Case Study

Electrophysiological characterization of functional and pharmacological effects of β subunit expression on BK channels

Introduction

Introducing the backbone of cellular regulation: Big Potassium (BK) channels. These gatekeepers of ion flow orchestrate many pivotal functions across diverse tissues within the human body.

BK channels, widely recognized as high-conductance voltage and calcium-activated potassium channels, are transmembrane proteins consisting of four pore-forming α subunits (Figure 1). Each α subunits is comprised of seven transmembrane segments, encompassing a voltage sensor domain and a pore-forming domain.

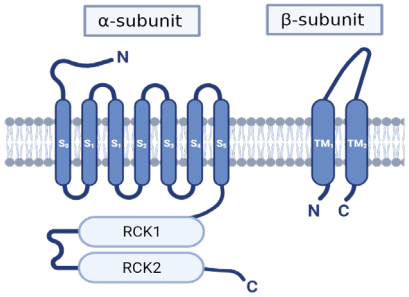


Figure 1: **Schematic representation of the BK channel structure composed of the alpha and beta subunits.**

Far from passive entities, BK channels have a unique dual activation mechanism, responding to both membrane depolarization and intracellular calcium concentrations. This distinctive trait enables BK channels to finely modulate cellular excitability and signalling pathways. Activation triggers a cascade where BK channels facilitate the efflux of potassium ions, inducing membrane hyperpolarization and effectively diminishing cellular excitability.

Beyond their primary duty in overseeing the smooth muscle tone across vital tissues such as blood vessels, urinary blader, and the gastrointestinal tract, BK channels play a pivotal role in modulating neuronal excitability and synaptic transmission.

SB Drug Discovery have developed a suite of comprehensive assays showcasing calcium sensitivity, kinetics, activator and inhibitor profiling, reproducibility benchmarks, and I-V assays. At SB Drug Discovery, we don't just offer customizable assays; we provide a pathway to novel insights, providing your research with data you can trust.

BK Channel Discovery Platform

- **Robust Stable Cell Lines**

Delivering premium, custom solutions with stable cell line generation that display strong co-expression of diverse beta subunits.

- **Customizable Assays**

Explore SB Drug Discovery's comprehensive suite of assays measuring calcium sensitivity, channel kinetics, and activator/inhibitor profiling.

- **End-to-End Support**

SB Drug Discovery's capabilities cover all aspects of ion channel drug discovery, providing flexible integrated solutions to your research.

Methods

HEK cells stably expressing BK alpha alone or co-expressed with a defined beta subunit were produced by SB Drug Discovery. Whole cell patch-clamp experiments were carried out at room temperature using single or multi-hole chips on the SynchroPatch 384i automated electrophysiology platform. For I-V experiments, 500 ms voltage steps from -50 mV to +150 mV, in 10 mV increments. Throughout a holding potential of -80 mV was maintained, with an additional 100 ms pre-pulse to -140 mV. Data analysis was performed using Data Control 384 V2.2 (Nanion) and GraphPad Prism V5.01.

