



# Application Note

Channel: hERG

Cells: HEK293

Tools: Port-a-Patch®

External Perfusion

Temperature Control

## Temperature controlled hERG recordings on the Port-a-Patch®

The electrophysiology team at Nanion Technologies GmbH, Munich.

Cells were kindly provided by Cytomyx Millipore, UK.

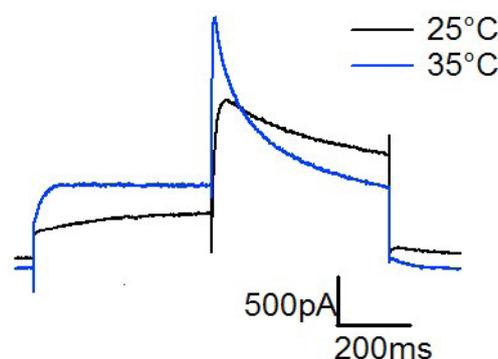
### Summary

The hERG gene encodes a potassium channel responsible for the repolarization of the IKr current in cardiac cells (Sanguinetti *et al*, 1995). This channel is important in the repolarisation of the cardiac action potential. Abnormalities in this channel can lead to long or short QT syndrome, leading to potentially fatal cardiac arrhythmia. Given the importance of this channel in maintaining cardiac function, and disturbances of channel activity by certain compounds such as anti-arrhythmias and anti-psychotics, it has become an important target in compound safety screening. It is a desirable option to study this channel at physiological temperature since compounds can display different actions or potencies at physiological temperature.

Here we present data collected on the Port-a-Patch® using the external perfusion system coupled with Nanion's temperature control device. Cells were captured and sealed at room temperature and control recordings made before raising the temperature to 35°C so that parameters such as current peak amplitude could be compared at the two different temperatures. Furthermore, the hERG active compound quinidine was used at physiological temperature and a full dose response curve was achieved. This demonstrates the stability of the recordings on the Port-a-Patch® at this temperature. The dose response curve generated an IC<sub>50</sub> similar to that obtained at room temperature, and similar to that published in the literature.

### Results

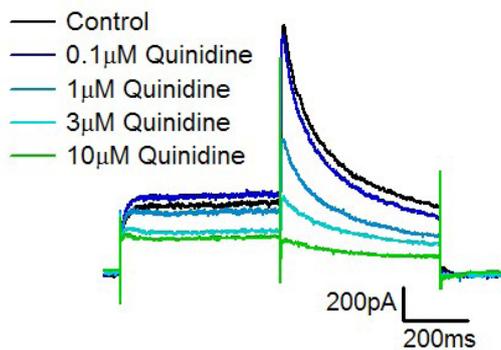
Current responses of an individual cell expressing the hERG channel to the voltage protocol (described in *Methods*) at room temperature (RT) and physiological temperature are shown superimposed in *Figure 1*. The most striking effect of raising the temperature to 35°C was that the peak amplitude was increased by almost 2-fold ( $63 \pm 9\%$  increase,  $n = 13$ ). Additionally, the rise time was decreased from  $15.7 \pm 0.7$  ms at RT to  $4.8 \pm 0.3$  ms at 35 °C. The decay phase could be fit using a single exponential equation and the time constant was decreased from  $251 \pm 18$  ms at RT to  $126 \pm 9$  ms at 35 °C.



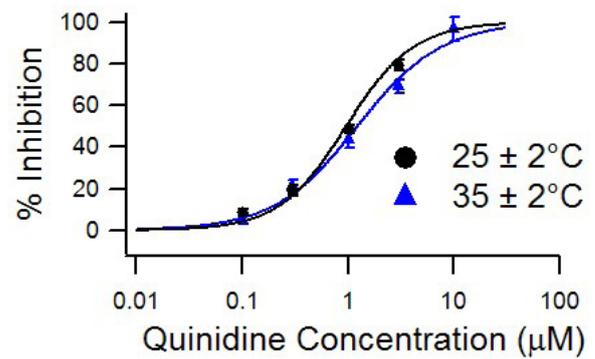
**Figure 1:** Example traces for hERG mediated currents at  $25 \pm 2$  °C and  $35 \pm 2$  °C. Peak amplitude was increased at 35 °C, the rise time and decay time constants were faster at physiological temperature compared to RT.

