Application Note

Channel: hERG
Cells: HEK293
Tools: Port-a-Patch®
External Perfusion

Characterization of hERG (HEK293 cells) on Nanion's Port-a-Patch®

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells were supplied by Millipore, USA

Summary

The hERG gene (KCNH2) encodes a potassium ion channel responsible for the repolarizing IKr current in the cardiac action potential (Sanguinetti et al., 1995).

Abnormalities in this channel may lead to either Long QT Syndrome (LQT2) (with loss-of-function mutations) or Short QT syndrome (with gain-of-function mutations), both potentially fatal cardiac arrhythmia, due to repolarization disturbances of the cardiac action potential.

Given the importance of this channel in maintaining cardiac function, it has become an important target in compound safety screening.

A large range of therapeutic agents with diverse chemical structures have been reported to induce long QT syndrome. These include antihistamines (e.g. terfenadine), gastrointestinal prokinetic agents (e.g. cisapride) and others.

In this report we present data that were collected on the Port-a-Patch®. Cells (HEK293 stably expressing hERG, supplied by Millipore) were tested. Current amplitudes, IVs and cisapride as well as quinidine dose-response curves were analyzed.

Results

Figure 1 shows current responses of an individual cell to a typical hERG voltage protocol. Potentials were stepped from the holding potential (-80 mV) for 500 ms to +40 mV followed by a 500 ms step to voltages ranging from -120 mV to +40 mV (20 mV steps), before stepping back to holding.
Figure 2 shows the average current-voltage relationship for recordings equivalent to the one shown in Figure 1. The averaged mean current at -40 mV of all recorded cells was 919 ± 57 pA (n=54).

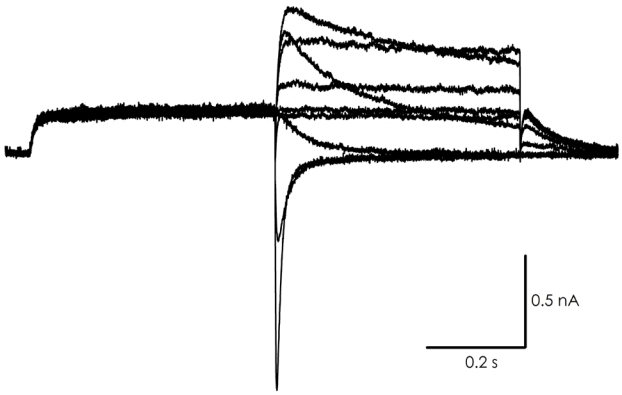

Figure 1:
Representative current responses of an individual cell to a hERG IV voltage protocol (for details see text above).

Application Note

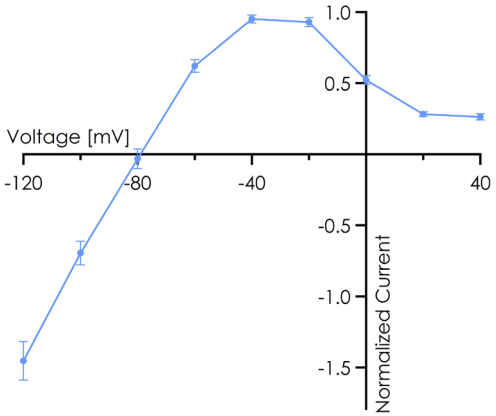

Figure 2:
Average normalized IV (n = 54) for HEK293 cells expressing hERG.

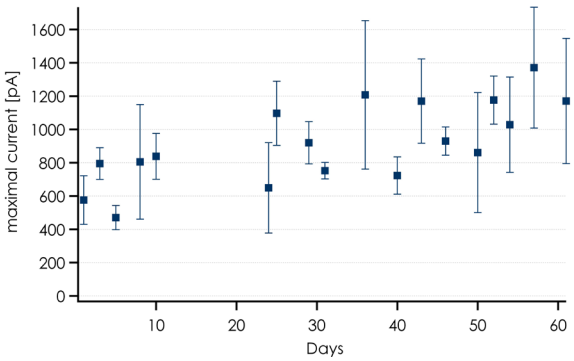

Figure 3:
Development of the peak current at -40 mV over time after thawing. Each day between three and five cells were measured.

Since cell lines permanently expressing hERG sometimes have rather small currents it was tested whether the culturing time after thawing had an effect on the current amplitudes. Results are shown in Figure 3.

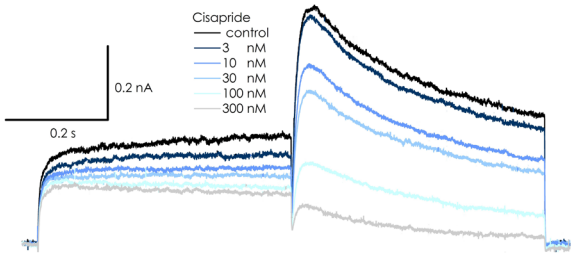

Figure 4:
Shown are raw current responses in the presence of increasing cisapride concentrations as indicated.

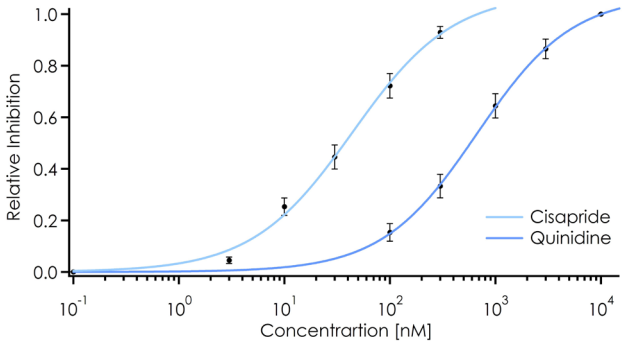

Figure 5:
Shown are average dose-response curves for cisapride quinidine.

With Nanion's External Perfusion System also cumulative does-response curves were collected for quinidine and cisapride. Raw current responses in the presence of different quinidine concentrations are shown in Figure 4. Average dose-response curves for quinidine and cisapride are shown in Figure 5. From these, IC_{50}s of 267 ± 68 nM and 31.5 ± 6.2 nM were determined for quinidine and cisapride, respectively.

Methods

Cells

HEK293 cells permanently transfected with hERG were supplied by 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 using a voltage step from a holding potential of -80 mV to +40 mV for 500 ms, then to the test potential for 500 ms and back to holding. Pulses were elicited every 20 seconds. External solution exchanges were done with Nanion's External Perfusion System.

Nanion Technologies GmbH
Erzgiessereistr. 4
80335 Munich, Germany

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