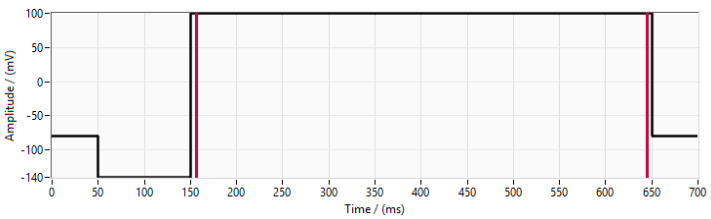
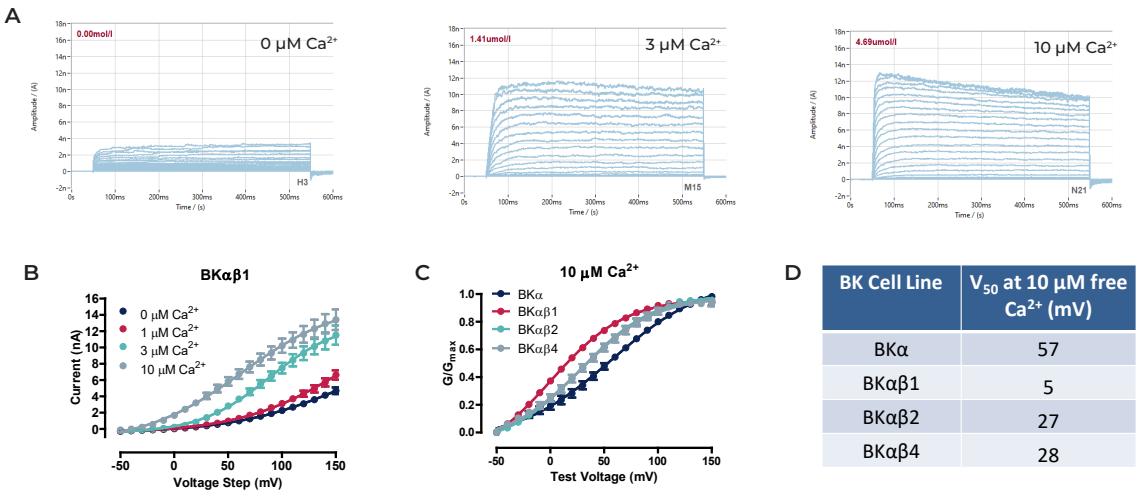


Figure 2: **Voltage protocol.**

Biophysical Properties

Ca^{2+} Sensitivity

Calcium sensitivity determines how likely BK channels are to open in response to changes in intracellular calcium concentration. As a result of their sensitivity to calcium, BK channels can act as molecular sensors, responding to changes in intracellular calcium concentration and influencing the overall physiology of the cell. Through understanding the calcium sensitivity of BK channels, researchers can gain insight into their physiological roles and how they might be involved in various disease states. SB Drug Discovery has designed and validated electrophysiological assays to measure BK calcium sensitivity (Figure 2), helping inform the development of new therapeutic interventions targeting these channels.



BK Cell Line	V_{50} at 10 µM free Ca^{2+} (mV)
BKα	57
BKαβ1	5
BKαβ2	27
BKαβ4	28

Figure 3: BK Ca^{2+} Sensitivity
(A) Representative current traces for the BKαβ1 subtype in the presence of 0 µM, 3 µM and 10 µM internal free Ca^{2+}. (B) I-V plot of BKαβ1 in the presence of increasing concentration of internal free Ca^{2+}. (C) The normalised I-V plot for each subtype in the presence of 10 µM internal free Ca^{2+}. Mean ± S.E.M. is shown for 2-8 cells per condition. (D) Calculated V_{50} values for each subtype. In line with literature, the BKαβ1 subtype displayed increased sensitivity to internal Ca^{2+}.

Kinetics

To elucidate the functional characteristics of the different BK channel subtypes, SB Drug Discovery has designed and validated electrophysiological assays to measure channel kinetics, comparing activation and inactivation under applied conditions. Figure 3 shows representative current traces for each BK subtype in the presence of 10 µM internal free calcium and associated activation and inactivation tau.

