits is strongly supported by the apparent rapid recovery from inactivation (<20 ms). (IonWorks HT Data).

K_V4.2/KChiP2

BACK

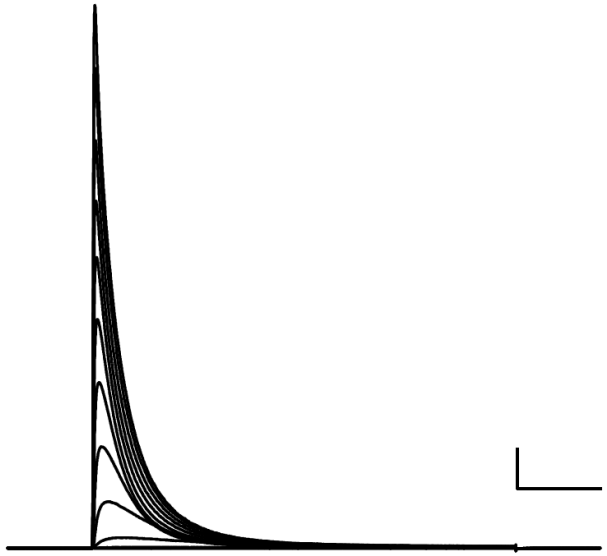




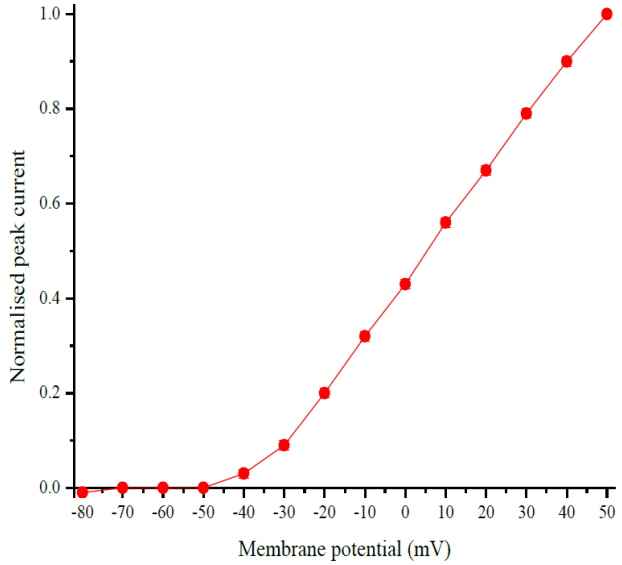
<u>Effect of Arachidonic Acid on the Gating Characteristics of the K_V4.2/KChIP2 Currents, K_V4.2/KChIP2 Pharmacology:</u> **Left:** Outward currents from hKv4.2/hKChIP2 expressing cells were evaluated on the IonWorks™ HT before and after the addition of arachidonic acid (10 µM, final concentration). Post-compound currents were obtained after a 3 minute exposure to test inhibitor. Data were best fit to a mono-exponential decay function (GraphPad PRISM). Arachidonic acid hastens the current inactivation thereby reducing the peak current magnitude. Control current peaks after approximately 13 ms of depolarising voltage. Using the same command voltage protocol in the presence of arachidonic acid, currents peak after approximately 4 ms. The inactivation time constant τ_{inac} was 31.27 ms in absence and 11.44 ms in the presence of arachidonic acid (see figure insert). These data provide further convincing evidence of the functional co-expression of both hKv4.2 and hKChIP2 subunits. **Right:** Inhibition of peak hKv4.2/hKChIP2 currents. Outward currents from hKv4.2/hKChIP2 expressing cells were evaluated before and after the addition of inhibitors at the indicated concentrations. Post-compound currents were obtained after a 3 minute exposure to test inhibitor. The data represent the mean ± SD of triplicate determinations from a representative experiment. IC50 values were arachidonic acid (2.82 µM), bepridril (11.8 µM), quinidine (16.1 µM) and nicardipine (3.78 µM) (IonWorks HT Data).

K_V4.3/KChiP1 (CYL3027)

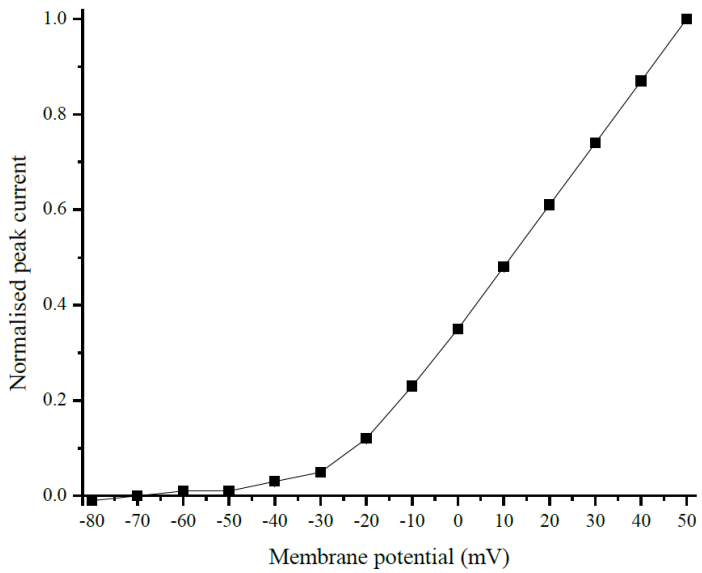
BACK



hKv4.3/hKChIP1



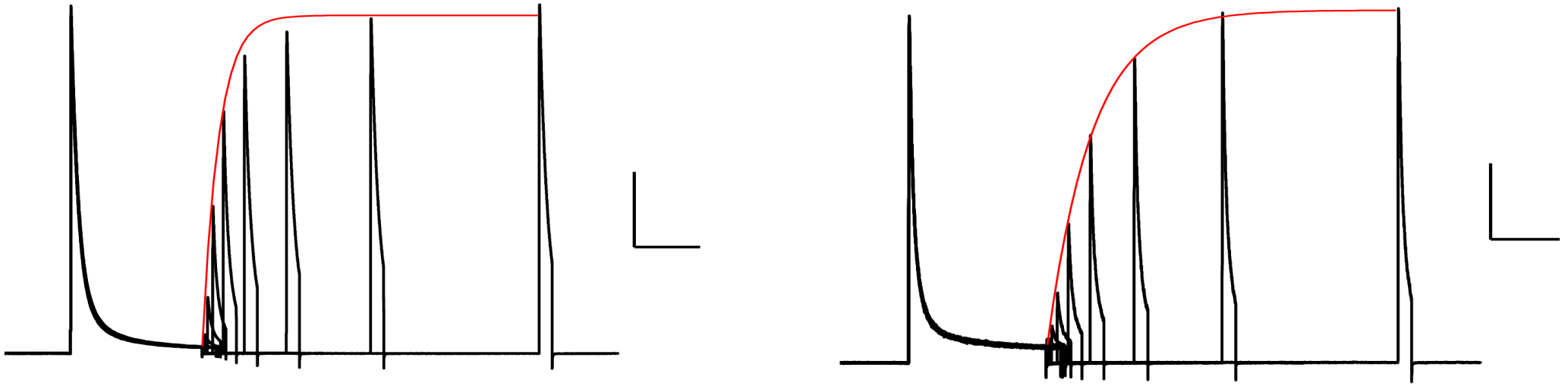
hKv4.3



K_V4.3/KChIP1 Current-Voltage (I/V) relationship and Biophysics: **Left:** Typical hKv4.3/hKChIP1 currents (upper panel) elicited by depolarising voltage pulses from –80 mV to +50 mV in 10 mV increments every 4 s from a holding potential of –80 mV. Scale bars represent 100 ms (x-axis) and 5 nA (y-axis). **Middle:** hKv4.3/hKChIP1: Peak current amplitudes were normalized to the current amplitude obtained at +50 mV. The mean current at +50 mV was 22.4 ± 6.5 nA (Mean ± SEM, n= 6). **Right:** hKv4.3: Peak current amplitudes were normalized to the current amplitude obtained at +50 mV. The mean current at +50 mV was 9.1 ± 4.7 nA (Mean ± SEM, n= 7) (Manual Patch Clamp Data)

K_V4.3/KChiP1

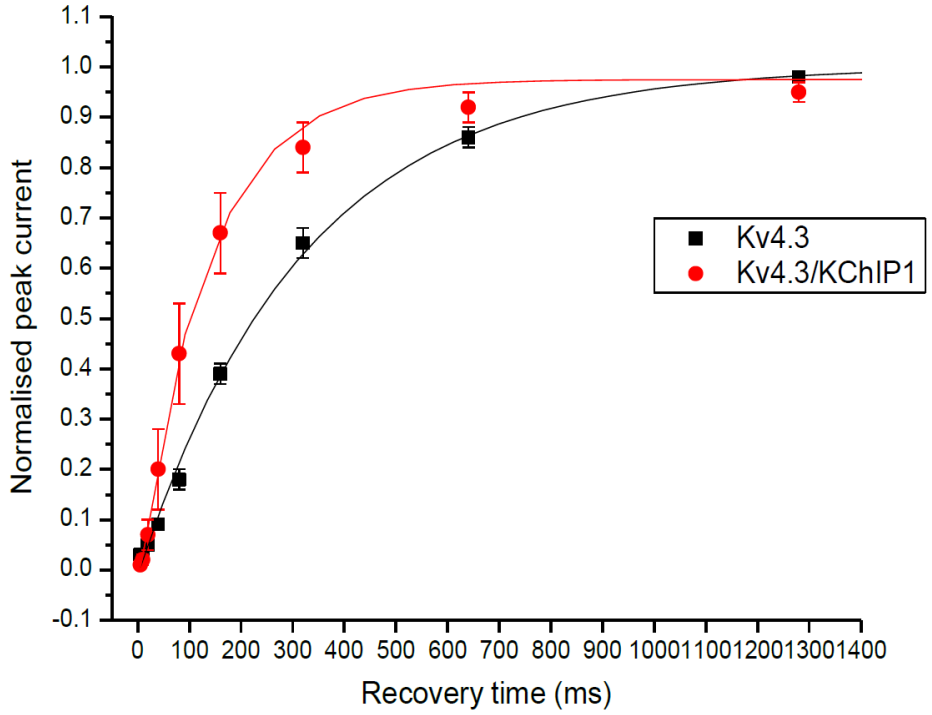
BACK



K_V4.3/KChIP1 and K_V4.3 Recovery From Inactivation: **Left:** hKv4.3/hKChIP1: Currents evoked by stepping from holding potential of –80 mV to 50 mV for 1 s for the 1st pulse and for 100 ms for the second pulse. Scale bars represent 500 ms and 5 nA. Red line is single exponential fit to peak of 2nd pulse peak currents. Second pulses after an interval of 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560 ms. **Right:** hKv4.3: Currents evoked as above. Scale bars represent 500 ms and 1 nA. Red line is single exponential fit to peak of 2nd pulse peak currents. Second pulses after an interval of 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2560 ms (Manual Patch Clamp Data).

K_V4.3/KChiP1

BACK



K_V4.3/KChIP1 Recovery From Inactivation: Normalised peak currents (y-axis) are plotted against the recovery time (x-axis). hKv4.3 alone (black) and hKv4.3 coexpressed with hKChIP1 (red). The values for recovery from inactivation (τ) were 319 ± 29 ms (n=7) and 141 ± 33 ms (n=4) for hKv4.3 and hKv4.3/hKChIP1 respectively (mean ± SEM) (Manual Patch Clamp Data).

K_V4.3/KChiP1

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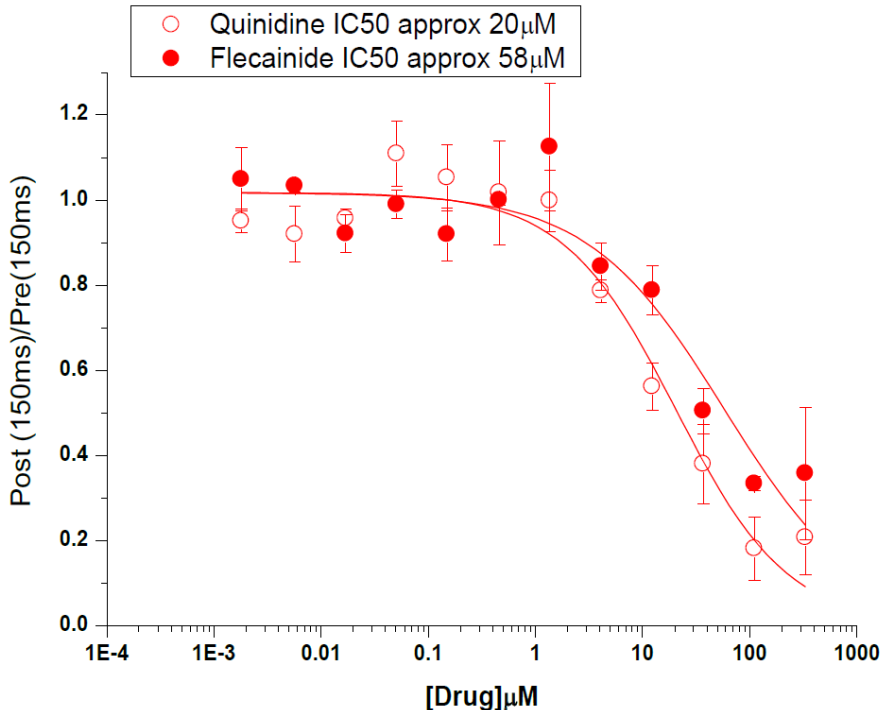
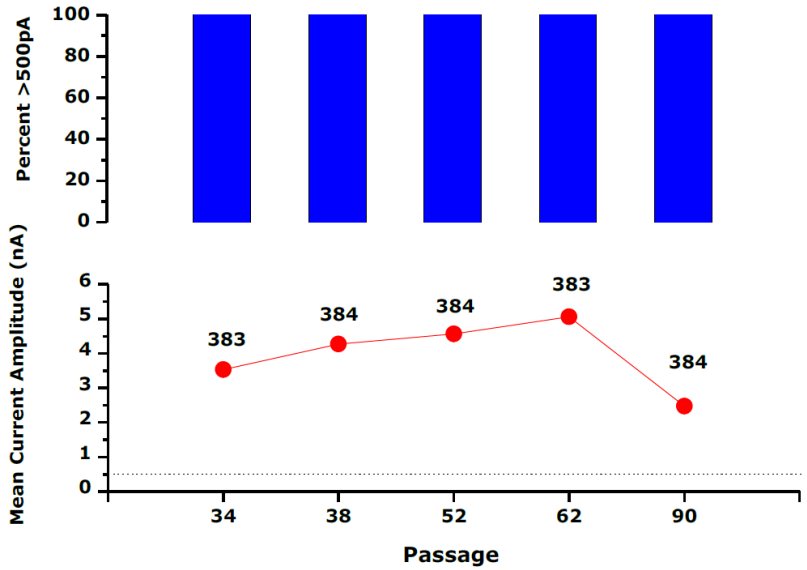
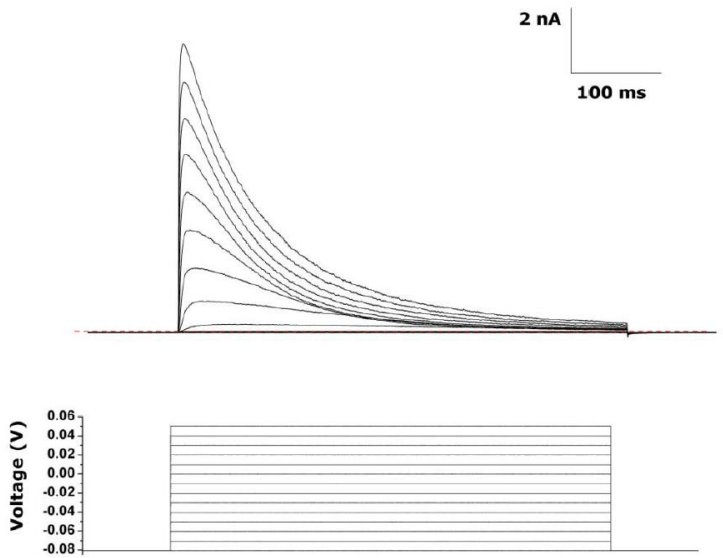


