Application Note

Channel: hERG
Cells: HEK293
Tools: Patchliner®

Effect of temperature on erythromycin action on hERG currents recorded on Nanion´s Patchliner®

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Cells were kindly provided by 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 repolarization 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.

Compounds can display different properties or different potencies at physiological temperature (35°C) *vs*. room temperature (RT) and therefore, it is a desirable option to be able to study this channel electrophysiologically at elevated temperature. One such compound which has been shown to have an increase in potency at physiological temperature is erythromycin. Erythromycin is a macrolide antibiotic which can cause QT prolongation and cardiac arrhythmia. Erythromycin has been shown to block hERG channels at physiological temperature with an IC_{50} of approximately 40 µM (Stanat *et al,* 2003; Duncan *et al*, 2005). However, at RT erythromycin is much less potent. At a concentration of 100 µM, erythromycin causes no significant block of hERG currents at RT but significantly blocks currents at physiological temperature (Guo *et al*, 2005).

Here we present data collected on an 8-channel Patchliner® with temperature control at RT and at 35°C and the effect this has on the potency of erythromycin.

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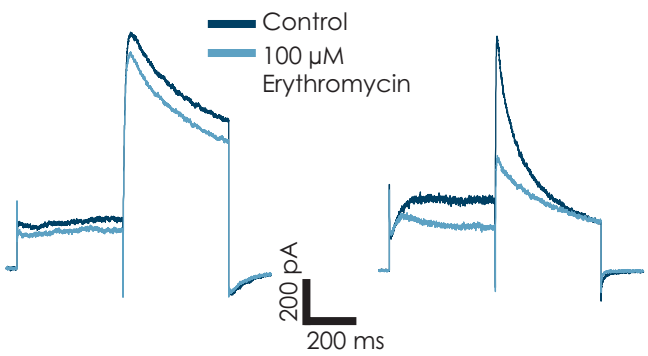
Results

Current responses of two individual cells to 500 ms voltage pulses to +40 mV and then -40 mV in the presence and absence of 100 µM erythromycin at RT and 35°C are shown in Figure 1. At RT (Panel A) 100 µM erythromycin caused little reduction in current amplitude (at -40 mV; approx. 15%) compared with an almost 50 % reduction of current amplitude at 35°C. This is in good agreement with the literature (Guo *et al*, 2005).



A. Room Temperature **B.** 35°C

Figure 1:
Effect of erythromycin on hERG-mediated currents at (A) room temperature and (B) 35°C. The graphs are shown on the same scale. 100 µM erythromycin significantly blocked hERG currents at 35°C but had little effect on hERG currents at RT.

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Application Note

Figure 2 shows the concentration response curves for erythromycin at RT and at 35°C. At higher concentrations (300 µM), erythromycin did block hERG currents at RT by approximately 40% and gave an IC_{50} of 427.5 µM calculated from the graph. At 300 µM, erythromycin blocked hERG currents at 35°C by 70% and the IC_{50} at this temperature was calculated to be 30.7 µM. This is in excellent agreement with values reported in the literature (Stanat *et al,* 2003; Duncan *et al*, 2005).

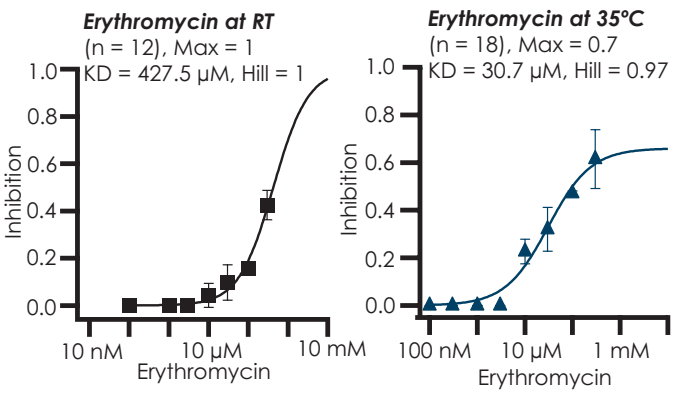

Figure 2:
Concentration response curves for erythromycin at room temperature and at 35°C. Erythromycin is approximately 10-fold more potent at 35°C compared with room temperature.

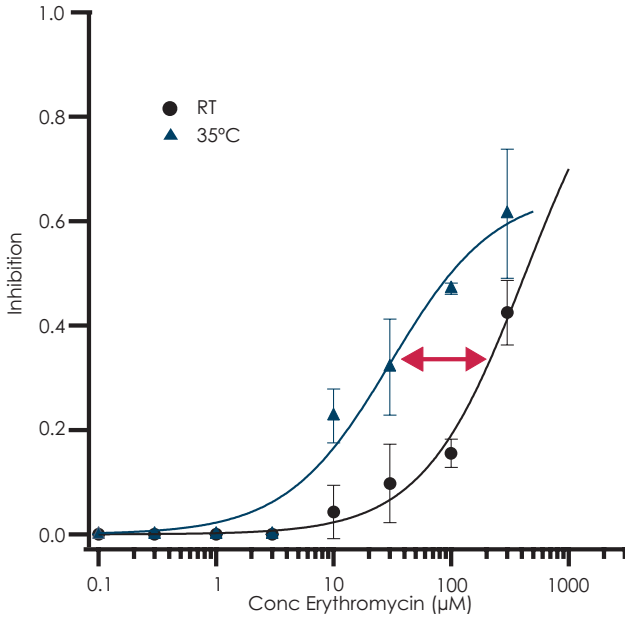

Figure 3:
The IC_{50} of erythromycin is strongly dependent on temperature. When the concentration response curves at room temperature and 35°C are overlaid, it is clear to see the shift in potency.

Typically, single concentrations of erythromycin were applied to each cell. Average concentration responses were then plotted using 12 (RT) and 18 (35°C) cells. The average concentration response curves are shown above overlaid which clearly demonstrates that erythromycin is 10-fold more potent at 35°C.

In summary, it is possible to perform whole cell recordings on the Patchliner® at physiological temperature. The expected IC_{50} value for erythromycin at 35°C was calculated. For safety screening, it may be critical to test compounds at 35°C and this makes the Patchliner® an ideal tool for such experiments.

References

1. Sanguinetti, M. C., Jiang, C., Curran, M. E., Keating, M. T., 1995. Cell. 81: 299-307
2. Stanat, S.J.C., Carlton, S.G., Crumb Jr, W.J., Agrawal, K.C., Clarkson, C.W., 2003. Mol. & Cell Biochem. 254 (1-2): 1-7
3. Duncan, R. S., Ridley, J.M., Milnes, J,T., Leishman, D.J., Hancox, J.C., Witchel, H.J., 2005. J. Physiol. 567P, PC8 (poster abstract)
4. Guo, J., Zhan, S., Lees-Miller, J.P., Teng, G.Q., Duff, H.J., 2005. Heart Rhythm. 2 (8): 860-866

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 Patchliner®. Currents were elicited every 20 s by 500 ms voltage steps to +40 mV followed by 500 ms step to -40 mV from the holding potential of -80 mV. Erythromycin was made as a 100 mM stock in DMSO and diluted in external solution at the indicated concentrations (max. DMSO concentration was 0.3%).

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