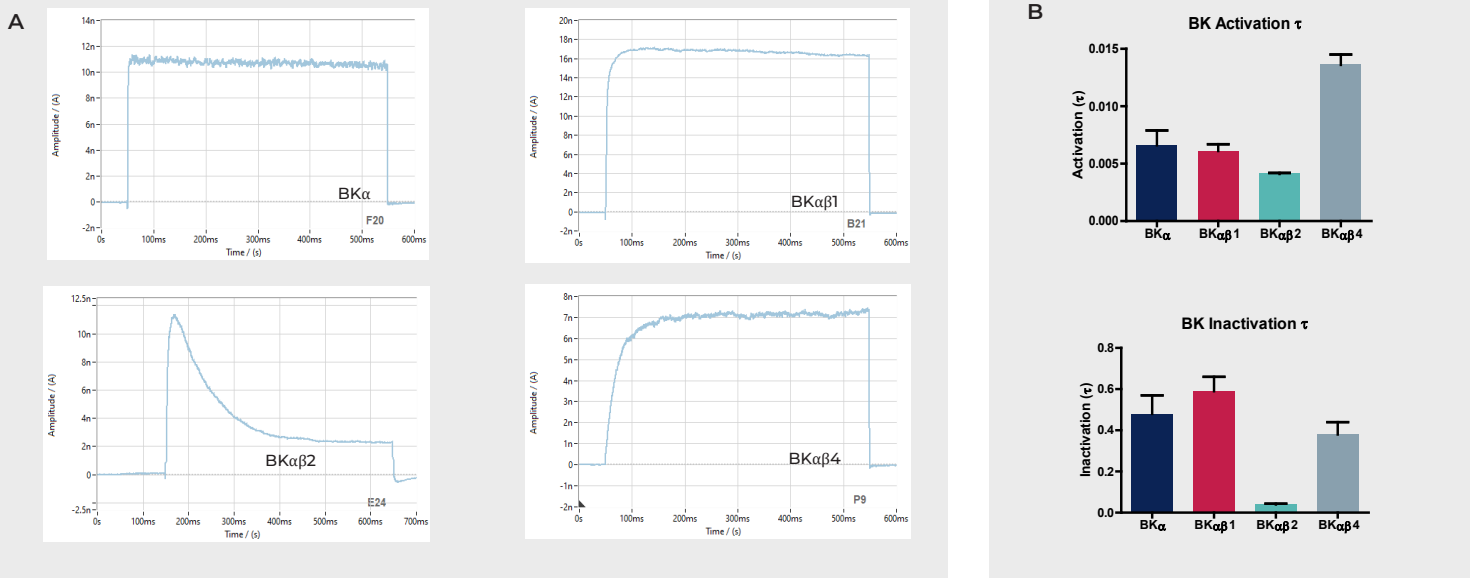


Figure 4: BK Channel Kinetics
(A) Representative current traces of the BK subtypes in the presence of 10 µM internal free Ca^{2+} monitored using a peak pulse. The activation (B) and inactivation (C) tau value of each BK subtype is shown in the presence of 10 µM internal free Ca^{2+}. As expected the BKαβ4 subtype displayed a slower activation rate and BKαβ2 exhibited the fastest inactivation rate.

