Case Study

Functional and Pharmacological Profiling of Kv1.x Ion Channels

Alexander Dickson

Lead Scientist, Electrophysiology

Introduction

Voltage-gated potassium (Kv) channels are integral membrane proteins that selectively conduct K^+ ions across cell membranes, playing a key role in modulating cellular excitability. These channels influence numerous physiological processes, including neuronal and cardiac signalling, muscle contraction, hormone secretion and intracellular signalling.

The Kv channel family comprises 12 subfamilies (Kv1-Kv12) each made up of multiple α subunits that assemble into tetrameric structures with unique biophysical and pharmacological properties. This diversity enables precise modulation of a broad variety of cellular responses.

Therapeutic Relevance

Kv channels, particularly the Kv1 subfamily, are increasingly recognized as valuable therapeutic targets across a broad spectrum of diseases. Kv1.3, a well-studied member of the Kv channels, is expressed in diverse tissues including immune cells and adipose tissue and is expressed in certain cancers, where it plays important roles in cell proliferation, inflammation and metabolic regulation.

Growing evidence also supports Kv1.3's contribution to type 2 diabetes and associated complications. In a 2021 study, pharmacological inhibition of Kv1.3 using the selective inhibitor PAP-1 improved insulin sensitivity, reduced systemic inflammation and prevented cardiac arrhythmias in diabetic rats, highlighting Kv1.3 as a promising target for both glycaemic control and cardiovascular protection (Zayas-Arrabal *et al*., 2021).

Interest in Kv1.3 has also intensified in the context of oncology. A 2023 study demonstrated that Kv1.3 inhibitors reduced glioma-induced astrocyte proliferation, suppressed tumour cell invasion and led to a reduction in tumour volume (Vitale *et al*., 2023). These findings underscore Kv1.3's dual relevance in both tumour cell biology and immune modulation.

In addition, other Kv1.x channels have been linked to cardiovascular, neurological and cancer-related disease, reinforcing their broader relevance in therapeutic development.

Methods

HEK cells stably expressing Kv1.x were produced by Sygnature Discovery. Whole-cell patch clamp experiments were carried out using single and multi-hole chips on the SyncroPatch 384i electrophysiology platform.

Currents were elicited by using repeated step pulses from a membrane potential of -80 mV, depolarising to +40 mV for 300 ms, returning to -80 mV, every 15 seconds.

Current amplitude was precisely monitored using an activation I-V curve, applying step pulses from -80 mV to +80 mV in +10 mV increments.

Data analysis was performed using DataControl 384 V3.2.1 (Nanion Technologies) and Prism V10.1 (GraphPad).

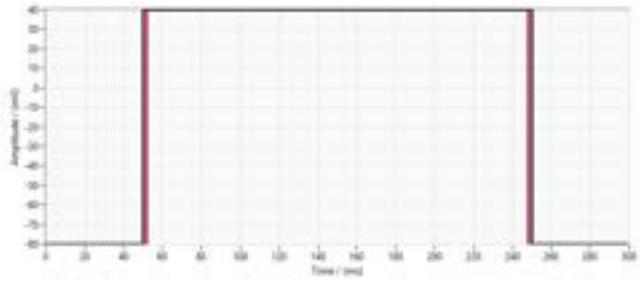

Figure 1.
Voltage protocol diagram showing Kv currents elicited by using repeated step pulses, increasing from a holding potential of -80 mV to +40 mV, over 300ms, repeated every 15 seconds.

References

- Zayas-Arrabal, J., *et al.* (2021). *Kv1.3 Channel Blockade Improves Inflammatory Profile, Reduces Cardiac Electrical Remodeling, and Prevents Arrhythmia in Type 2 Diabetic Rats*. Cardiovascular Drugs and Therapy, 37, 63–73. https://doi.org/10.1007/s10557-021-07264-1
- Vitale, A., *et al*. (2023). *Voltage-gated potassium channel 1.3: A promising molecular target in glioma therapy*. Médecine et Maladies Infectieuses. https://doi.org/10.1016/j.medmal.2023.104089

Kinetic Diversity Across Kv1.x Channel Family

Assay fidelity and isoform discrimination were examined by analysing activation and inactivation kinetics of the Kv1.x family. Each channel displays a distinct electrophysiological signature, consistent with literature and driven by differences in gating mechanisms.

