



Application Report

CHO-K_v1.3 and current clamp using Qube 384: another perspective of ion channel behavior

A robust current clamp assay for investigating CHO-K_v1.3 pharmacology using Qube 384

Summary

Current clamp recordings provide more physiologically relevant measurements of ion channel activity (in comparison to voltage clamp), allowing the contribution of different ion channel subtypes to resting membrane potential to be evaluated.

Here we demonstrate that:

- Due to their mode of action, some compounds display different potencies in voltage clamp and current clamp.
- Qube 384 enables drug screening in current clamp mode, here demonstrated in a cell line stably expressing the K_v1.3 ion channel (success rate out of 384 wells of up to 65%).
- Compounds blocking the K_v1.3 channel, are evaluated for their ability to depolarize the cell membrane.

Introduction

K_v1.3 (KCNA3) is a voltage-gated potassium channel belonging to the Shaker related subfamily (1). K_v1.3 is best known for its role in activation of immune cells such as T lymphocytes and microglial cells, where high expression is found (2; 3). However, more recently, K_v1.3 channels have also been demonstrated to be present in the mitochondria and nuclei of cancer cells (2; 4).

K_v1.3 is a promising target for treating autoimmune disorders through development of novel immunosuppressants (5; 2): specifically, K_v1.3 channels are upregulated in effector memory T cells (TEM) following repeated antigen challenge (6) and have a crucial role in controlling T cells resting membrane potential (RMP) and, hence the driving force for Ca²⁺ influx (7), which is critical for T cell activation.

The K_v1.3 channel displays fast activation kinetics and rapid C-type inactivation. The biophysical properties of K_v1.3 give rise to a steady-state conductance over a small range of voltages (the window current). In T cells, K_v1.3 regulates the RMP at the voltage range of this window current region (1).

Combining both voltage clamp and current clamp recording techniques allows a better understanding of the physiological effects of compounds targeting ion channels. In this report, we investigated pharmacological effects of K_v1.3 inhibitors on K_v1.3 currents alongside RMP of a CHO-K_v1.3 cell line with our automated patch clamp system, the Qube 384. Current clamp mode significantly expands the automated electrophysiology toolset for understanding pharmacological and physiological effects of ion channel modulators.

This study is the result of a productive and successful collaboration with **Metrion Biosciences**, where Qube experiments were performed in parallel at Sophion and Metrion.

Results and discussion

In the Qube 384 experiments, two known tool compounds were evaluated, applying both voltage clamp and current clamp protocols as shown in Methods, Fig. 5. The Qube 384 amplifiers allow recordings of both voltage- and current clamp steps in the same protocol, where the K_v1.3 current clamp protocol was designed to include a pre-step in voltage clamp, close to the physiological resting membrane potential (approximately -40 mV), before switching to current clamp mode (injecting I=0). Success rate was calculated after application of quality filters, based on membrane resistance (R_m > 500 MΩ) at the first and last liquid application. Success rates values evaluated in this report were obtained from the average of three QChips.

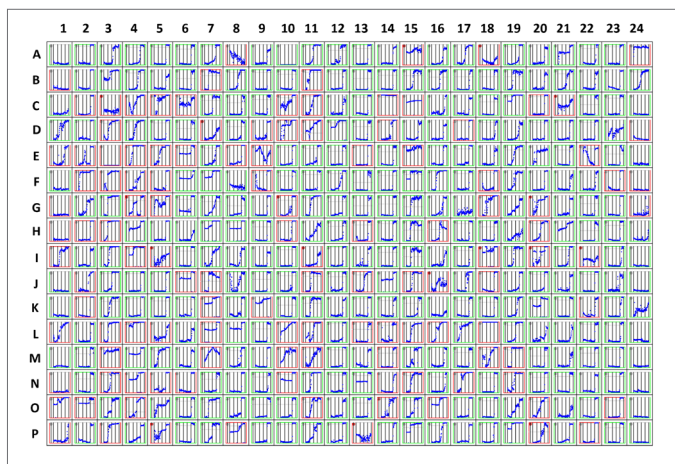


Fig. 1: Example of success rate in a typical QChip (384 wells). Green sites represent recordings that have passed the quality filters (membrane resistance, $R_m > 500 \text{ M}\Omega$ for the first and last liquid application).