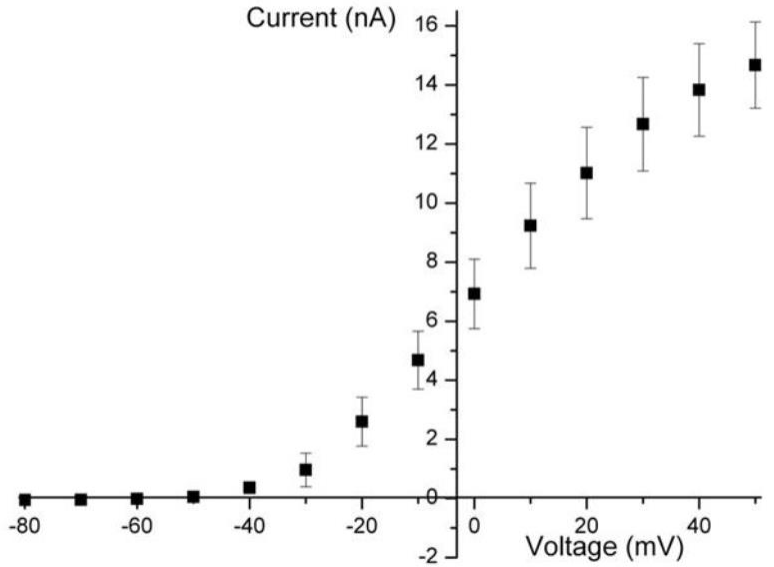


Effect of Quinidine and Flecainide on K_V4.3/KChIP1 Currents, Stability of Expression over Passage Number: **Left:** The antagonists quinidine and flecainide are both known antagonists of Kv4.3 currents. In these studies, the pre-compound and post-compound currents elicited by the double pulse protocol were recorded and the post-/pre-compound ratio plotted against antagonist concentration Dose-response curves for the reduction in post-/pre-compound ratio by the antagonists quinidine and flecainide. **Right:** Stability of expression over passage. The upper panel shows the percentage of cells expressing a mean peak current >500 pA at cell passages 34, 38, 52, 62, and 90. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of these cells (numbers above red circles). (IonWorks HT Data)

K_V4.3/KChiP2 (CYL3069)

BACK

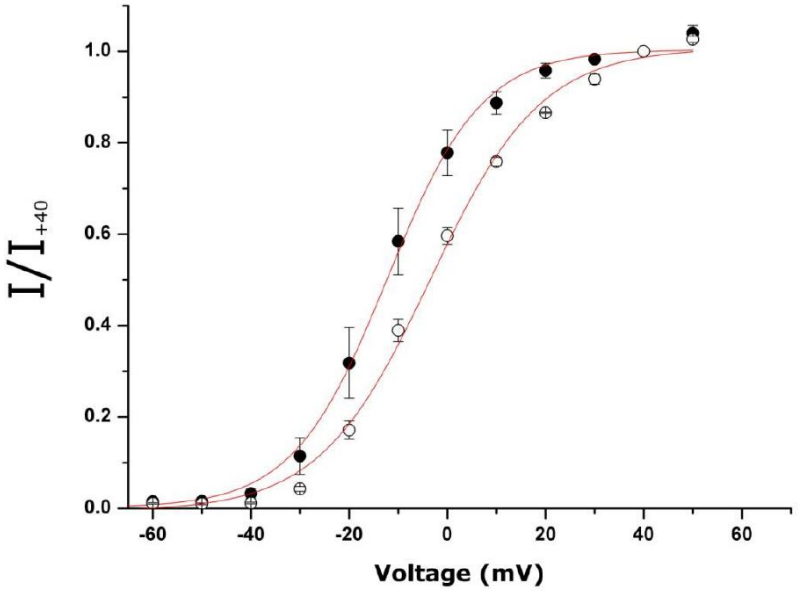


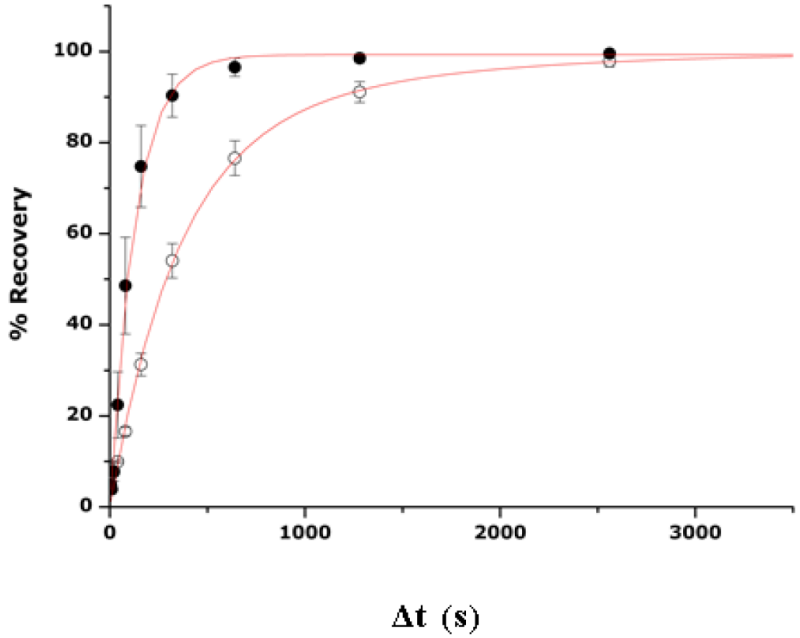


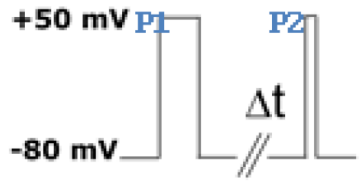
<u>hK$_V$4.3/hKChIP2 Raw Data Currents and Current-Voltage (I/V) relationship</u>: **Left:** Cells were voltage-clamped (Left) at a holding potential of –80 mV and 500 ms depolarizing steps were applied in 10 mV increments up to +50 mV. **Right:** The mean peak I/V relationship (Right) is plotted (n= 5) (Manual Patch Clamp Data).

K_V4.3/KChiP2

BACK







<u>hK$_V$4.3/hKChIP2 vs hK$_V$4.3 Current Activation and Recovery from Inactivation:</u> Current Activation curves **(Left), t**he data can be described by a Boltzmann equation where the V½ of activation and slope were –3.5 mV and 11.3 mV respectively for hKv4.3 (open circles) and –12.2 mV and 9.6 mV for hKv4.3/hKChIP2 (n = 5). Comparison of the recovery from inactivation **(Right)** for hKV4.3/hKChIP2 vs hKV4.3 using the two pulse protocol **(inset),** the expression of hKChIP2 speeds the recovery from inactivation. Peak amplitudes (P2) were measured for various recovery intervals and plotted as percent amplitude of P2 vs P1. For both hKv4.3 (open circles) and hKv4.3/hKChIP2 the data could be described by a single exponential where the recovery from inactivation time constant (τ) was 450 ± 25 ms and 125 ± 14 ms (n=6) respectively. (Manual Patch Clamp Data).

K_V4.3/KChiP2

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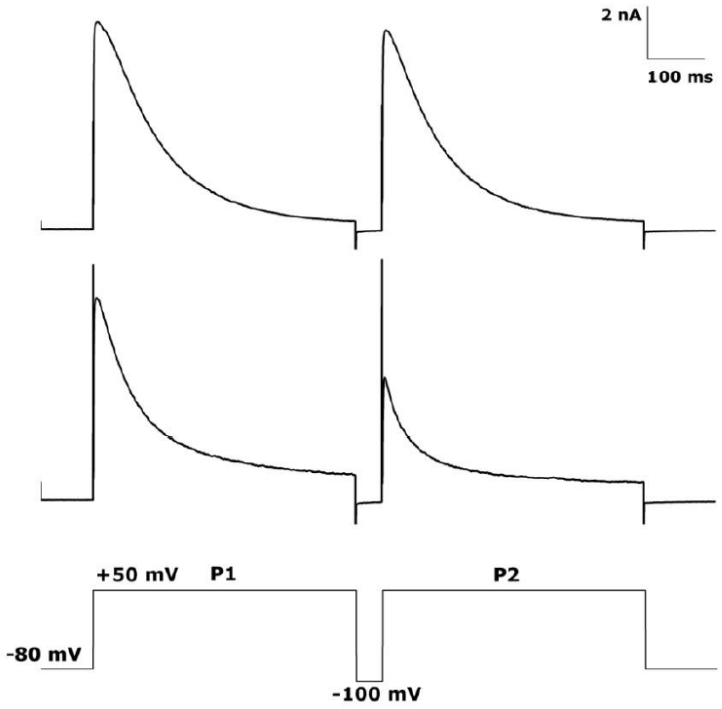
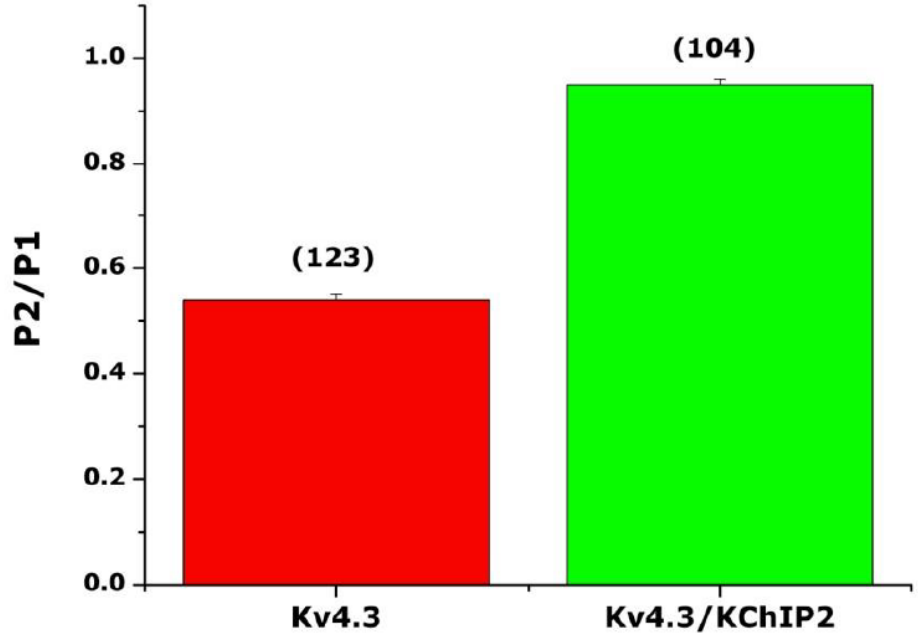
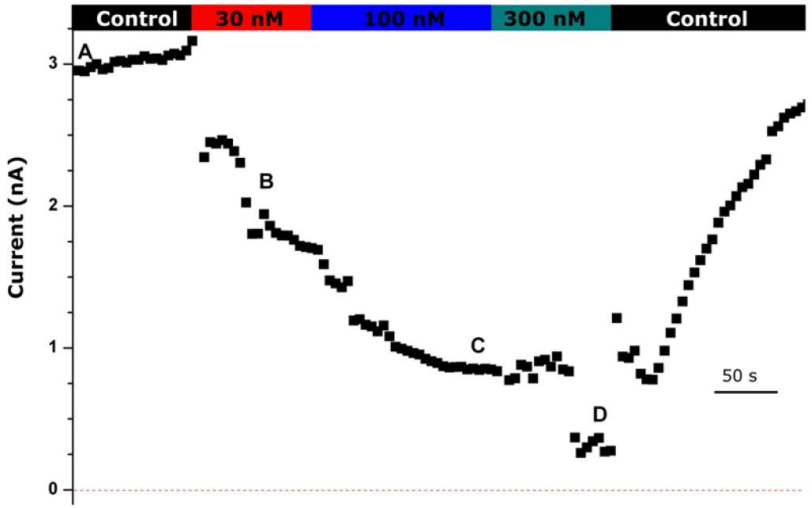


