Application Note

Channel: hERG
Cells: HEK293
Tools: Port-a-Patch

Temperature controlled hERG recordings on Nanion's Port-a-Patch

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells kindly provided by EMD Millipore.

Summary

The hERG gene encodes a potassium channel responsible for the repolarization of the IKr current in cardiac cells[1]. This channel is important in the repolarization of the cardiac action potential. Abnormalities in this channel can cause 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[2,3].

Here we present data collected on the Port-a-Patch using the External Perfusion System coupled with Nanion's Temperature Control. 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 concentration response curve generated an IC_{50} similar to that obtained at room temperature, and similar to that published in the literature[2,4-7].

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 ± 9 % increase, n = 13). Additionally, the rise time was decreased from 15.7 ± 0.7 ms at RT to 4.8 ± 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 ± 18 ms at RT to 126 ± 9 ms at 35°C.

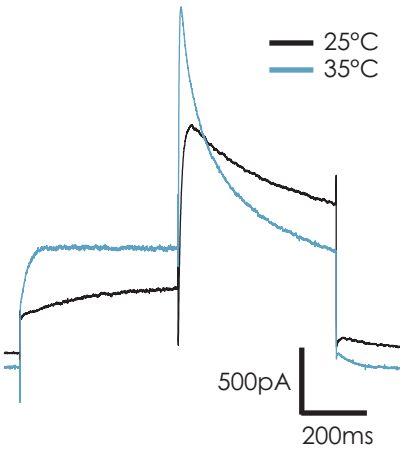

Figure 1: Example traces for hERG-mediated currents at 25 ± 2°C and 35 ± 2°C. Peak amplitude was increased at 35°C, the rise time and decay time constants were faster at physiological temperature compared to RT.

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

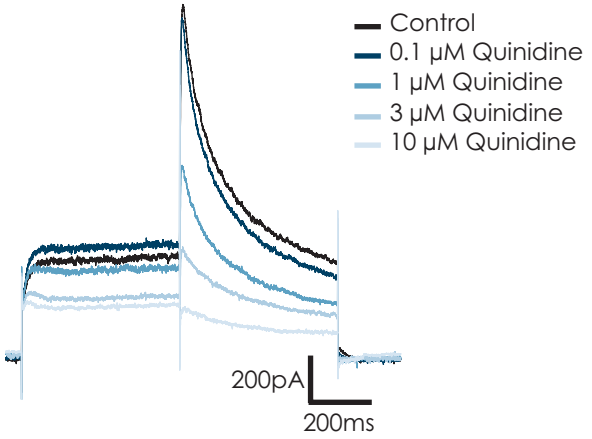

Figure 2: A full concentration response curve to quinidine acting on the hERG channel was achieved at physiological temperature. Example traces, in the absence and presence of increasing concentrations of quinidine, from one cell are shown here.

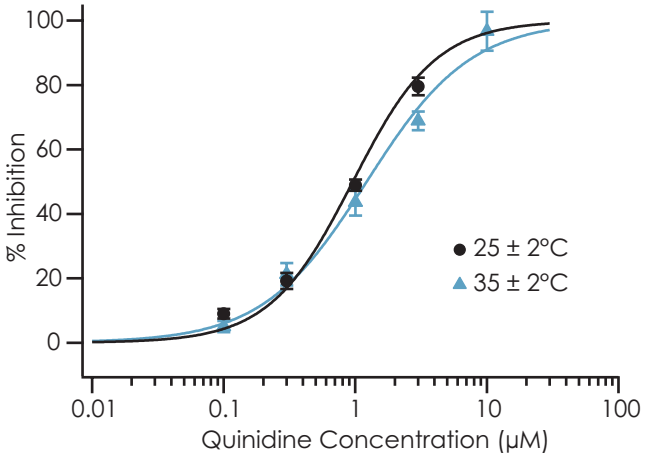

Figure 3: : Concentration response curves at 25°C and 35°C for quinidine. The IC_{50} for quinidine at physiological temperature was estimated to be 1.3 ± 0.2 µM (n = 5), similar to that obtained at RT (1.00 ± 0.03 µM, n = 3).

Concentration response curves for quinidine at RT and 35°C are shown superimposed in Figure 3. The two concentration response curves are almost identical. The IC_{50} for quinidine at 35°C was estimated to be 1.3 ± 0.2 µM (n = 5) and at RT was estimated to be 1.00 ± 0.03 µM (n = 3). The estimation of the IC_{50} is in good agreement with those reported in the literature[2,4-7].

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 IC_{50} of quinidine, as expected[2].

References

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Methods

Cells

HEK293 cells stably expressing hERG were supplied by EMD Millipore.

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 mV to +40 mV for 200 ms followed by a step to -40 mV for 200 ms and back to -80 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 ± 2°C was reached within 2 mins and maintained. In the case of quinidine concentration response curves, a 1 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.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de