The kinetic diversity underpins the functional versatility of Kv1 channels across tissues. Fast-activating and inactivating isoforms such as Kv1.4 and Kv1.7, found in neurons and muscle, enable rapid repolarization and support high-frequency firing. Slower-kinetic channels such as Kv1.1 generate more sustained currents, stabilizing membrane potential, particularly in the CNS, where fine control over excitability is essential. These differences in gating help explain the tissue-specific distribution and specialized roles of Kv1 family members.

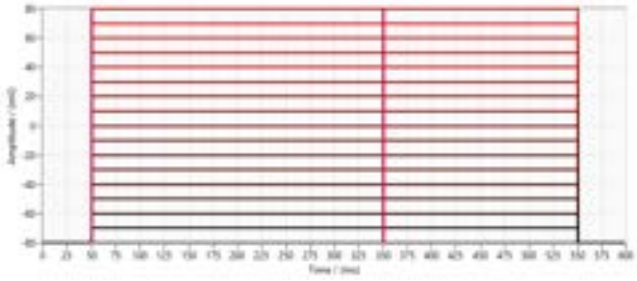

Figure 2.
Activation I-V curve protocol diagram showing Kv currents elicited by using repeated step pulses, increasing from a holding potential of -80 mV to +80 mV, in +10 mV increments over 500ms, repeated every 15 seconds.

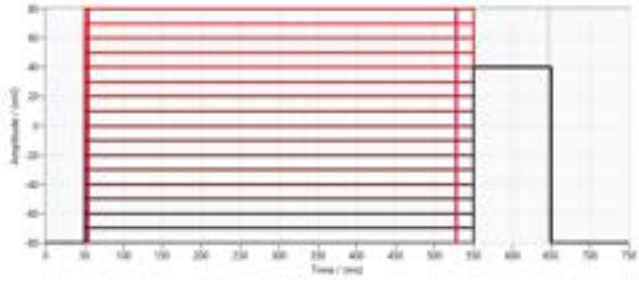

Figure 3.
Inactivation I-V curve protocol diagram showing Kv currents elicited by using repeated step pulses, increasing from a holding potential of -80 mV to +80 mV, in +10 mV increments over 500ms, then at held at +40 mV for 100ms, before returning to -80 mV, repeated every 3 seconds.

