Application Note

Channel: $K_V1.3$
Cells: Jurkat
Tools: Port-a-Patch®
Internal Perfusion

Automated internal perfusion of Jurkat cells expressing $K_V1.3$ channels

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Summary

$K_V1.3$ is a voltage-gated potassium channel which plays a role in human T cell activation and proliferation, cell-mediated cytotoxicity and volume regulation. It is therefore an important target for the therapeutic control of T cell responses. The $K_V1.3$ channel is endogenously expressed in Jurkat cells, an immortalised T lymphocyte cell line. The channel is activated by membrane depolarisation at voltages typically more positive than -40 mV with rapid activation kinetics and slower inactivation.

In common with many other voltage-gated potassium channels, $K_V1.3$ can be blocked by replacing K^+ with Cs^+ ions in the pipette solution. Using Nanion's Internal Perfusion System, we were able to record stable $K_V1.3$ currents from Jurkat cells and reliably block this potassium current by perfusing the internal side of the chip with a Cs^+-containing solution. The $K_V1.3$ mediated current could also be completely recovered by perfusing the inside of the chip with K^+-containing solution.

$K_V1.3$ mediated currents can also be blocked by internal perfusion with tetraethylammonium (TEA). We present data here showing block of $K_V1.3$ by increasing concentrations of TEA using the Internal Perfusion System. The IC_{50} that we obtained for TEA applied internally was in good agreement with the literature.

Results

Current responses of an individual cell to 200 ms voltage pulses (+40 mV) in the presence of K^+ and subsequent block by internal perfusion of Cs^+ ions are shown in Figure 1. The block of $K_V1.3$ currents by Cs^+ using the internal exchange device was rapid and complete, and could be easily reversed by removal of Cs^+.

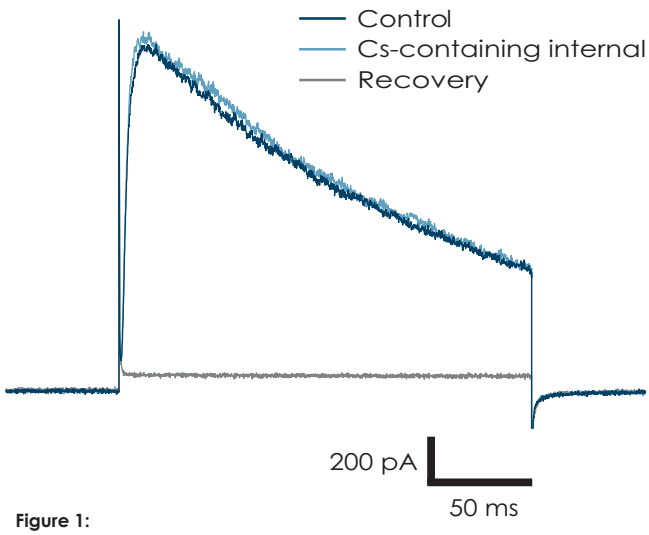

Figure 1:
Example traces for $K_V1.3$ mediated currents recorded from a Jurkat cell in the presence of K^+ and the subsequent block with internal Cs^+. $K_V1.3$ currents were blocked by the internal perfusion of Cs^+ and the current could be completely recovered by replacement back to K^+.

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Figure 2 shows the time course of the peak $K_V1.3$ current in the presence of K^+ or Cs^+ in the internal solution. The recording using the internal solution exchange device was highly stable where the internal solution could be exchanged from K^+ to Cs^+ over 19 times, with full recovery upon return to K^+-containing solution. In this example, the recording lasted over 35 minutes.

$K_V1.3$ mediated currents recorded from Jurkat cells could also be blocked by increasing concentrations of TEA on the internal side. Figure 3 shows traces recorded from a single cell in control conditions and subsequent block by increasing concentrations of TEA in the internal solution.

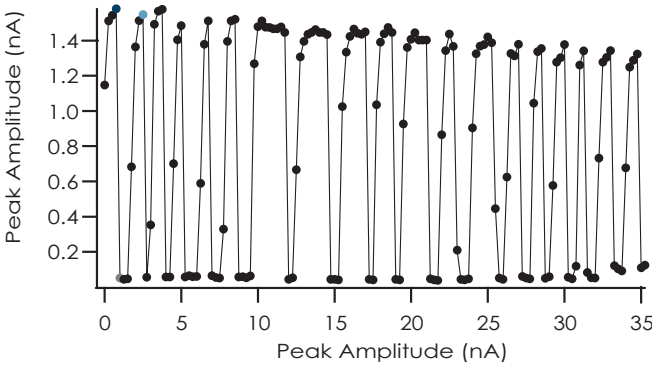

Figure 2:
Time course of the peak $K_V1.3$ current in a single experiment in the presence of K^+ or Cs^+ in the internal solution. The first exchange is indicated by the blue/grey circles (see Figure 1). Exchange to Cs^+ was possible over 19 times with full recovery and the recording lasted over 35 min.

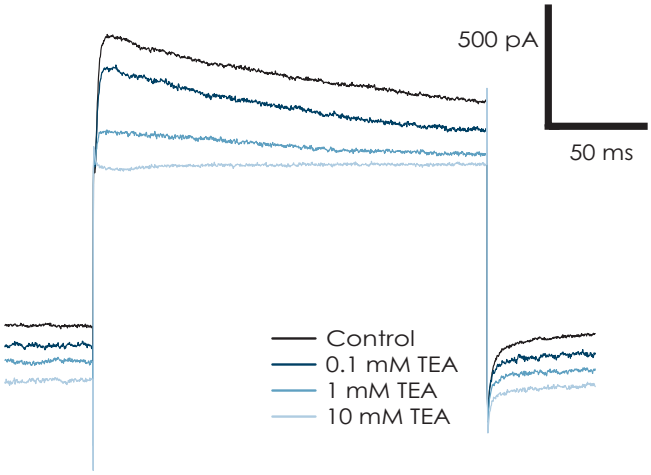

Figure 3:
Example traces for $K_V1.3$ mediated currents recorded from a Jurkat cell in the absence and presence of increasing concentrations of internal TEA. $K_V1.3$ mediated currents were blocked by the internal perfusion of TEA in a concentration dependent manner.

The estimated IC_{50} value for TEA was 0.9 ± 0.3 mM (n = 3) which is in good agreement with that found in the literature (0.63 ± 0.18 mM; Aiyer *et al.*, 1994).

In conclusion, the internal exchange device from Nanion can be used for the relatively rapid administration of ions and small molecules to the internal surface of the cell membrane. Recordings using the internal solution exchange device on the Port-a-Patch® are stable and full exchange can be achieved within a few seconds.

References

1. Aiyar, J., Nguyen, A.N., Chandy, K.G., and Grissmer, S. 1994. The P-Region and S6 of Kv3.1 contribute to the formation of the ion conduction pathway. Biophys. J. 67: 2261-2264

Methods

Cells

Jurkat cells endogenously expressing $K_V1.3$ were used.

Cell culture

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

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch®. Currents were elicited using a voltage step from a holding potential of -80 mV to +40 mV for 200 ms every 20 seconds. Nanion's Internal Perfusion System was used to constantly perfuse the inside surface of the chip with standard internal solution containing K^+, Cs^+ or TEA.

TEA was made as a 1M stock solution in water and diluted in standard K^+-containing internal solution accordingly.

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