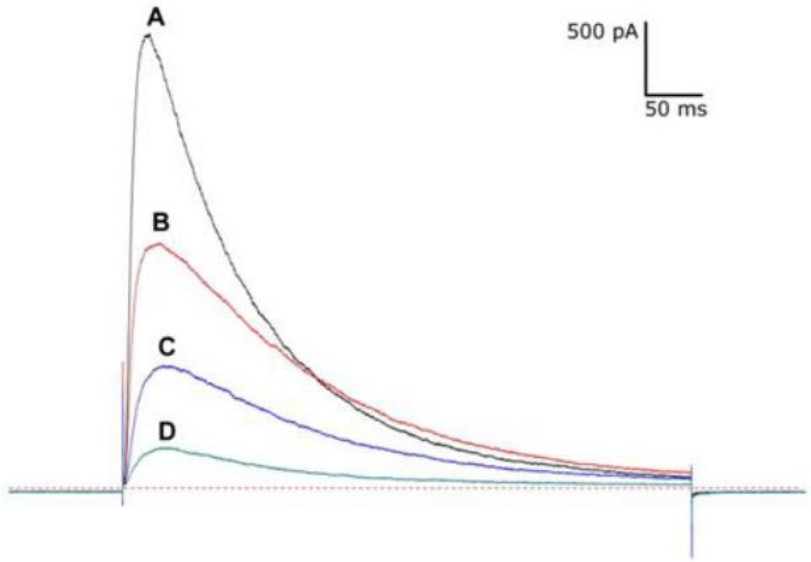


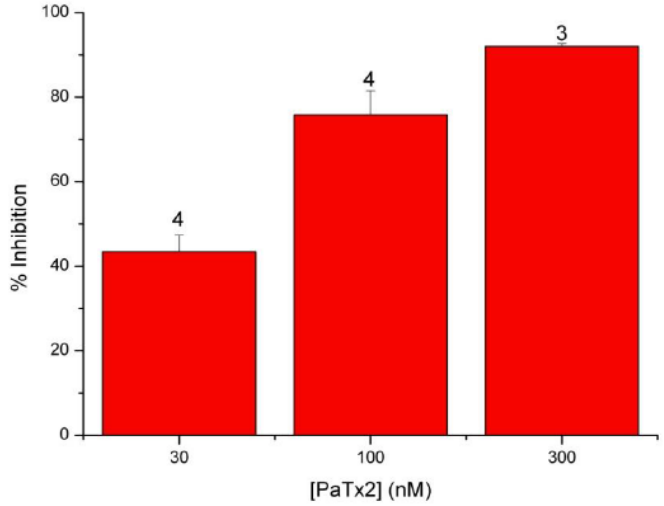
Assessing Recovery from Inactivation Using IonWorks HT: **Left:** Typical traces obtained using the voltage protocol shown in the lowest panel using with hKv4.3 co-expressed with hKChIP2 (A) or hKv4.3 expressed alone (B). Note the almost complete recovery from inactivation in A but only partial recovery in B. **Right:** P2/P1 rations for the hKv4.3 and hKv4.3/hKChIP2 cell lines (IonWorks HT Data).

K_V4.3/KChiP2

BACK



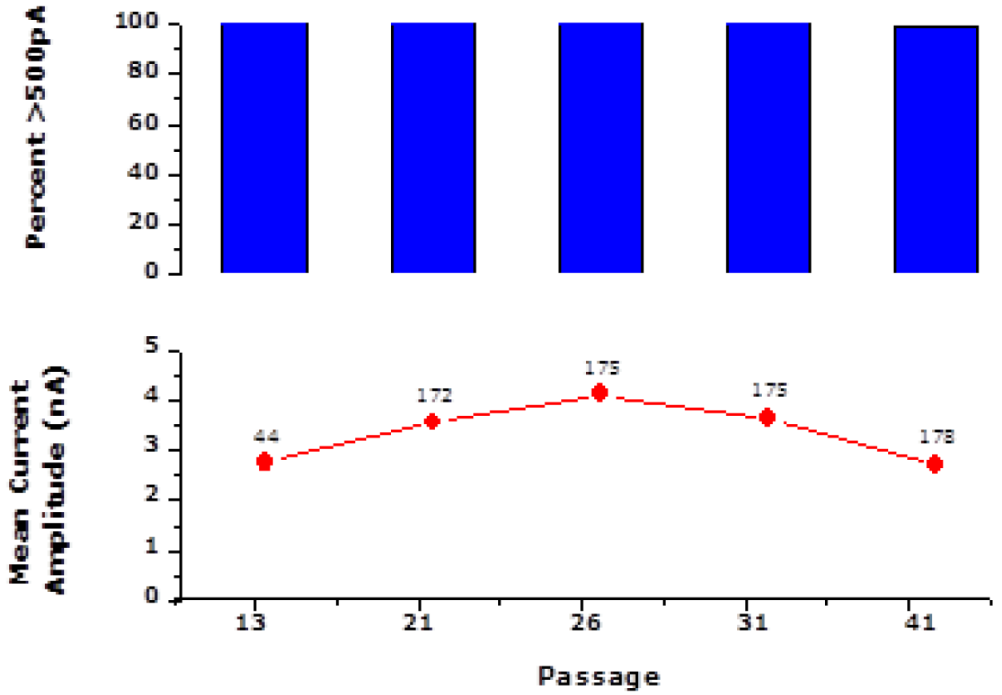




Effect of Phrixotoxin-2 on hK_V4.3/hKChIP2 currents: **Left:** Current-time plot of currents evoked by 500 ms steps to -10 mV before (Control) and in the presence of 30 nM (red bar), 100 nM (blue bar) and 300 nM (green bar) phrixotoxin-2. Consecutive measured peak amplitudes at -10 mV are shown as black squares. **Middle:** Current traces recorded at A, B, C and D. **Right:** Mean data showing percent inhibition relative to control. The numbers above the error bars refer to the number of replicates (Manual Patch Clamp Data).

K_V4.3/KChiP2

BACK



Stability of expression over passage: The upper panel shows the percentage of cells expressing a mean peak current ≥500 pA on first stepping to 0 mV from a holding potential of –80 mV (P1) at cell passages 13, 21, 26, 31 and 41. The lower panel shows the mean current amplitude (mean ± SEM, red circles) and the number of cells (numbers above red circles – out of 64 cells at passage 13 and out of 192 cells for all other passages) (IonWorks HT Data).

hKCNQ1/hminK (K_V7.1/KCNE1) (CYL3007)

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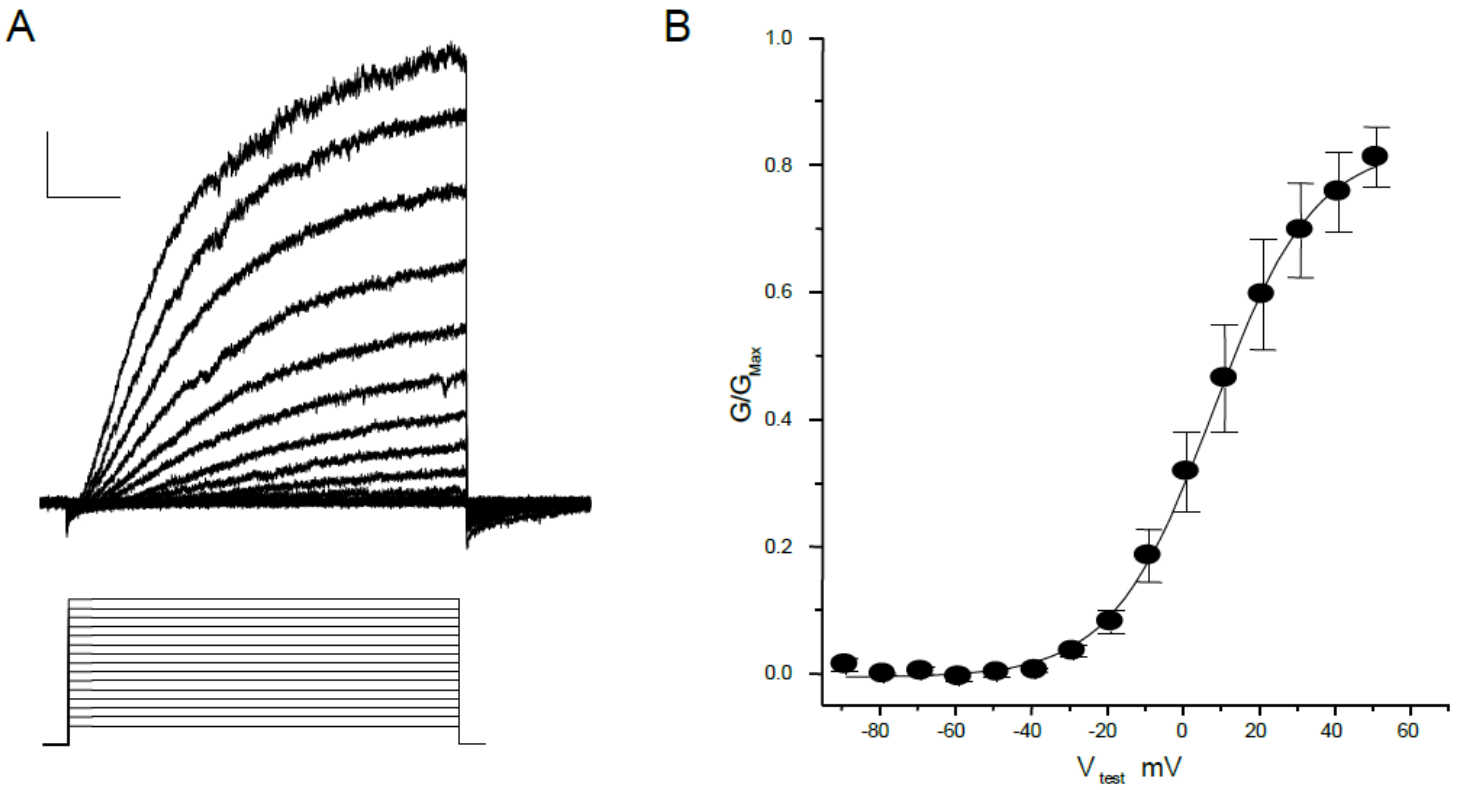
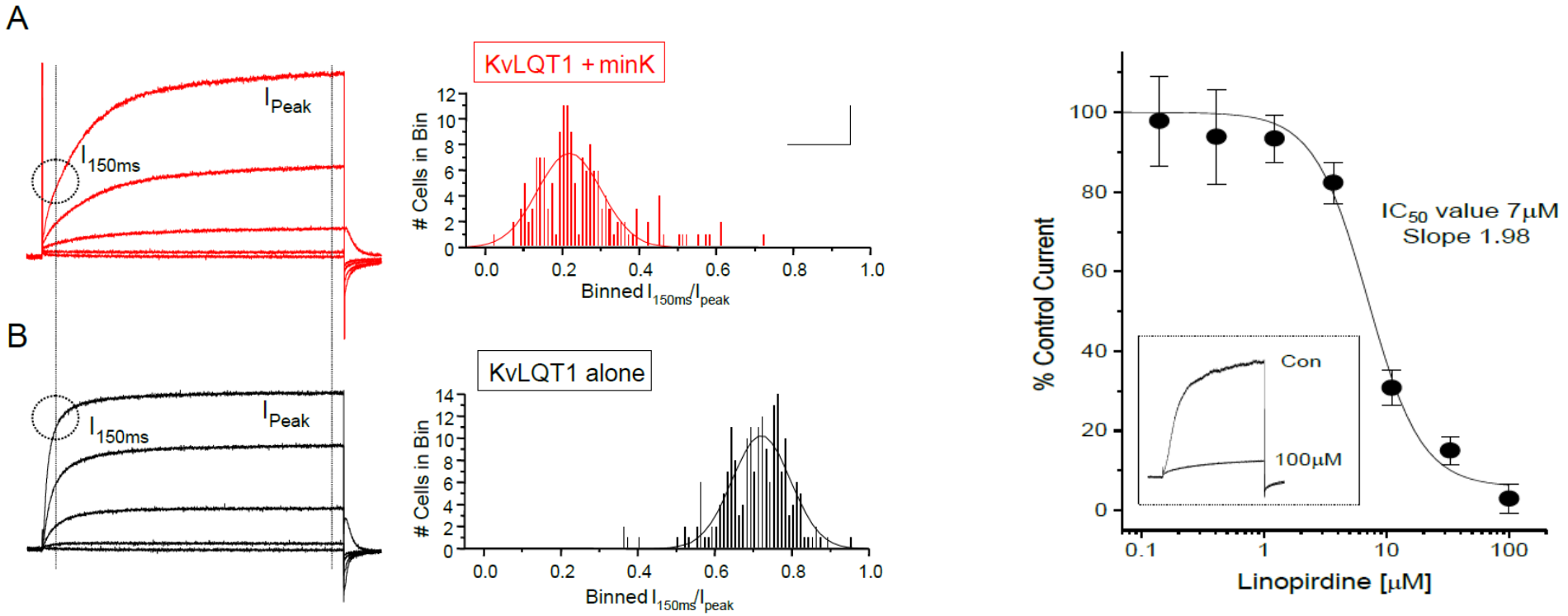


hKCNQ1/hminK Raw Data Currents and Current Voltage Relationship. Raw data currents **(Left)** evoked from Vh of –80 mV to test potentials ranging from -60 to +60 mV. Calibration bars 200 pA, 1s. **Right:** Voltage vs normalised conductance plot (G/Gmax) for mean data from 6 cells [mean V1/2 for activation of +7.8 mV with a k value (Manual Patch Clamp Data).

hKCNQ1/hminK (K_V7.1/KCNE1)