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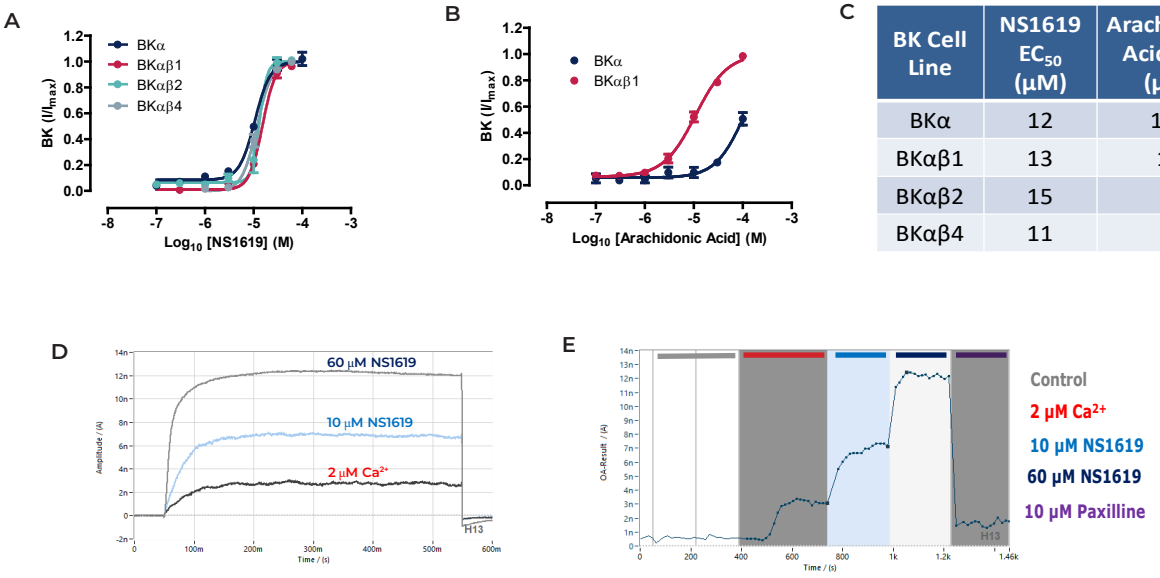
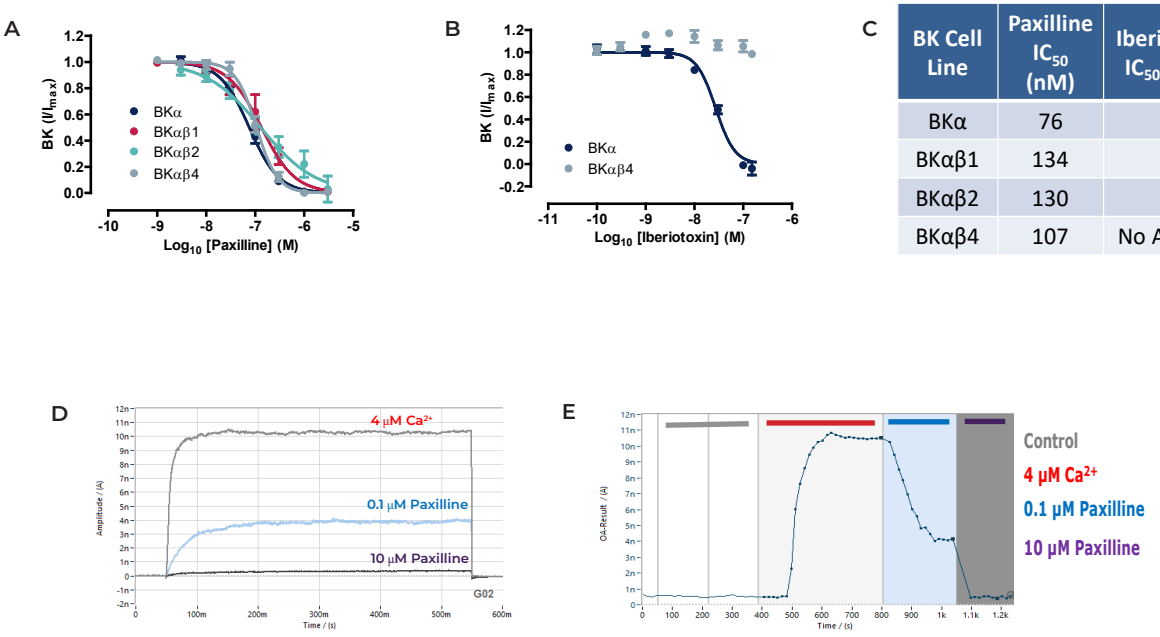
BK Channel Modulation

To assess the effect of activators and inhibitors on various BK subtypes, the effects of Arachidonic Acid and NS1619 (Figure 4), as well as Paxilline and Iberiotoxin (Figure 5), were evaluated. Arachidonic Acid's selectivity for the β1 subunit was clearly visible while Paxilline's known non-selective profile was also observed. Iberiotoxin's selectivity for BK α over BK α+ β4 was also confirmed.



BK Cell Line	NS1619 EC_{50} (µM)	Arachidonic Acid EC_{50} (µM)
BKα	12	108
BKαβ1	13	11
BKαβ2	15	-
BKαβ4	11	-

Figure 5: **BK Activator Assay**
(A) Normalised concentration response curves showing the effect of NS1619 on the four BK subtypes.
(B) Arachidonic Acid, demonstrated the expected selectivity for the β_1 subunit over BKα alone. (C) Calculated EC_{50} values for reference activtors against each BK subtype. (D) Example current trace and (E) time-course of BKαβ1 in the presence of 2 µM free internal Ca^{2+} in combination with 10 µM NS1619.



BK Cell Line	Paxilline IC_{50} (nM)	Iberiotoxin IC_{50} (nM)
BKα	76	27
BKαβ1	134	-
BKαβ2	130	-
BKαβ4	107	No Activity

Figure 6: **BK Inhibitor Assay**
(A) Normalised concentration response curves showing the effect of Paxilline on the four BK subtypes.
(B) The normalised response of Iberiotoxin which is inactive in cells expressing the β_4 subunit over BKα alone.
(C) Calculated IC_{50} values for reference inhibitors against each BK subtype. (D) Example current trace and (E) time-course of BKαβ4 in the presence of 4 µM free internal Ca^{2+} in combination with 0.1 µM Paxilline.