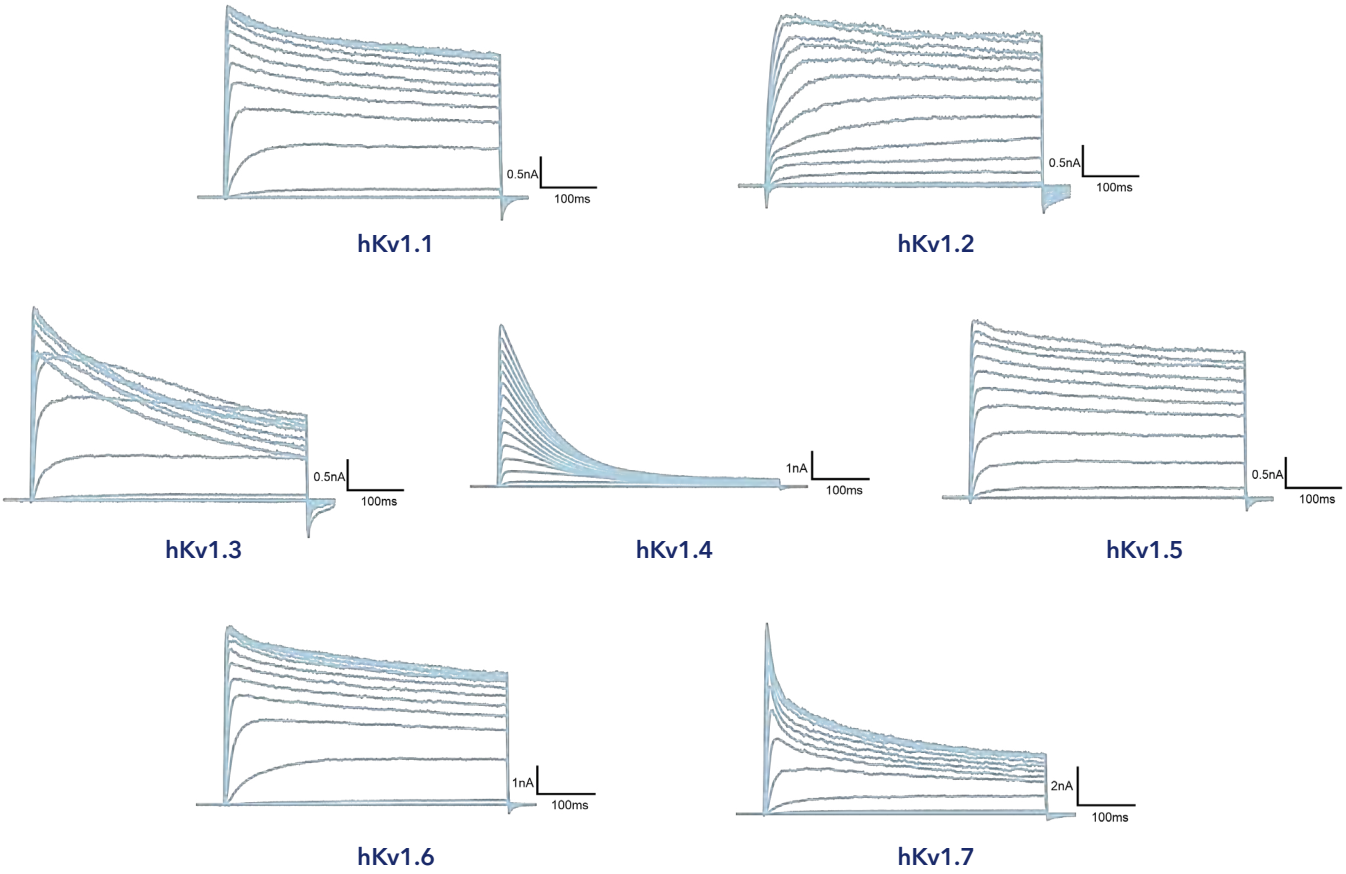

Figure 4.
Representative current traces of Kv1.x, illustrating the distinct deactivation kinetics across the Kv1.x family.

Pharmacology

Pharmacological profile was assessed using Quinidine, a broad-spectrum Kv channel inhibitor, as a reference antagonist. Quinidine's known activity across multiple Kv1 subtypes makes it an ideal benchmark for assessing assay responsiveness and pharmacological robustness.

Across all Kv1.x channels, Quinidine produced a robust and reproducible inhibitory effect, confirming:

- Consistent reduction in current amplitude
- Expected dose-dependent response profiles
- Stable assay windows suitable for screening or mechanistic studies

While overall inhibition was consistent, minor differences in response onset and inhibition depth were observed between isoforms, reflecting known pharmacological variation within the family.

These results demonstrate that the Kv1.x assays are sensitive, pharmacologically responsive and ready for use in both hit identification and compound profiling settings.

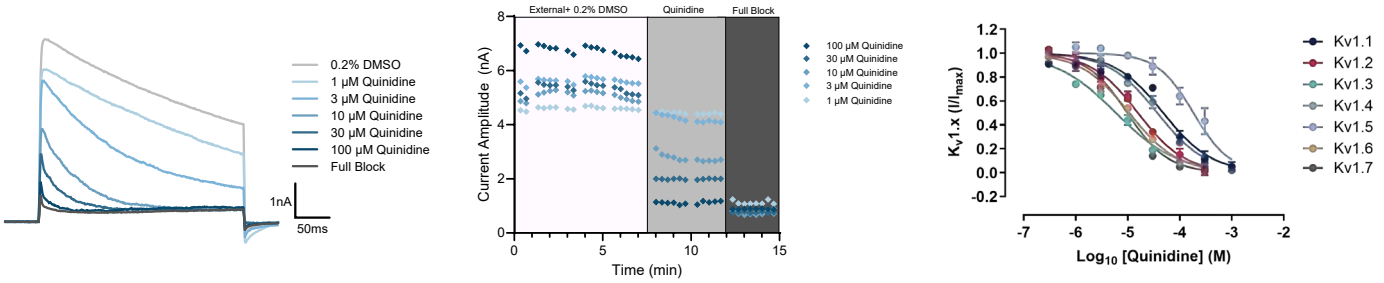

Figure 5.
A) Representative current trace and corresponding time course of Kv1.3, first under control conditions, followed by progressive inhibition by increasing concentrations of the antagonist, Quinidine, culminating in a complete channel block at a saturating concentration. B) Concentration-response curves illustrating the activity of Quinidine across the Kv1 panel.