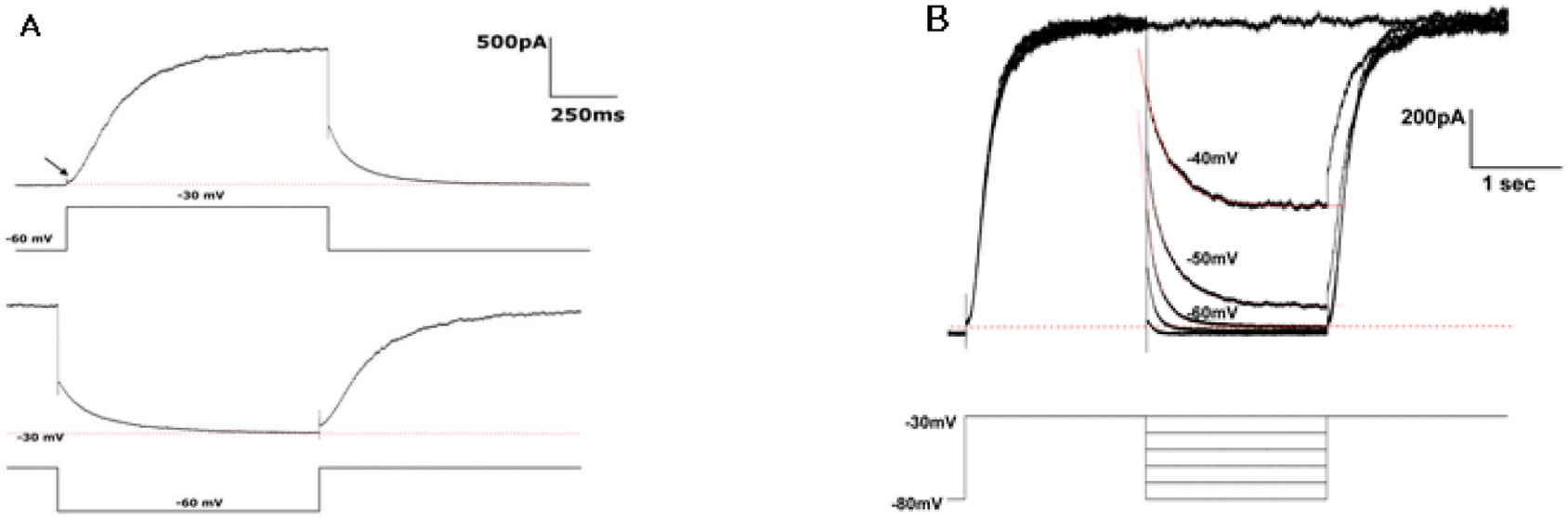
BACK



<u>Functional Expression of the hminK Subunit from Kinetic Analysis Using IonWorks and hKCNQ1/hminK Pharmacology.</u> The hminK subunit slows activation of ionic currents for hKCNQ1/hminK **(Left Top)** vs hKCNQ1 alone **(Left Bottom).** Note the slower activation of hKCNQ1/hminK compared to hKCNQ1 alone and hence the smaller I150/Ipeak value of 0.25 vs 0.80. **(Middle Top and Bottom).** Population histograms for I150/Ipeak values and illustrate that the vast majority of cells in the hKCNQ1/hminK cell line have kinetics that are substantially slower than hKCNQ1 alone (i.e. that hminK is expressed in almost every cell). Calibration bars 1s and 200pA. **Right:** Concentration-response curve and representative current record (inset) for inhibition of hKCNQ1/hminK currents by linopirdine in stably expressing CHO-K1 cells. (IonWorks HT Data).

$K_V7.2/K_V7.3$ (CYL3059)

BACK



Kinetics of hKv7.2/hKv7.3 currents: Left: hKv7.2/hKv7.3 currents were either opened by a depolarizing step from -60 mV to -30 mV (upper panel) or closed by a hyperpolarizing step from -30 mV to -60 mV (lower panel). **Right:** The time course of current deactivation was assessed by stepping the membrane voltage from -30 mV to various negative potentials (lower panel). Decaying currents (upper panel) were fitted with exponentials (Manual Patch Clamp Data).

$K_V7.2/K_V7.3$

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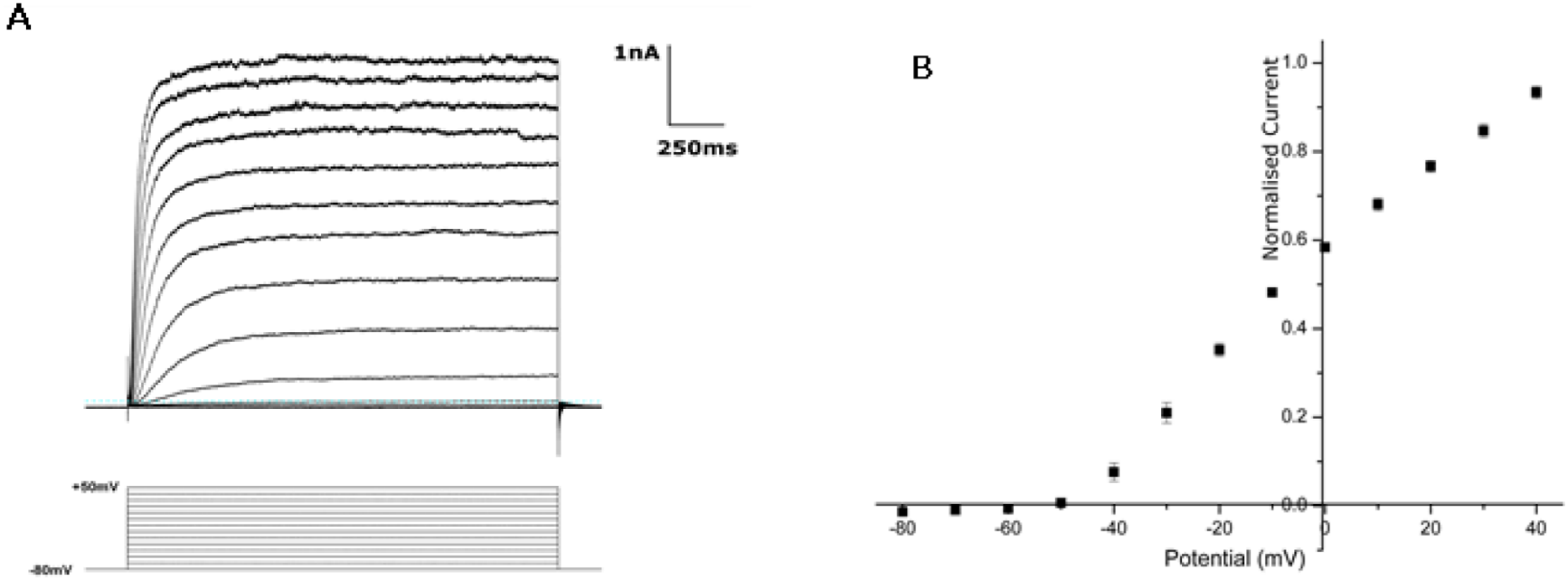
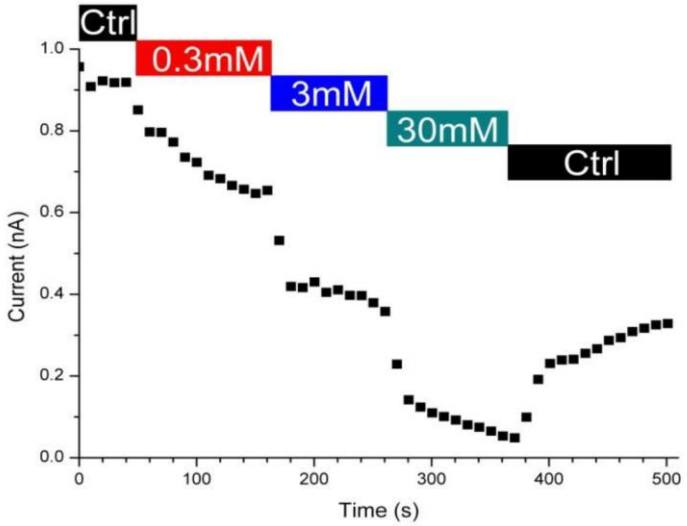
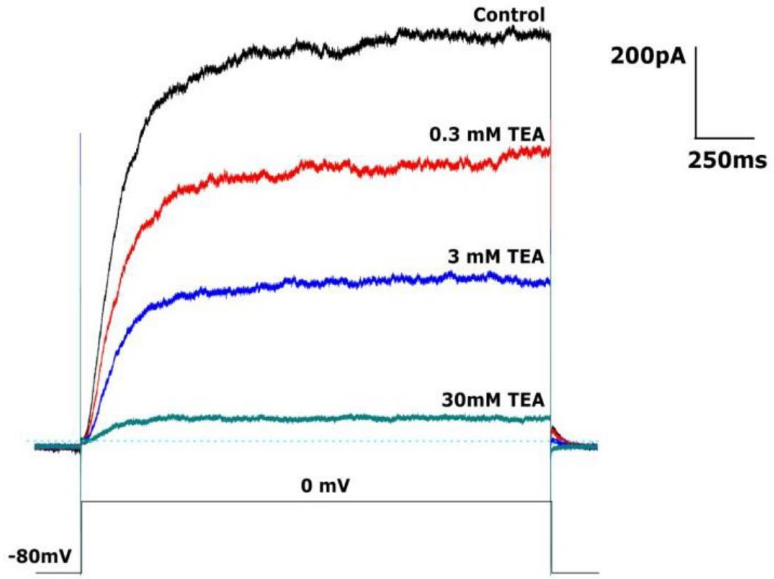


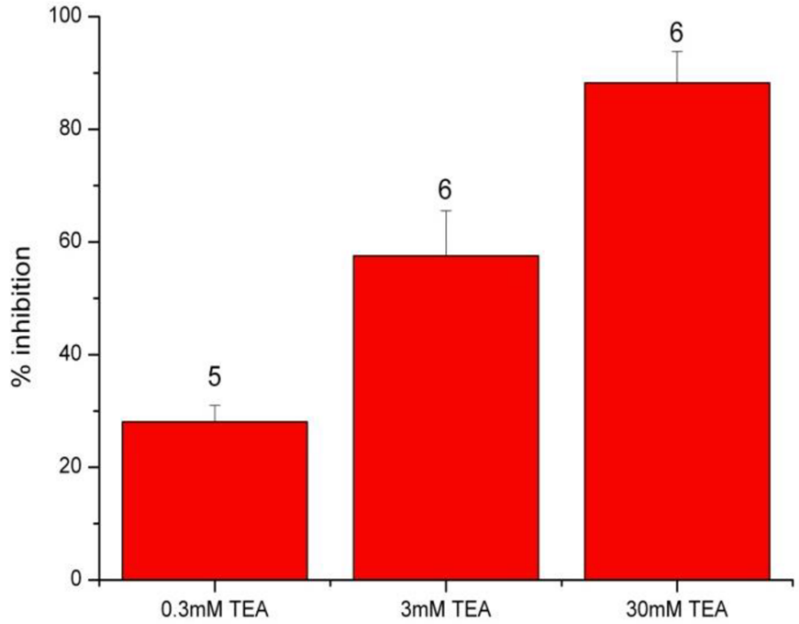
Raw Data Currents and Current-Voltage (I/V) relationship of hKv7.2/hKv7.3 currents: Left: Currents (upper panel) were evoked by 2000 ms depolarising voltage pulses stepped in 10 mV increments from -80 mV to +50 mV from a holding potential of –80 mV once every 10 seconds (lower panel). The green dotted line indicates zero current level. **Right:** The steady state current amplitudes elicited by the voltage protocol shown were normalised to the current evoked by the +50 mV voltage step for each cell. The mean data (n=3) is shown plotted against the step potential (mV) (Manual Patch Clamp Data)

$K_V7.2/K_V7.3$

BACK



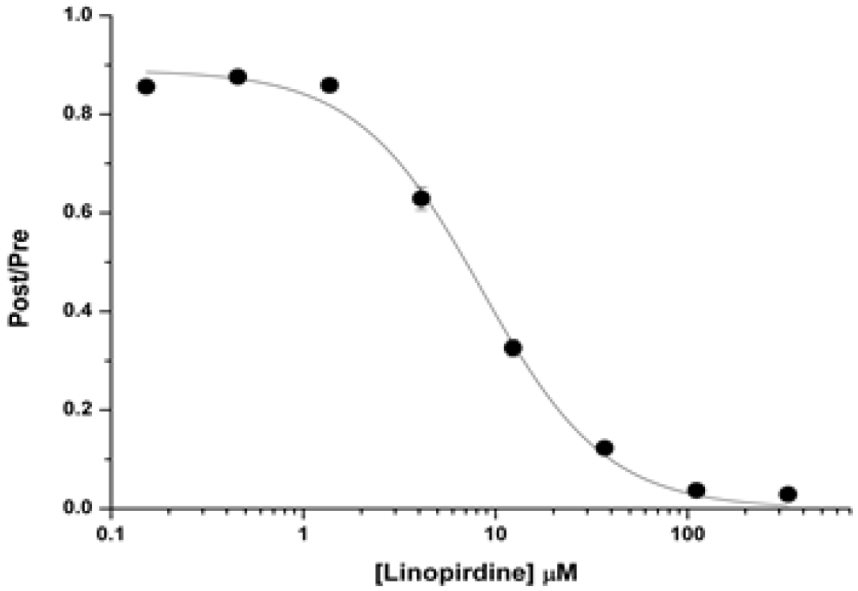


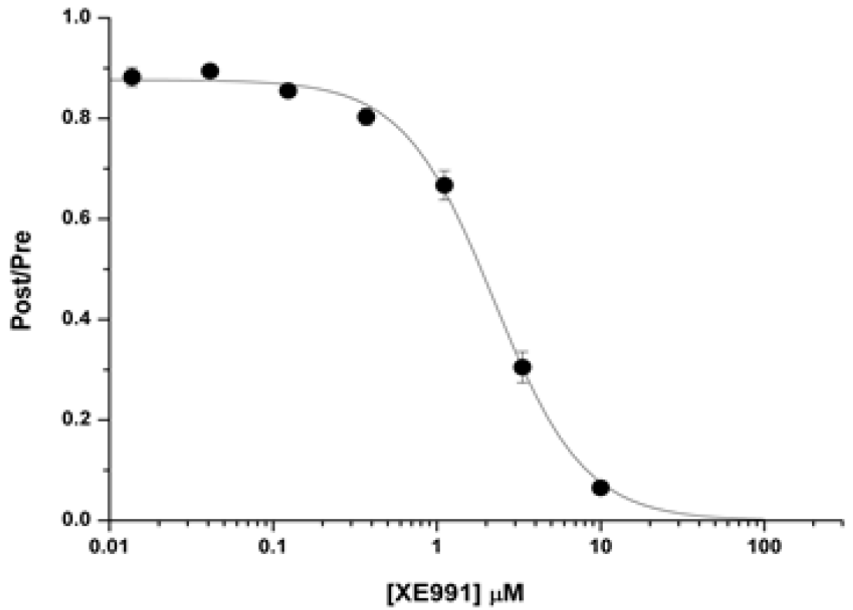


Effect of TEA on hKv7.2/hKv7.3 currents: **Left:** Current-time plot, Cells were pulsed to a potential of 0 mV for 2 seconds from a holding potential of -80 mV and repeated every 10 seconds. Once stable hKv7.2/hKv7.3 current amplitudes at 0 mV were achieved under control conditions, increasing doses of TEA were cumulatively applied to the cell, allowing each concentration to achieve a stable reduction in current amplitude prior to addition of a subsequent dose. Outward current is measured at the end of the depolarizing pulse to 0 mV. **Middle:** Typical current records obtained prior to addition (Control, black trace) and after 0.3 mM (red trace), 3 mM (blue trace) and 30 mM (green trace) TEA. The blue dotted line is the zero current level. **Right:** The mean percent inhibition relative to the control amplitude prior to addition for each concentration of TEA is shown (n = 5-6). (Perforated Manual Patch Clamp Data)