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Reproducibility

The effect of NS1619 and Paxilline on BK channel subtypes was shown to be reproducible over repeat experiments (Figure 6), highlighting the reliability of this assay for drug discovery screening.

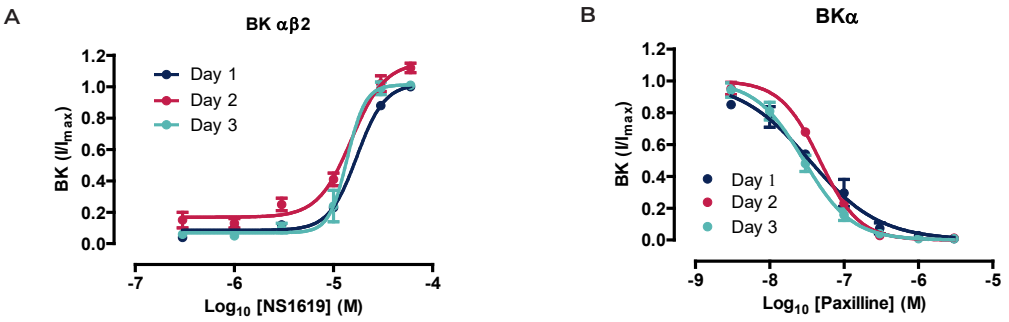

Figure 7: Assay Reproducibility
(A) Normalised concentration response curves showing the effect of the activator NS1619 on the BKαβ2 subtype over several days. (B) Normalised concentration response curves showing the effect of the inhibitor Paxilline on the BKα subtype over several days.

Characterization of Conductance Properties

I-V studies were conducted on two different BK channel subtypes in the presence of a specific concentration of internal free calcium (Figure 7). The results demonstrate that the presence of the agonist NS1619 led to an increased responsiveness of the channels to voltage changes, as evidenced by a leftward shift in the I-V curve. Conversely, the presence of the inhibitor Paxilline reduced BK channel activity. These studies provide valuable insight into the modulatory effect of test compounds on BK channels.

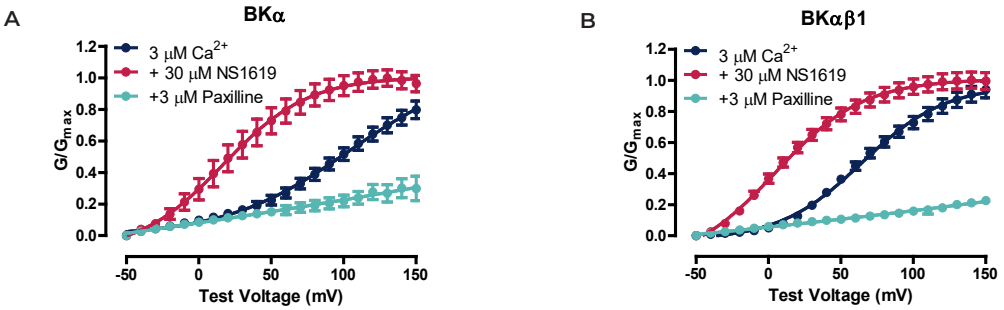

Figure 8: I-V Assay
(A) Normalised I-V relationship of BKα in the presence of 3 μM internal free Ca^{2+}. (B) Normalised I-V relationship of BKαβ1 in the presence of 3 μM internal free Ca^{2+}. Mean ± S.E.M. is shown for 2-12 cells per concentration.

Conclusions

SB Drug Discovery's comprehensive suite of assays helps elucidate the intricate biophysical properties of BK channels. Through calcium sensitivity assays, kinetic studies, and modulator assessment, SB Drug Discovery provides a pathway to understanding BK channel behaviour under diverse conditions and offers valuable insights for potential therapeutic interventions and the development of subtype-selective compounds.