Application Note

Channel:	$hK_{Ca}3.1$
Cells:	CHO
Tools:	Patchliner

Modulation of $hK_{Ca}3.1$ by internal Ca^{2+} performed on Nanion's Patchliner

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Cells kindly provided by Charles River.

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Summary

The intermediate-conductance calcium-activated K^+ channel, also known as $K_{Ca}3.1$, $IK_{Ca}1$ or SK4, is a member of the large family of potassium channels gated by calcium[1]. It can be distinguished from the other calcium-activated K^+ channels by differences in channel conductance, calcium sensitivity, voltage dependence and pharmacological properties[1]. The $hK_{Ca}3.1$ channel is encoded by the KCNN4 gene[1]. It is primarily expressed in peripheral tissues, including those of the hematopoietic system, colon, lung, placenta, and pancreas[1] and has been proposed to play an important role in a variety of physiological processes including volume regulation in erythrocytes[2], proliferation and differentiation of B- and T-lymphocytes[3,4,5], and tissue protection following spinal cord injury[6]. Importantly, the hKCa3.1 channel is a promising therapeutic target for a variety of health disorders including sickle cell anaemia[7] and immunological disorders[4,5,8]. The Ca^{2+}-binding protein, calmodulin (CaM), is required for the activation of $hK_{Ca}3.1$[9]. The Ca^{2+}-CaM complex is proposed to bind to an intracellular domain of the C terminus of all subunits in the tetramer, inducing conformational changes to open the channel[9].

In this study the Patchliner was used to perform a biophysical and pharmacological characterization of $hK_{Ca}3.1$ channels expressed in CHO cells. $hK_{Ca}3.1$ was activated by exchanging the internal solution to a solution containing free-Ca^{2+} and blocked by external application of non-selective ($BaCl_2$) and selective (TRAM-34) inhibitors with an IC_{50} value consistent with that reported in the literature[4,8].

Results

To compare channel activity in the absence and presence of internal Ca^{2+}, exchange of the internal solution is required. The cells worked well on the Patchliner with a success rate (seal resistance >500 MΩ) of 78% before and 66% after exchange of the internal solution (n = 32 cells). Currents mediated by $hK_{Ca}3.1$ were activated by application of 1 µM free-Ca^{2+} in the internal solution. Figure 1 shows the current-voltage relationship of a cell in the absence (dark blue trace) and presence (light blue trace) of 1 µM internal free-Ca^{2+}. The $hK_{Ca}3.1$ current was reversibly blocked by external application of 5 mM $BaCl_2$ (grey trace). The timecourse of activation of the current following internal application of Ca^{2+} and blockade by external $BaCl_2$, including washout, is shown in Figure 1B. $BaCl_2$ blocked the current in a reversible and voltage-dependent manner.

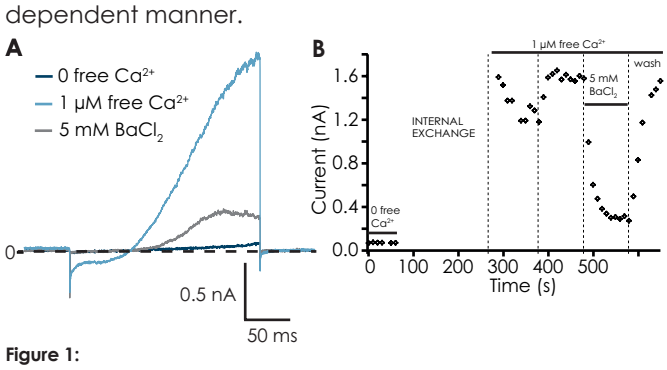

Figure 1:
A Raw traces from an exemplar cell in the absence (dark blue trace) and presence (light blue trace) of internal calcium and subsequent block by external $BaCl_2$. **B** Timecourse of the experiment.

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To further characterize $hK_{Ca}3.1$ channels the pharmacological effect of TRAM-34 was investigated. TRAM-34 is a membrane permeable lipophilic compound, designed to be a highly selective inhibitor of $K_{Ca}3.1$ currents[8]. Figure 2A shows raw current traces of an individual cell in the absence (control, 1 µM internal free-Ca^{2+}) and presence of increasing concentrations of TRAM-34. The timecourse of the experiment is also shown (Figure 2B). The average concentration response curve for TRAM-34 (Figure 3) reveals an IC_{50} of 24.3 ± 6.0 nM (n = 7), in excellent agreement with values reported in the literature[4,8].

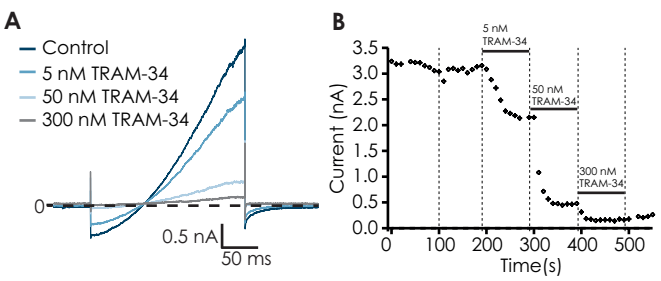

Figure 2:
A Raw traces of an individual cell in the absence (control) and presence of increasing concentrations of TRAM-34. **B** Timecourse of the experiment.

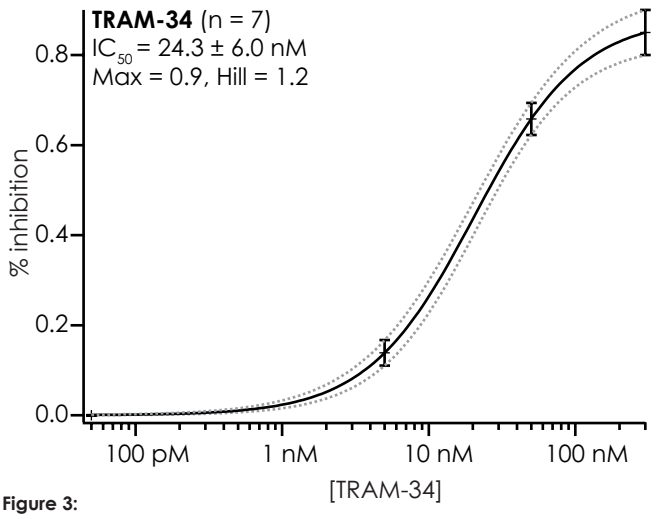

Figure 3:
The concentration response curve for TRAM-34 for an average of 7 cells, revealing an IC_{50} of 24.3 ± 6.0 nM (n = 7).

In conclusion, the Patchliner, in combination with $hK_{Ca}3.1$ expressing CHO cell line (Charles River), is a robust electrophysiological assay for activation by internal solution exchange and subsequent block of $hK_{Ca}3.1$ for drug discovery. The assay would also be transferable to the higher throughput SyncroPatch 384PE device.

References

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Methods

Cells

CHO cells stably expressing $hK_{Ca}3.1$ (Catalog # CT6123) were supplied by Charles River.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocols.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner with standard solutions. Currents were elicited using a voltage ramp protocol from -140 to +60 mV over 200 ms, interval 10 s. Intersweep holding potential was -100 mV. To activate $hK_{Ca}3.1$ currents, the chip wagon was removed from the measurehead, the internal solution exchanged and the recording continued. The compounds $BaCl_2$ and TRAM-34 were applied via the external solution. Cells and solutions were stored at 15°C throughout the experiments, using the Patchliner CoolingPlate. This improves cell quality thus extending duration of the recording and cell viability.

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