$K_V7.2/K_V7.3$

BACK





Effect of Linopirdine and XE991 on hKv7.2/hKv7.3 currents: The effect of a 10 min incubation of various concentrations of either linopirdine (Left) or XE991 (Right) was assessed on the amplitude of hKv7.2/hKv7.3 currents using IonWorksTM Quattro automated electrophysiology. Each data point represents the mean of 12 cells. (IonWorks Quattro Population Patch Clamp Data)

$K_V7.2/K_V7.3$

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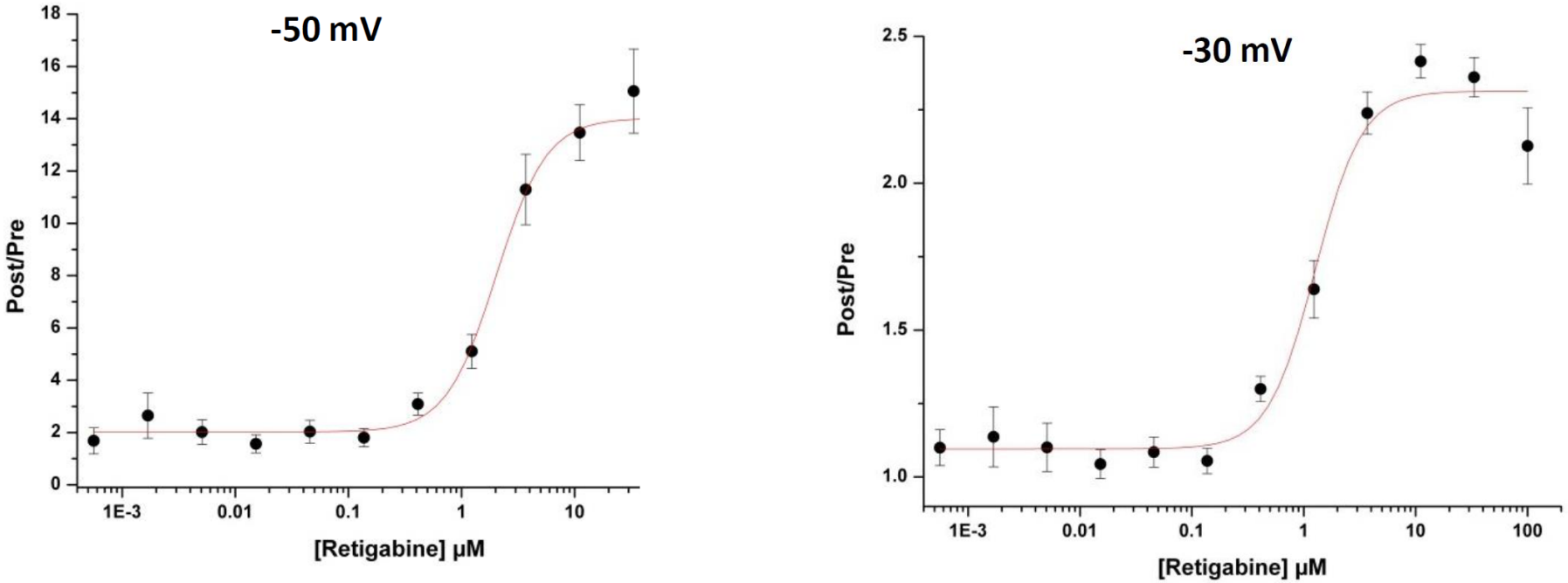

Effect of Retigabine on hKv7.2/hKv7.3 currents: The effect of a 10 min incubation of various concentrations of retigabine on the amplitude of hKv7.2/hKv7.3 currents evoked by a voltage step to either -50 mV (Left) or -30 mV (Right). Each data point represents the mean of 5-8 cells.

$K_V7.3/K_V7.5$ (CYL3060)

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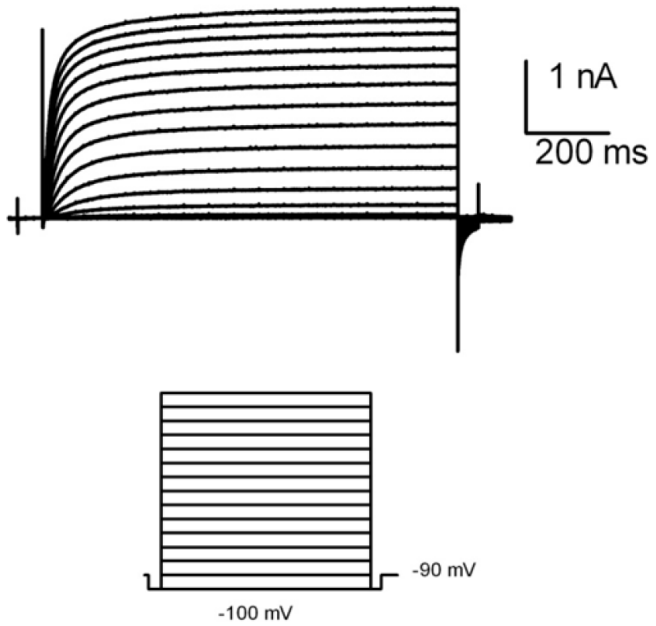

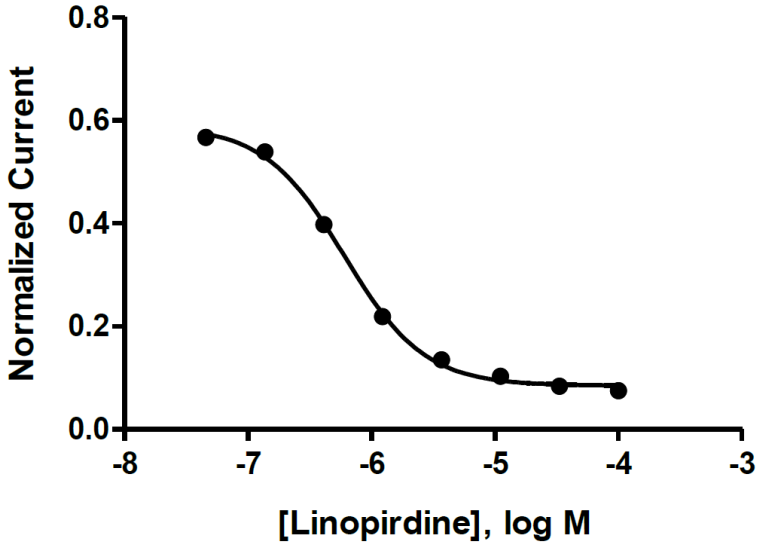

Raw hKv7.3/hKv7.5 Currents and Linopirdine Pharmacology: **Left:** Example currents. hKv7.3/7.5-CHO cells were held at -90 mV, and stepped for 1 s from -100 mV to +40 mV in 10 mV increments. Time-dependent outward currents were observed for steps positive to -80 mV and increased steadily to the +40 mV test potential. **Right:** We observed higher potency than the reported IC_{50} of 7.7 µM, with an IC_{50} value of 0.6 µM and a Hill slope of -1.3. Because we normalized the current to the maximum for each cell before a test application of DMSO, which elicits some rundown of the current, the apparent block at the lowest concentration of linopirdine is overestimated. Estimates of the IC_{50} value and Hill slope from currents normalized to the first DMSO application gave identical values (IonWorks HT Data).

$K_V7.3/K_V7.5$

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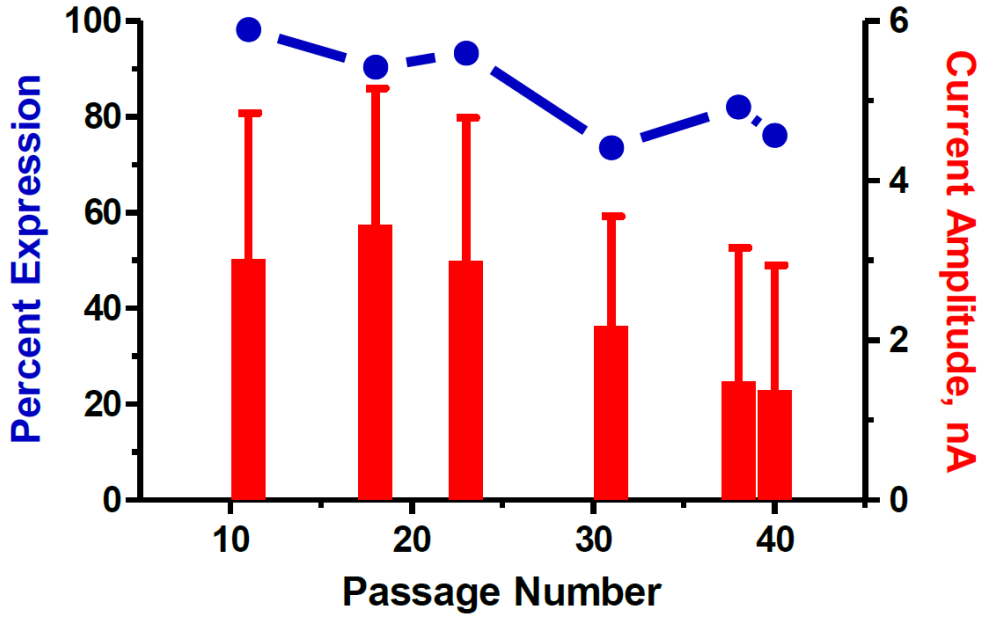

Stability of Expression Over Passage Number: The blue line shows the percentage of cells expressing a mean peak inward current >0.25 nA at +40 mV at cell passages 11, 18, 23, 31, 38 and 40. The red bars show the current amplitude (mean ± SD) for 87-251 cells per experiment (IonWorks HT Data)

K_V7.4 (CYL3092)

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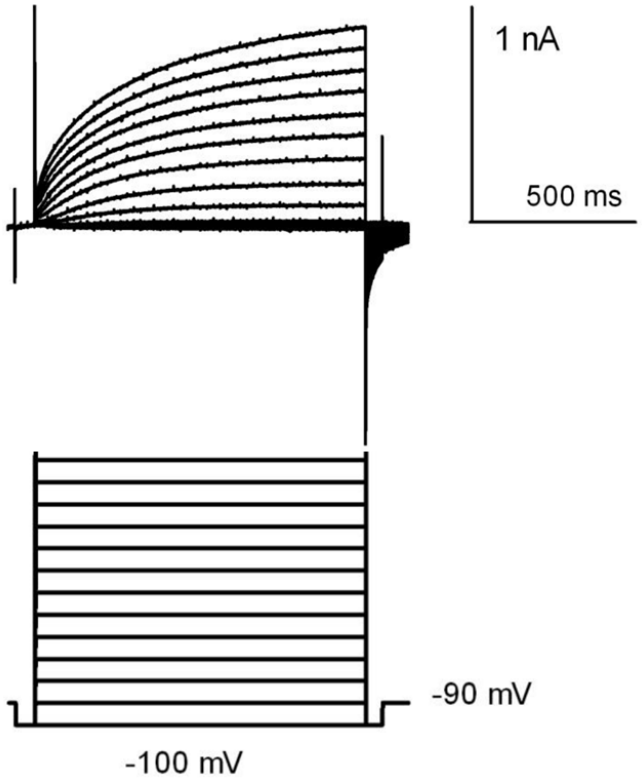
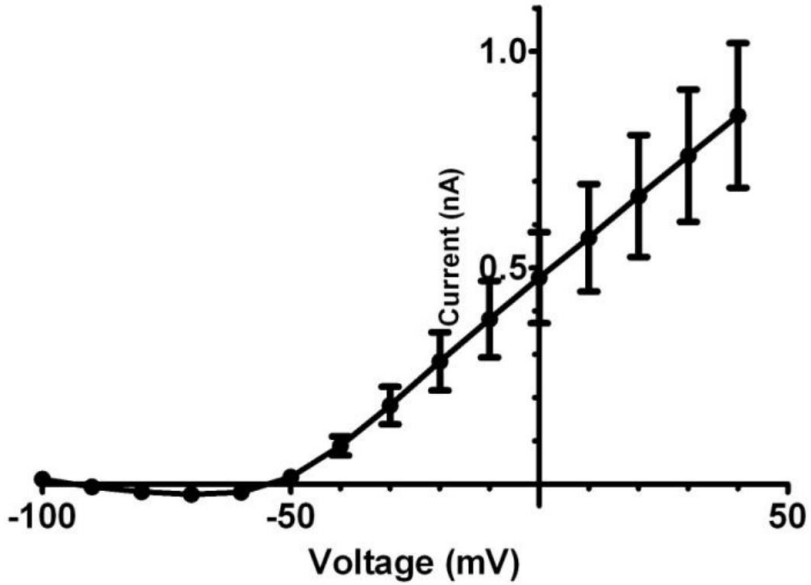
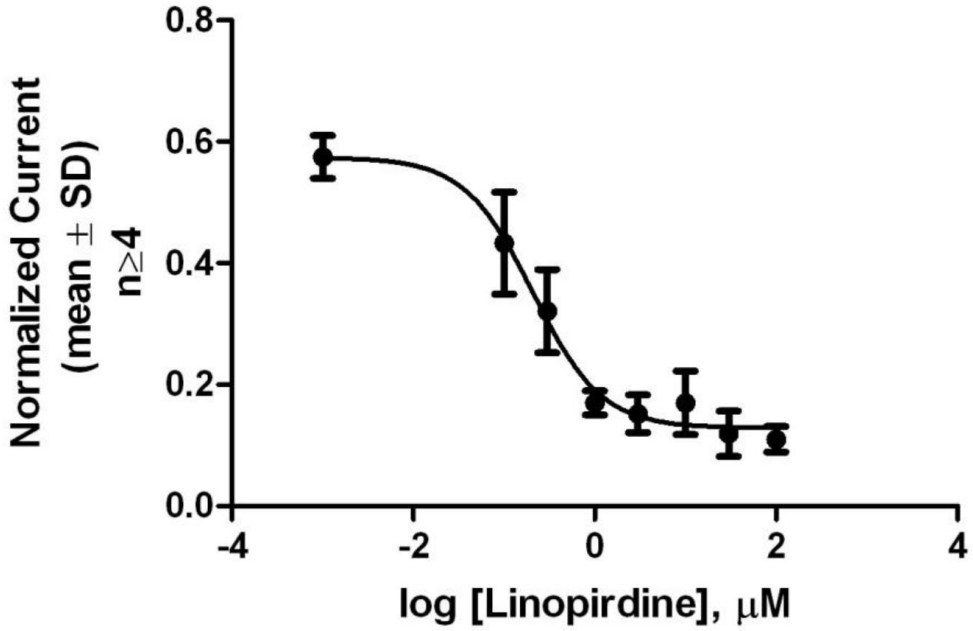


