

Modulation of hK_{Ca}3.1 by internal Ca²⁺ performed on Nanion's Patchliner

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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 calcium¹. It can be distinguished from the other calcium-activated K⁺ channels by differences in channel conductance, calcium sensitivity, voltage dependence and pharmacological properties¹. The hK_{Ca}3.1 channel is encoded by the KCNN4 gene¹. It is primarily expressed in peripheral tissues, including those of the hematopoietic system, colon, lung, placenta, and pancreas¹ and has been proposed to play an important role in a variety of physiological processes including volume regulation in erythrocytes², proliferation and differentiation of B- and T-lymphocytes^{3,4,5}, and tissue protection following spinal cord injury⁶. Importantly, the hK_{Ca}3.1 channel is a promising therapeutic target for a variety of health disorders including sickle cell anaemia⁷ and immunological disorders^{4,5,8}. The Ca²⁺-binding protein, calmodulin (CaM), is required for the activation of hK_{Ca}3.1⁹. The Ca²⁺-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 channel⁹.

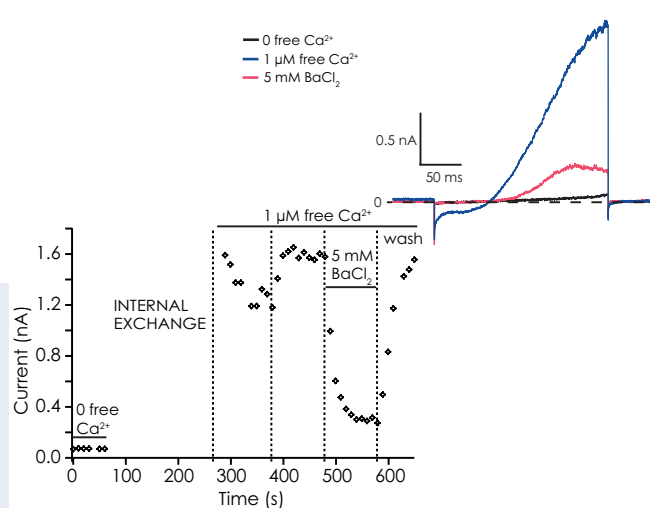
In this study the Patchliner was used to perform a biophysical and pharmacological characterization of hK_{Ca}3.1 channels expressed in CHO cells. hK_{Ca}3.1 was activated by exchanging the internal solution to a solution containing free-Ca²⁺ and blocked by external application of non-selective (BaCl₂) and selective (TRAM-34) inhibitors with an IC₅₀ value consistent with that reported in the literature^{4,8}. The exchange of the internal

solution utilized an optimized procedure on the Patchliner.

Results

To compare channel activity in the absence and presence of internal Ca²⁺, 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-Ca²⁺ in the internal solution. Figure 1 shows the current-voltage relationship of a cell in the absence (black trace) and presence (blue trace) of 1 μM internal free-Ca²⁺. The hK_{Ca}3.1 current was reversibly blocked by external application of 5 mM BaCl₂ (red trace). The timecourse of activation of the current following internal application of Ca²⁺ and blockade by external BaCl₂, including washout, is shown in Figure 1 (top). BaCl₂ blocked the current in a reversible and voltage-dependent manner.

Figure 1: Top: Raw traces from an exemplar cell in the absence (black trace) and presence (blue trace) of internal calcium and subsequent block by external BaCl₂ (red trace). **Bottom:** Timecourse of the experiment.



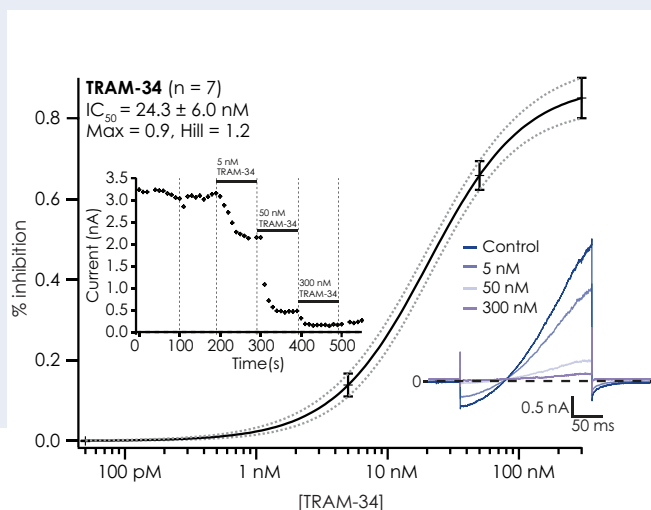


Figure 2: The concentration response curve for TRAM-34 for an average of 7 cells, revealing an IC_{50} of 24.3 ± 6.0 nM ($n = 7$). The timecourse of the experiment and traces of an individual cell in the absence (control) and presence of increasing concentrations of TRAM-34 are shown in the insets.

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 $K_{Ca}3.1$ currents⁸. Figure 2 shows raw current traces of an individual cell in the absence (control, 1 μ M internal free- Ca^{2+}) and presence of increasing concentrations of TRAM-34. The timecourse of the experiment is also shown (Figure 2). The average concentration response curve for TRAM-34 (Figure 2) reveals an IC_{50} of 24.3 ± 6.0 nM ($n = 7$), in excellent agreement with values reported in the literature^{4,8}.

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.

References

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Methods

Cells

CHO cells stably expressing $K_{Ca}3.1$ were used, kindly provided by Charles River.

Electrophysiology measurements

Cells were cultured and harvested according to Nanion's standard protocols. Cells were resuspended in external recording solution and stored in the CellHotel of the Patchliner before being dispensed into each well of the NPC-16 chip. Internal and external solution compositions are available upon request. 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. an optimized protocol was used for this procedure. 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.