Figure 2 shows representative traces from an individual cell in the absence and presence of increasing concentrations of quinidine at  $35 \pm 2^\circ\text{C}$ . Quinidine was applied using the perfusion system and the temperature maintained at  $35 \pm 2^\circ\text{C}$  during perfusion of the drug. The recording was stable and a full concentration response of quinidine ranging from  $0.1 \mu\text{M}$  to  $10 \mu\text{M}$  was achieved. The effect of quinidine was reversible and approximately 60-70 % of the current could be recovered upon washout (data not shown).

Dose response curves for quinidine at RT and  $35^\circ\text{C}$  are shown superimposed in Figure 3. The two dose



**Figure 2:** A full dose response curve to quinidine acting on the hERG channel was achieved at physiological temperature. Example traces in the absence and presence of increasing concentrations of quinidine in one cell are shown here.



**Figure 3:** Concentration response curves at  $25^\circ\text{C}$  and  $35^\circ\text{C}$  for quinidine. The  $\text{IC}_{50}$  for quinidine at physiological temperature was estimated to be  $1.3 \pm 0.2 \mu\text{M}$  ( $n = 5$ ), similar to that obtained at RT ( $1.0 \pm 0.03 \mu\text{M}$ ,  $n = 3$ ).

response curves are almost identical. The  $\text{IC}_{50}$  for quinidine at  $35^\circ\text{C}$  was estimated to be  $1.3 \pm 0.2 \mu\text{M}$  ( $n = 5$ ) and at RT was estimated to be  $1.0 \pm 0.03 \mu\text{M}$  ( $n = 3$ ). The estimation of the  $\text{IC}_{50}$  is in good agreement with those reported in the literature, which range between  $0.6$  and  $2 \mu\text{M}$  (Yang *et al.*, 2004; Paul *et al.*, 2002; Limberis *et al.*, 2006).

In conclusion, we have demonstrated stable recordings of the hERG channel at physiological temperature on a planar patch clamp system. The effect of temperature had a dramatic effect on peak amplitude but did not affect the  $\text{IC}_{50}$  of quinidine.

## References

1. Limberis, J.T., Su, Z., Cox, B.F., Gintant, G.A., and Martin, R.L. 2006. Altering extracellular potassium concentration does not modulate drug block of human ether-a-go-go-related gene (hERG) channels. *Clin. Exp. Pharmacol. Physiol.* 33 (11): 1059 – 65.
2. Paul, A.A., Witchel, H.J., and Hancox, J.C. 2002. Inhibition of the current of heterologously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine. *Br. J Pharmacol.* 136: 717 - 729.
3. Sanguinetti, M. C., Jiang, C., Curran, M. E., Keating, M. T., 1995. A mechanistic link between an inherited and an acquired arrhythmia: HERG encodes the IKr potassium channel. *Cell.* 81: 299-307
4. Yang, B., Xu, D., Xu, C., Li, Z., Du, Z., Wang, H., and Dong, D. 2004. Inactivation gating determines drug potency: a common mechanism for drug blockage of HERG channels. *Acta Pharmacol. Sin.* 25 (5): 554 - 560.

## Methods

### Cells

HEK293 cells stably expressing hERG 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 every 20 s stepping from a holding potential of  $-80 \text{ mV}$  to  $+40 \text{ mV}$  for 200 ms followed by a step to  $-40 \text{ mV}$  for 200 ms and back to  $-80 \text{ mV}$ . Currents were recorded at RT until they were stable. The temperature control device from Nanion was then switched on. A stable temperature of  $35 \pm 2^\circ\text{C}$  was reached within 2 mins and maintained. In the case of quinidine concentration response curves, a  $1 \text{ mM}$  quinidine stock was diluted in external solution and applied via the perfusion system. Quinidine was applied for at least 2 mins at each concentration.