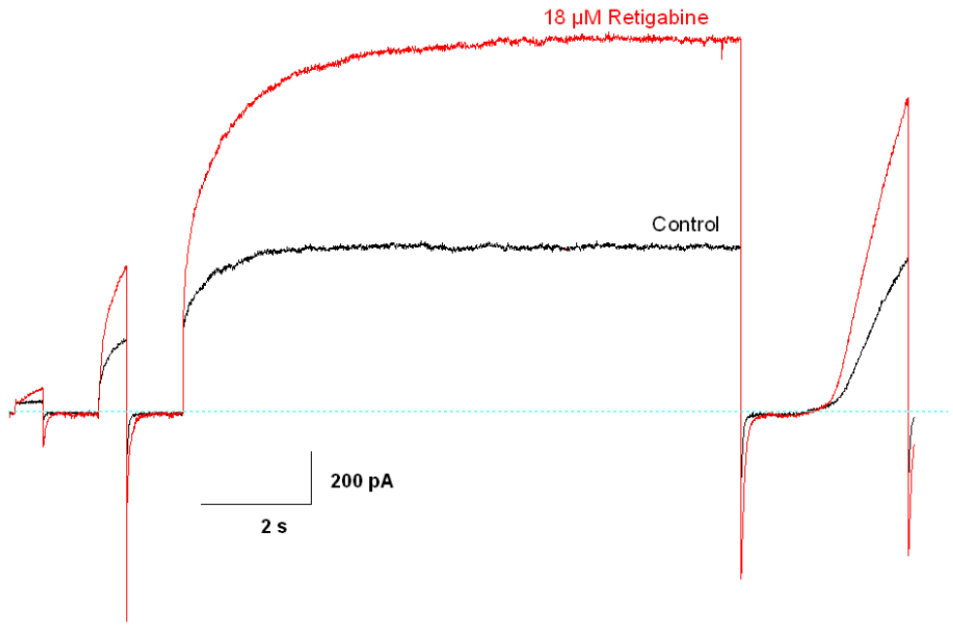


K_V7.4 Raw Data Currents and Current-Voltage (I/V) Relationship: **Left:** Example currents. hKv7.4-HEK cells were held at -90 mV, and stepped for 1 s from -100 mV to +40 mV in 10 mV increments. Time-dependent outward currents were observed for steps positive to -50 mV and increased steadily to the +40 mV test potential. The voltage protocol is shown below the raw currents. **Right:** The mean (+/- SEM) current from 5 cells is shown in the plot (IonWorks HT Data).

$K_V7.4$

BACK

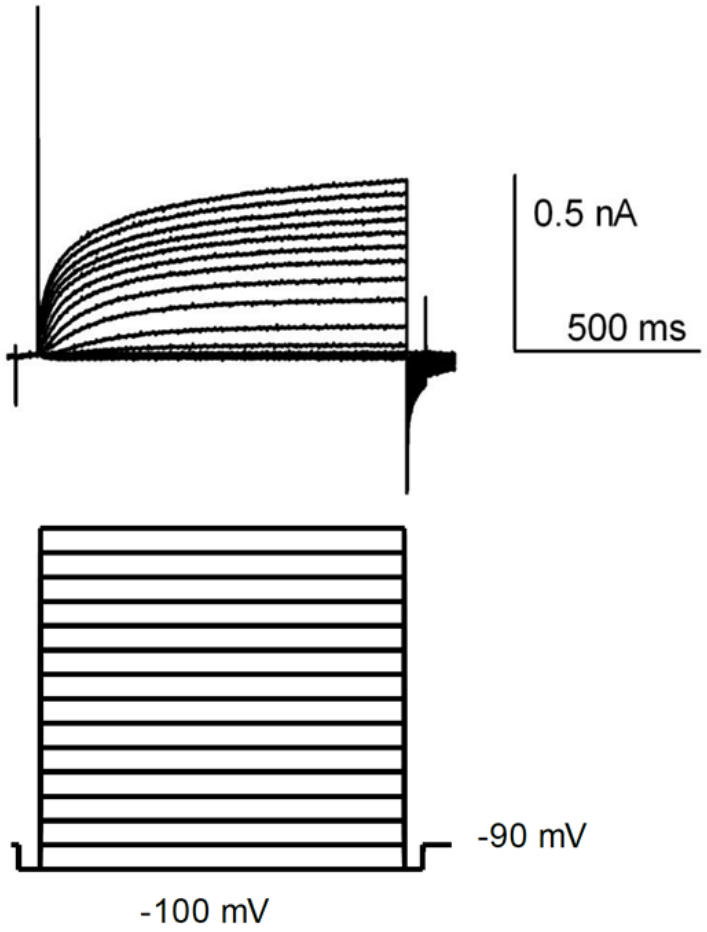


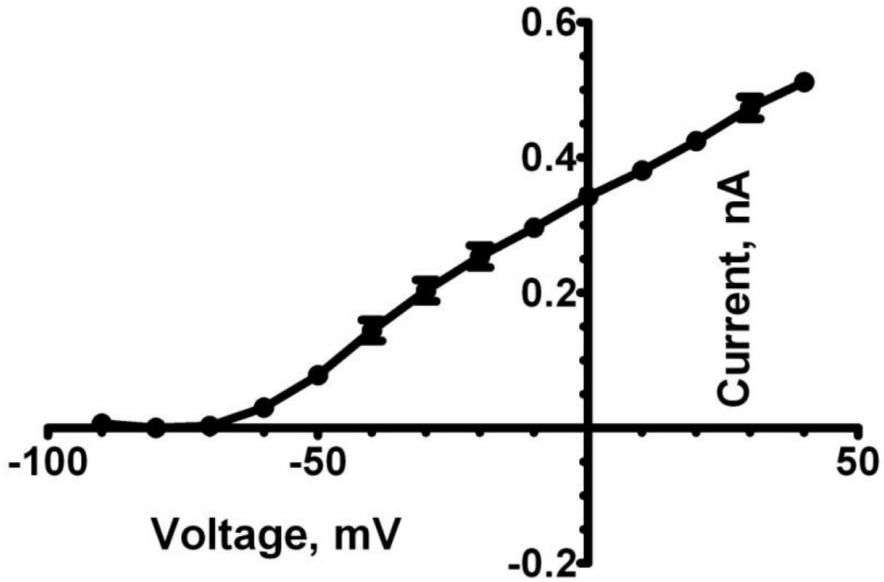


Blockade of $K_V7.4$ Currents with Linopridine, Enhancement of Currents by Retigabine: **Left:** Application of Linopridine inhibited the Kv7.4 current with an IC_{50} of 165 nM, with a Hill slope estimate of 0.5. (Ionworks HT Data). **Right:** Effect of 18 μM retigabine on currents recorded from a Kv7.4/Kv7.5 cell. The dotted line represents the zero current level. Retigabine is a well-characterized opener of all Kv7 channels, with the exception of Kv7.1-minK (KCNQ1/minK). Individual Kv7.4/Kv7.5 cells were held at -100 mV, stepped to -50 mV for 500 ms, to 0 mV for 500 ms, and then to +60 mV for 10 sec. There was a 1 s recovery at the initial holding potential after each pulse. The cells were then ramped from -100 mV to +60 mV in 2 s. Using this protocol, 18 μM retigabine increased the current during the pulse to -50 mV, 0 mV or to +60 mV, and at positive potentials during the ramp. This concentration of retigabine potentiated the current during the pulse to +60 mV by 125%, and by 103% when the ramp reached +60 mV. Similar results were seen in one additional cell. (Manual Patch Clamp Data)

$K_V7.4/K_V7.5$ (CYL3096)

BACK

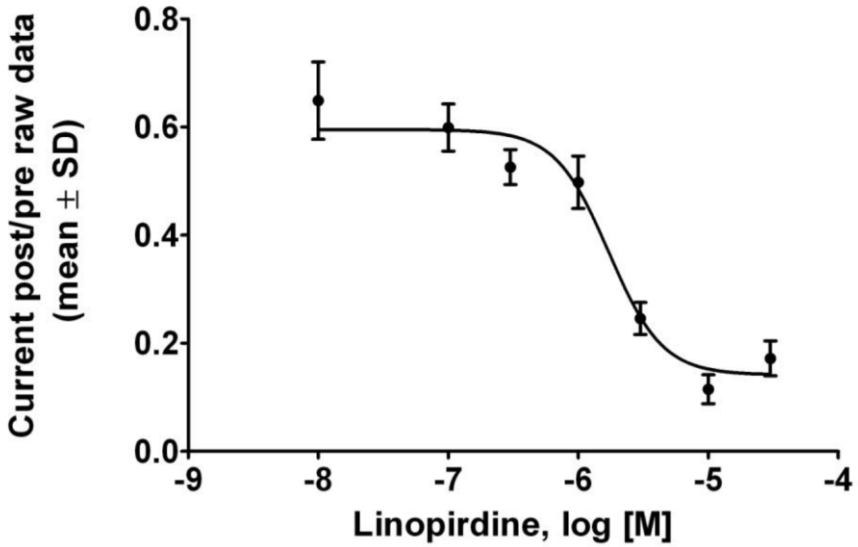




$hK_V7.4/hK_V7.5$ Raw Data Currents and Current-Voltage (I/V) Relationship: **Left:** hKv7.4/hKv7.5-HEK cells were held at -90 mV, and stepped for 1 s from -100 mV to +40 mV in 10 mV increments. Time-dependent outward currents were observed for steps positive to -60 mV and increased steadily to the +40 mV test potential. The voltage protocol is shown below the current traces. **Right:** Current-voltage relationship obtained from the protocol used above. Each point is the mean peak outward current (± SD) from 5 wells.

$K_V7.4/K_V7.5$

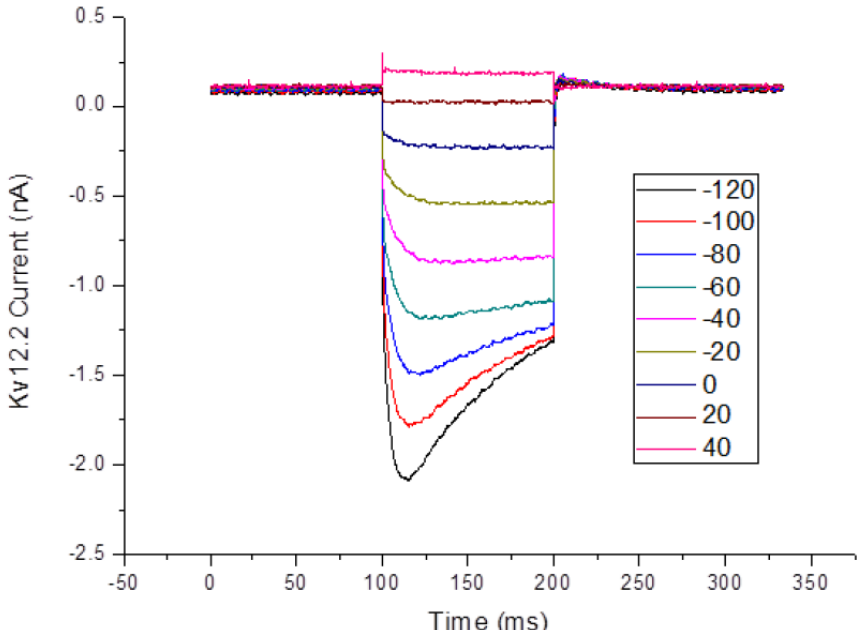
BACK

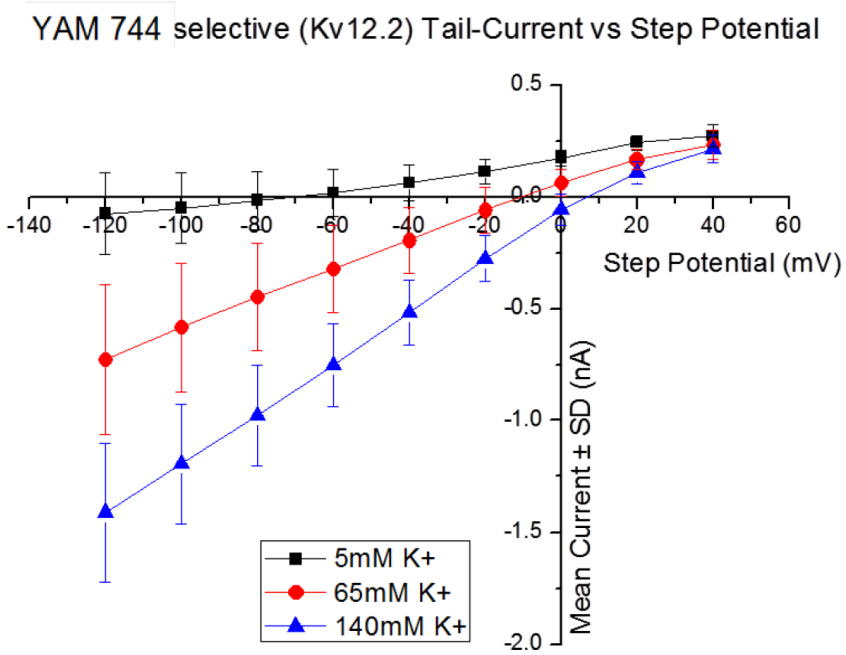


Blockade of $K_V7.4/K_V7.5$ with Linopiridine: Concentration-response curve for Linopirdine on Kv7.4/Kv7.5 cells. Application of Linopirdine inhibited the current amplitude with an IC50 of 1.7 µM, with a Hill slope estimate of -2.1. Data are the mean current ratio (± SD) obtained for each concentration from 9-11 wells (IonWorks HT Data)