Voltage clamp

Two compounds, Trans-DSC and phenoxyalkoxypsoralen-1 (PAP-1), which are known C-type inactivated state blockers for the $K_v1.3$ channel, were tested in both voltage clamp and current clamp (Fig. 2 and 4). Corresponding concentration-response curves and IC_{50} values are shown in Fig. 2 and Table 1 respectively. For each concentration tested, "Peak" and "End" values were determined from currents evoked at the beginning and end of the voltage pulse.

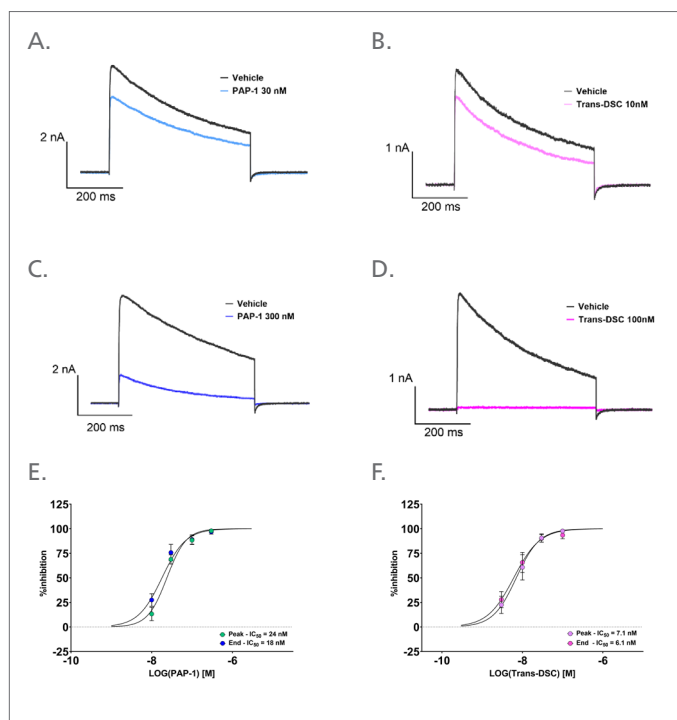


Fig. 2: Voltage clamp recordings of the $K_v1.3$ channel with compound effects (A, C: PAP-1; B, D: Trans-DSC). Concentration response curves for PAP-1 (E) and Trans-DSC (F). Data are shown as mean \pm SD ($n = 5-11$).

Table 1: IC_{50} values of PAP-1 and Trans-DSC taken at the beginning (peak) and end (end) of the square pulse.

Compound	IC_{50} Peak (nM)	IC_{50} End (nM)	Literature values (nM)
PAP-1	24	18	2 (9); 2 (10); 2 (11)
Trans-DSC	7.1	6.1	50 (12)

Differences between Qube and literature IC_{50} values for PAP-1 and TRANS-DSC could reflect differences in measurement technique, and/or cellular models.

Current clamp

Current clamp recordings were performed following completion of the voltage clamp protocol (Fig. 3 and 4).

The average resting membrane potential (RMP) in vehicle (DMSO 0.3%) was approximately -38 mV (Fig. 3, A and D). Both PAP-1 and Trans-DSC depolarized the RMP (Fig. 3) in a concentration-dependent manner (Fig. 4).