Known subtype-selective blockers were evaluated to confirm assay fidelity. These compounds provided clear pharmacological resolution between closely related channel isoforms.

- TEA (tetraethylammonium) showed selective inhibition of Kv1.1 and Kv1.6, with no measurable effect on Kv1.4, Kv1.5 or Kv1.7, supporting its known differential sensitivity across the family
- Dendrotoxin-K produced a potent inhibition of Kv1.1, with no measurable response against Kv1.5, confirming subtype selectivity

These findings complement the broad-spectrum inhibition seen with Quinidine and demonstrate the platform's ability to detect both pan-Kv activity and isoform-selective pharmacology.

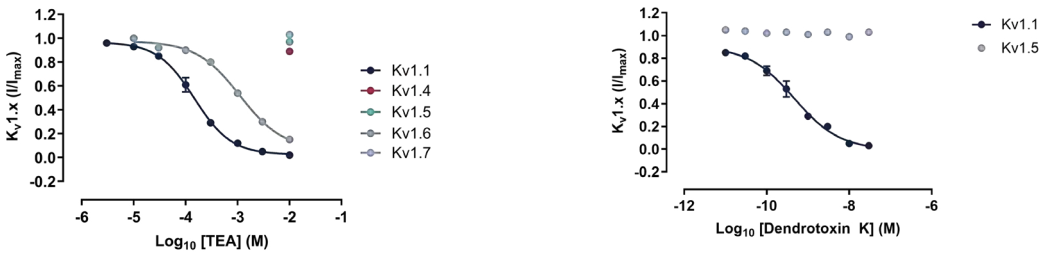

Figure 6.
Concentration-response curves illustrating the effect of two reference antagonists, TEA and DTXk, against the Kv1 panel, emphasising compound selectivity.

Additionally, I-V profiling of Kv1.2 in the presence of reference antagonist Spinoxin and Quinidine (Figure 7) showed a marked rightward shift in the voltage required for half-maximal activation (V_{50}), consistent with reduced channel sensitivity. This provides further validation of assay responsiveness to modulators that affect channel gating kinetics, in addition to current amplitude.

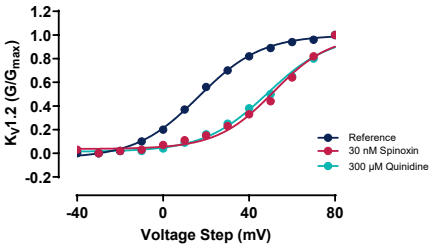

Figure 7.
Current-voltage (I-V) curves illustrating Kv1.2 curved under control conditions and in the presence of 30 nM Spinoxin or 300 µM Quinidine.

Condition	V_{50}
Reference	16.6
30 nM Spinoxin	51.3
300 µM Quinidine	48.5

Table 1.
V_{50} value for Kv1.2 activation under control conditions and in the presence of Spinoxin and Quinidine.

Comprehensive Coverage of Potassium Channel Targets

Sygnature Discovery offers an expansive range of potassium channel capabilities to support hit finding, lead optimization and mechanistic profiling for ion channel drug discovery. Our potassium channel portfolio includes:

- Voltage-gated channels
- Calcium-activated channels
- Two-pore domain channels
- Inward rectifying channels

All assays are developed and run in-house using validated, proprietary cell lines generated by our expert cell line generation team. Leveraging multiple platforms, including 384-well automated, manual and TEVC electrophysiology and fluorescence-based screening assays, we deliver robust, reproducible data across diverse channel classes.

Our ion channel platform enables :

- High-throughput compound screening
- Mechanistic and kinetic assessment
- Potency and selectivity studies

From neuroscience to cardiovascular, whatever your therapeutic indication, we provide the consistency and flexibility needed to accelerate your ion channel drug discovery.

Advance your research with Sygnature Discovery's electrophysiology services. Offering end-to-end ion channel screening capabilities, we deliver the results you need to advance your ion channel drug discovery research.

Contact us today to explore how our electrophysiology services can accelerate your drug discovery efforts and drive meaningful advancements in new therapeutics.

Visit the Sygnature Discovery website.