K_V12.2 (CYL3089)

BACK

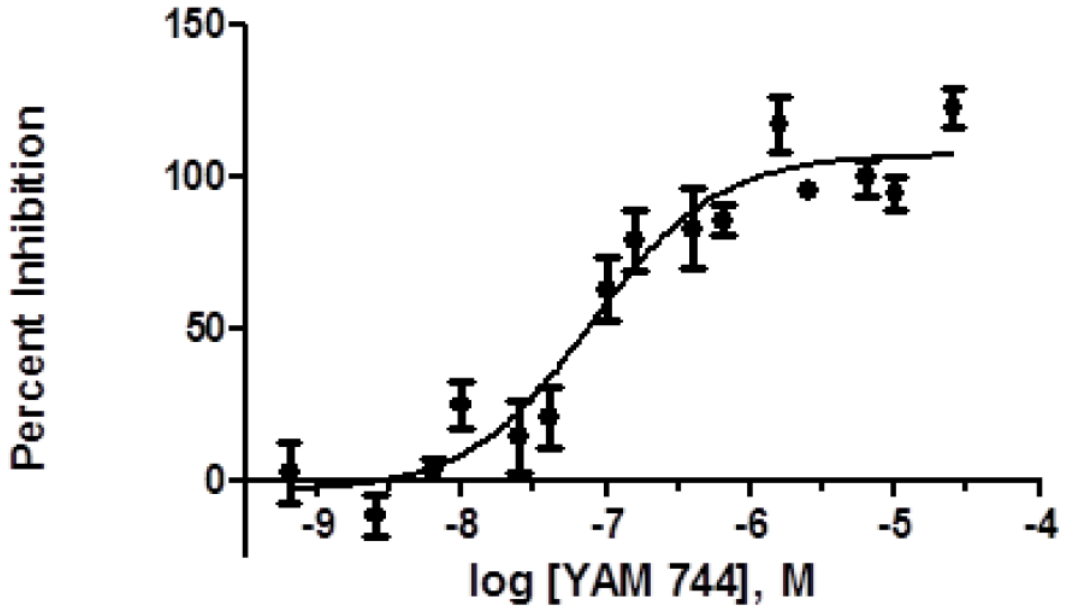




hK$_V$12.2 Raw Data Currents and Current-Voltage (I/V) relationship at various bath K$^+$ concentrations: Currents recorded **(Left)** in 140 mM external K+, evoked by 100 ms voltage steps from –120 mV to +40 mV in 20 mV increments, applied from a holding potential of +30 mV. Activation of voltage-and time-dependent currents was clear at the step to 0 mV, and these currents became progressively larger with more negative steps. The currents shown were obtained by subtracting the current in the presence of YAM 744 from the total current. Right: Current-voltage relationship of hKv12.2 currents recorded in 5 mM external K+ (black symbols & lines), 65 mM external K+ (red symbols & lines) or 140 mM external K+ (blue symbols & lines). Each symbol indicates the current obtained by subtracting the current in the presence of YAM 744 from the total current. The calculated reversal potential for a K+-selective current with 140 mM internal K+ is -83.9 mV in 5 mM external K+ , -19.3 mV in 65 mM external K+ , and 0 mV in 140 mM external K+ . The Kv12.2 currents in each case reversed close to the calculated reversal potential.

$K_V12.2$

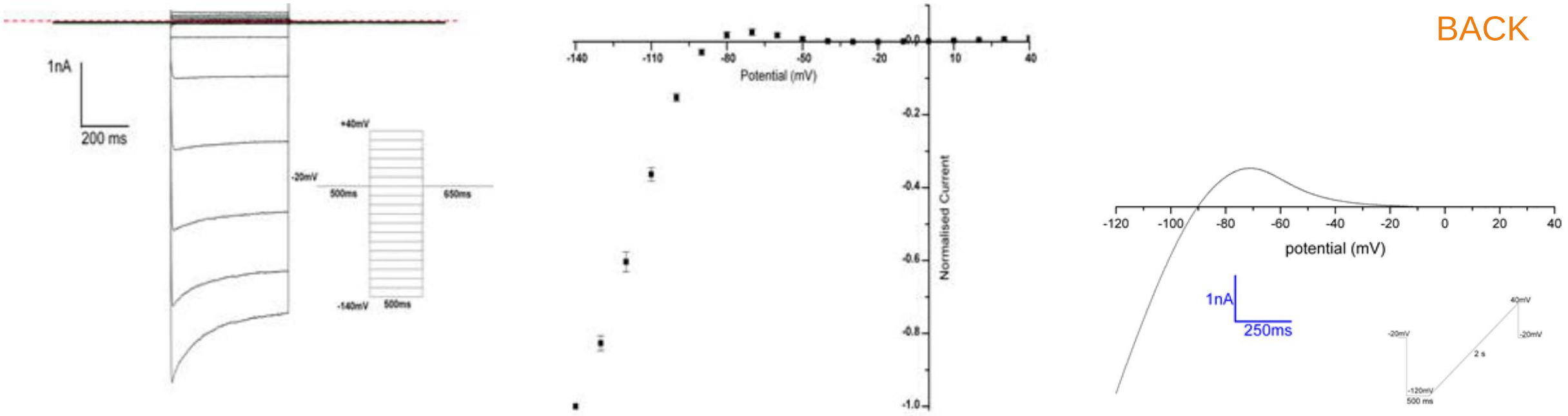
BACK



$K_V12.2$ Blockade by YAM 744: YAM744 is a potent hKv12.2 channel blocker with a reported IC50 value in the low nanomolar range. In this study, bath application of YAM 744, (Figure 3) inhibited the current amplitude with an IC50 of 78.4 nM, with a Hill slope estimate of 1.0.

$K_{ir}2.1$ (CYL3032)

BACK



hK$_{ir}$2.1 Raw Data Currents and Current-Voltage Relationship Showing Inwardly Rectifying Current Properties: hKir2.1 currents **(Left)** were evoked by 500 ms depolarising voltage pulses stepped in 10 mV increments from +40 mV to -140 mV from a holding potential of –20 mV once every 5 seconds (inset). The red dotted line indicates zero current level. Current-voltage relationship **(Middle)** showing the steady state current amplitudes elicited by the voltage protocol shown in A were normalised to the current evoked by the –140 mV voltage step for each cell. The mean data is shown plotted against the step potential in mV (n=5). **(Right).** I/V Relationship generated by a voltage ramp. Cells were held at –20 mV and stepped to –120 mV for 500 ms followed by a 2 second depolarizing ramp to +40 mV (Manual Patch Clamp Data).

$K_{ir}2.1$

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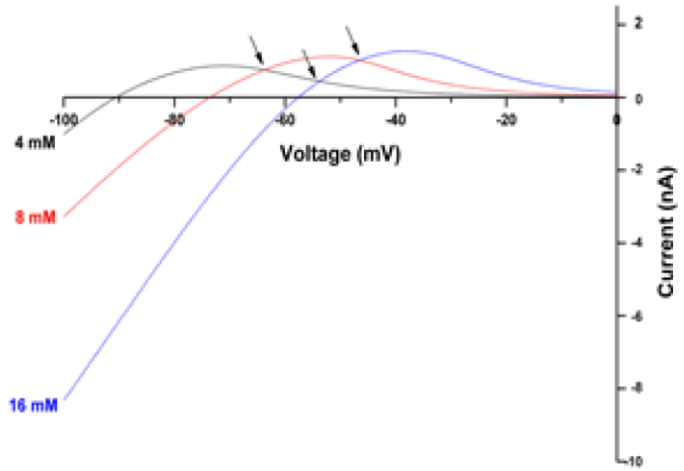

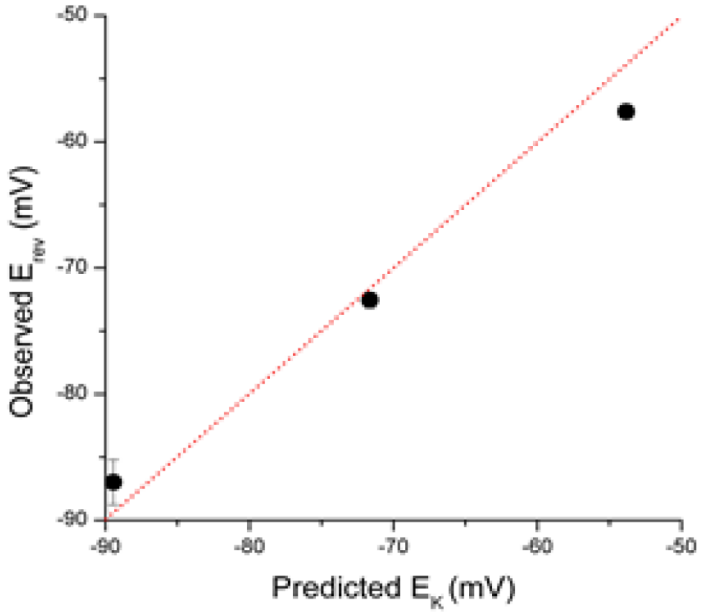

hK$_{ir}$2.1 Effect of Extracellular K$^+$: Typical traces **(left)** illustrating a section of the ramp protocol. The section shows the ramp from –100 mV to 0 mV carried out in the three different external K+ concentrations (4, 8 and 16 mM) cumulatively applied to the cell. Note the cross-over points (arrows) and the voltage regions beyond this point where the Kir2.1 conductance is greater in the higher external K$^+$. **Right:** The predicted mean of E_K (n=3) according to the Nernst equation has been plotted against the observed E_{rev} from the left hand panel. The red dotted line illustrates the line of unity (Manual Patch Clamp Data).

$K_{ir}2.1$

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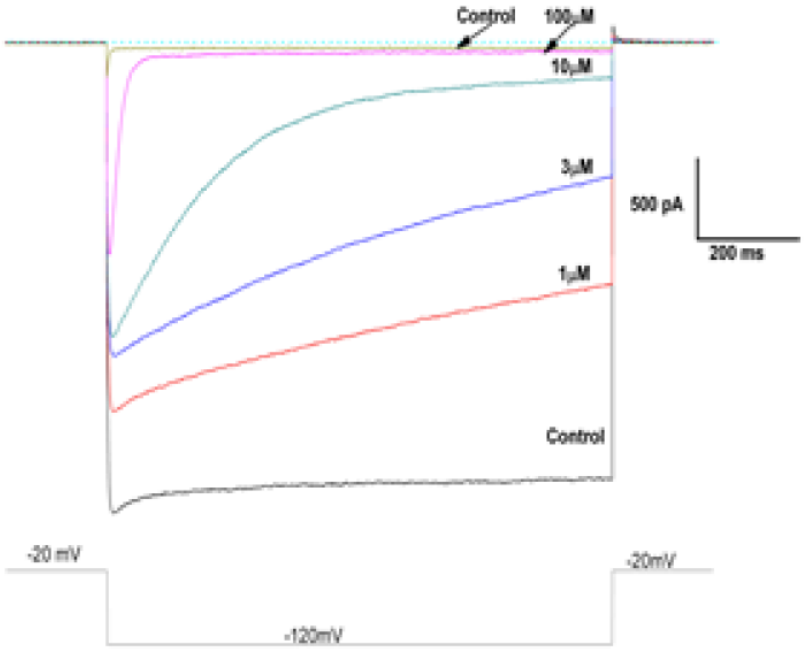
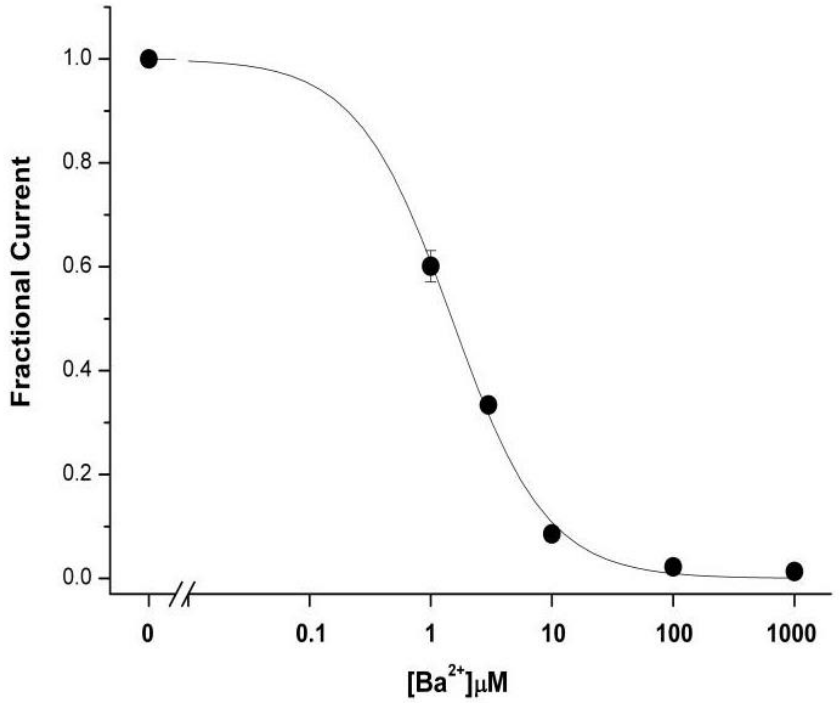
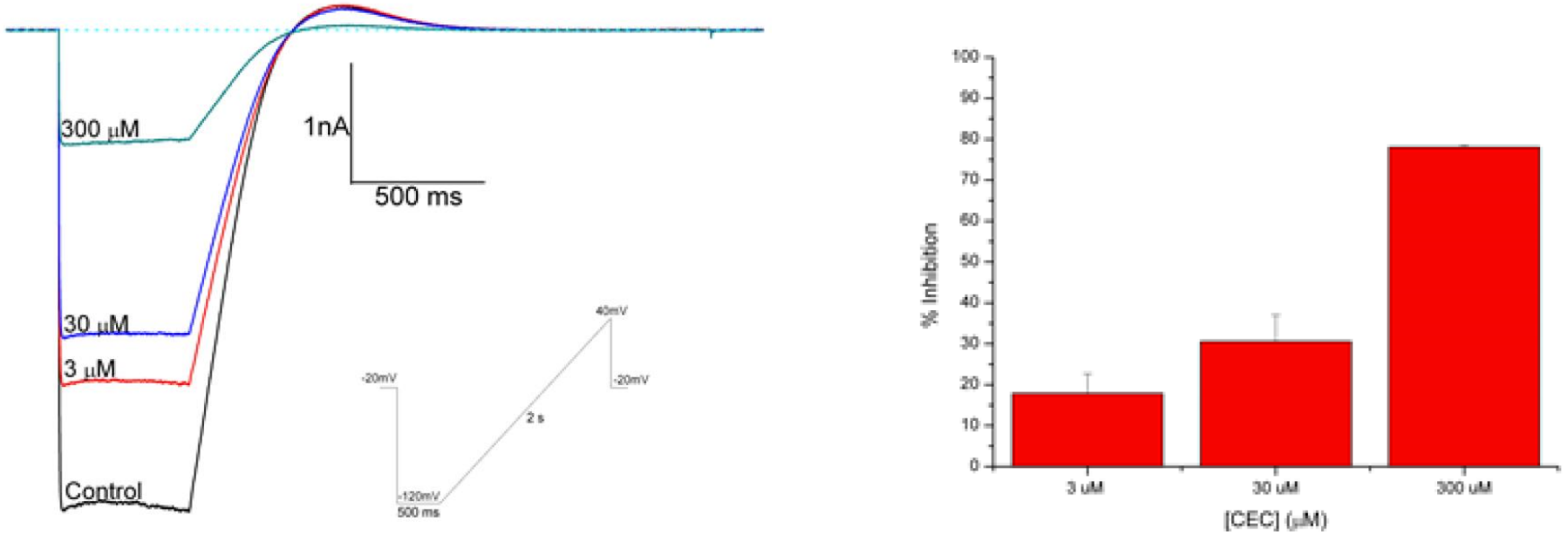