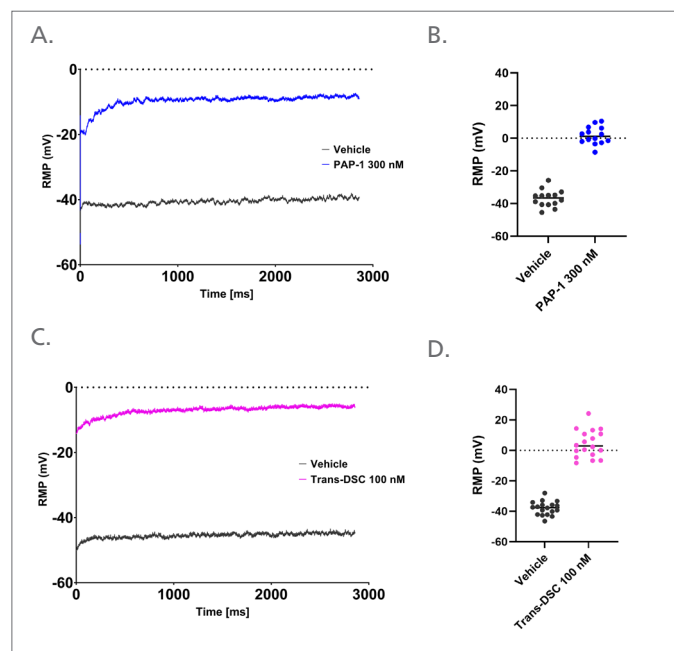


Fig. 3: Representative traces recorded in current clamp mode showing resting membrane potential (RMP) before and after addition of $K_v1.3$ inhibitors (A, C). Data are shown in the presence of vehicle (DMSO 0.3%, black) and after compound application (A, B PAP-1 300 nM, blue; C, D Trans-DSC 100 nM, pink). Scatter-plot of median values are analyzed from peak current (B, D) ($n = 14-18$).

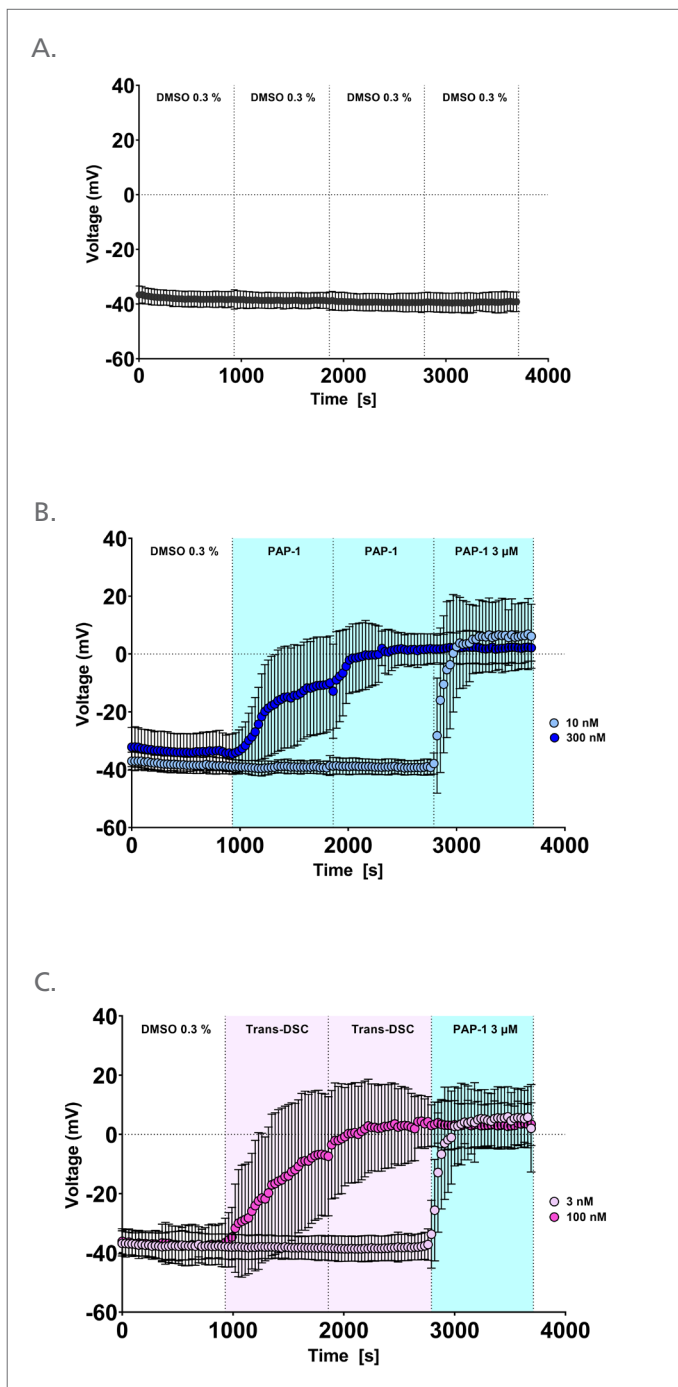


Fig. 4: Representative current-time (IT) plot in current clamp mode showing the effect of vehicle (DMSO 0.3%, A) and increasing concentrations of PAP-1 (10 nM, light blue; 300 nM, blue, B) and Trans-DSC (3 nM, light pink; 100 nM, pink, C). Data are normalized to the highest concentration of PAP-1 (3 μ M). Data are shown as mean \pm SD (n = 14-25).