hK$_{ir}$2.1 Blockade by Barium at -120 mV. Left: Cells were pulsed to a potential of –120 mV for 1 second from a holding potential of -20 mV and repeated every 10 seconds. Once a stable current amplitude was achieved under control conditions, increasing doses of Ba2+ were cumulatively applied to the cell, allowing each concentration to achieve a stable reduction in current amplitude prior to addition of a subsequent dose. **Right:** The amplitude of the current at the end of the hyperpolarizing step to -120 mV in various concentrations of Ba2+was measured and expressed as relative current remaining compared to the control response, prior to addition. These values were plotted against concentration to obtain the dose-response curve. This could be described by a Hill equation with an estimated IC50 value of 1.5 μM (Manual Patch Clamp Data).

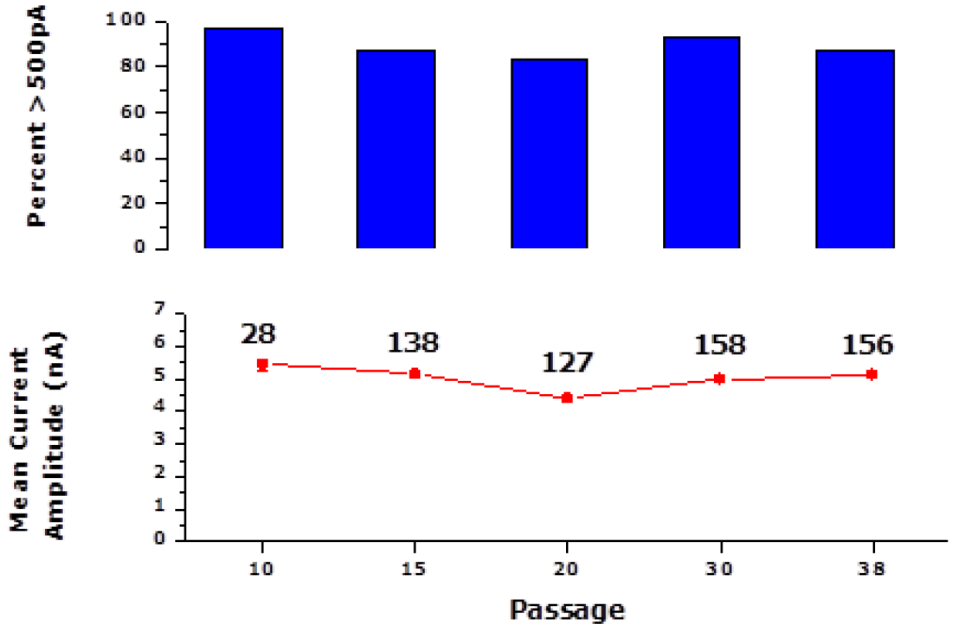
$K_{ir}2.1$



Effect of chloroethylclonidine (CEC) on hK_{ir}2.1 currents. hKir2.1 current traces **(Left)** evoked by stepping to -120 mV for 500 ms from a holding potential of –20 mV followed immediately by a 2 second ramp from –120 mV to +40 mV in the presence of various concentrations of CEC. A pulse interval of 10 seconds was used. **(Right)** Inhibition values (n=3) obtained during the steady state current at –120 mV have been plotted against CEC concentration. (Manual Patch Clamp Data).

$K_{ir}2.1$

BACK



$hK_{ir}2.1$ Currents Stability of Expression Over Passage: The upper panel shows the percentage of cells expressing a mean peak current >500 pA at -60 mV at cell passages 10, 15, 20, 30, and 38. The lower panel shows the mean current amplitude (mean SEM, red circles) and the number of cells (numbers above red circles - out of 32 cells for passage 10 and out of 192 cells for all other passages) (IonWorks HT Data).

K_{ir}6.2/SUR2A (CYL3099)

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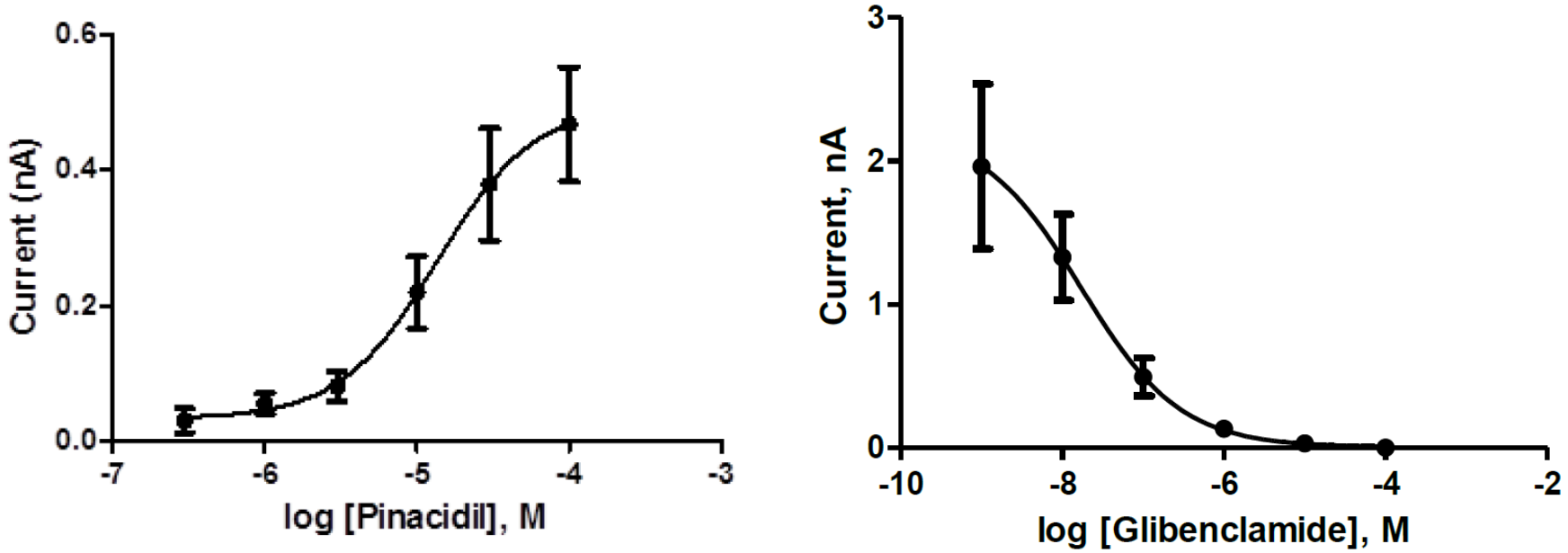

Pinacidil activation and Glibenclamide block of hK_{ir}6.2/Sur2A currents: **Left:** The EC_{50} for pinacidil, each point indicates the mean (±SD) response for 5 cells. We obtained an EC_{50} of 13.5 µM and a Hill slope of 1.36. **Right:** The IC_{50} for glibenclamide was determined in the presence of 30uM pinacidil in the hKir6.2/Sur2A-HEK cells. Each point indicates the mean (±SD) response for 4 cells. We obtained an IC_{50} of 17.2 nM and a Hill slope of -0.71 (PatchXpress Data).

$K_{ir}6.2/SUR2A$

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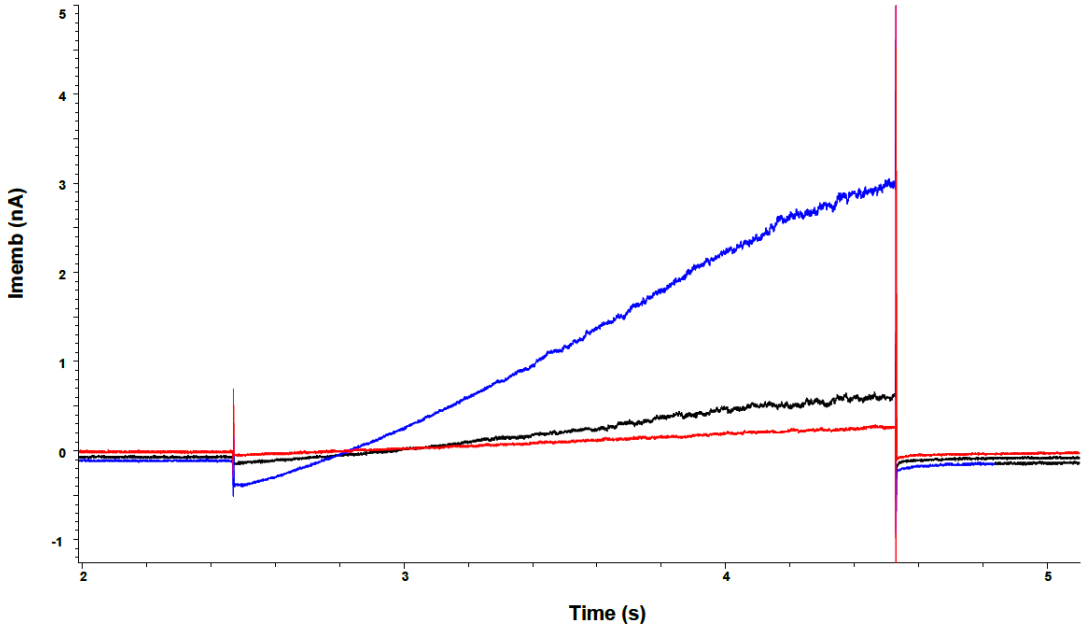
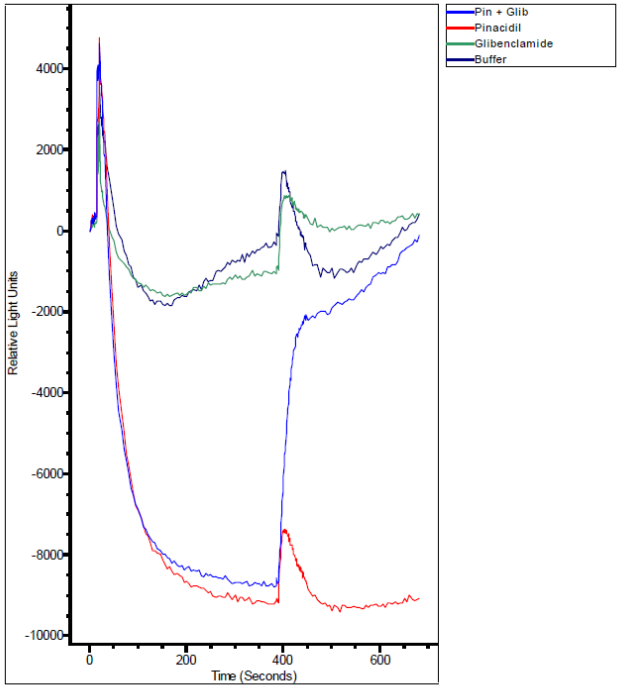


Effect of pinacidil and glibenclamide on hKir6.2/Sur2A currents. The black trace shows the response to addition of external buffer alone. The addition of 30 µM pinacidil stimulated current (blue trace) of approximately 3 nA . After washout, 10 µM glibenclamide was added alone for 3 minutes and had no effect (not shown). After the incubation with glibenclamide, the response to the addition of 30 µM pinacidil + glibenclamide (red trace) was now inhibited. In eleven cells, 10 µM glibenclamide completely blocked the pinacidil-induced increase in current (PatchXpress Data).

K_{ir}6.2/SUR2A



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K_{ir}6.2/Sur2A Membrane Potential Assay: The cells were pre-loaded with the blue membrane potential dye (Molecular Devices, Inc.) for 60 minutes at 30□C. On the instrument the cells were initially treated with buffer (black trace), 10μM (final) glibenclamide (green traces), 30 μM (final concentration) pinacidil (red and blue traces) for 6.3 minutes. The second addition at approximately 400 seconds was either buffer (black trace), pinacidil (red trace), glibenclamide (green trace) or pinacidil+glibenclamide (blue trace). The data show the channel was activated by the initial addition of pinacidil (red and blue traces) resulting in a decrease in fluorescence indicative of hyperpolarization of the cell. This response was reversed by the addition of the blocker glibenclamide (10 μM) in the presence of pinacidil (blue trace). The response to glibenclamide alone was similar to that of buffer alone. (FLIPR^{Tetra} Data)