As shown in Fig. 5, the effects of both PAP-1 and TRANS-DSC are concentration dependent, where the highest concentrations of both compounds (PAP-1=300 nM and Trans-DSC=100 nM) lead to a consistent depolarization of RMP.

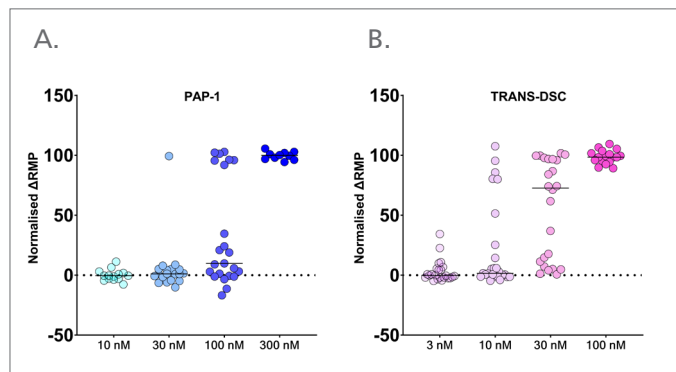


Fig. 5: Normalized resting membrane potential (RMP) data recorded in current clamp mode with increasing concentration of PAP-1 (A) and TRANS-DSC (B). Scatter-plots are shown as median values (n = 14-25).

Conclusion

Within this application report we demonstrate that current clamp experiments can be performed on the Qube 384 with a success rate up of to 65% - after the application of quality filters for membrane resistance ($R_m > 500 \text{ M}\Omega$). Comparison of concentration-dependent effects of compounds in voltage clamp and current clamp modes can be exploited to understand the direct relationship between compound potency and physiological effects on membrane potential. For both PAP-1 and Trans-DSC, concentrations $>10 \times IC_{50}$ (measured in voltage clamp) are required to observe a robust and consistent depolarization in current clamp recordings.

In summary, combining voltage clamp with current clamp recordings provides key data aligning potency and physiological effects of compounds, which is important for the design of future efficacy studies.

Methods

- CHO-Kv1.3 cell line kindly provided by Metrion Biosciences
- Voltage and current clamp protocol used:

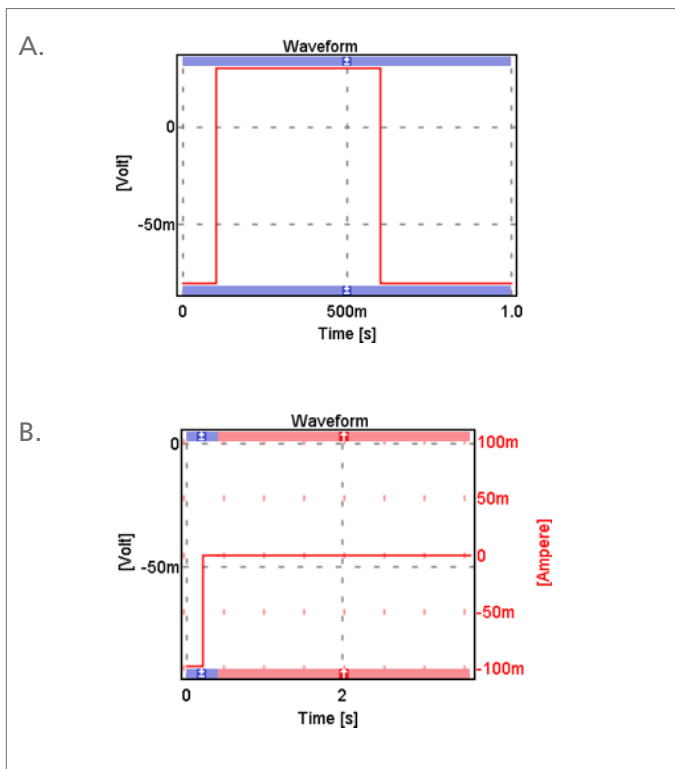


Fig. 6: Voltage (A) and current clamp (B) protocol used. B) A pre-step in voltage clamp from -90 mV to -45 mV was applied before switching to current clamp mode ($I=0$).

- Compounds tested were PAP-1 and Trans-DSC, which are both inactivated state blockers:
 - PAP-1 concentrations: 3 μ M, 300 nM, 100 nM, 30 nM, 10 nM
 - Trans-DSC concentrations: 100 nM, 30 nM, 10 nM, 3 nM

References

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