Application Note

Channel: hERG
Cells: CHO
Tools: Port-a-Patch®

Pharmacology of hERG recorded on Nanion's Port-a-Patch®

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Cells were kindly provided by Cytomyx Millipore, UK.

Summary

The hERG gene (KCNH2) encodes a potassium ion channel responsible for the repolarizing I_{Kr} 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.

Here we present data collected on the Port-a-Patch®. Astemizole, Terfenadine, Cisapride and Flunarizine dose-response curves on hERG expressed in CHO cells are shown. The mean current amplitude in these cells was 1076 ± 79 pA (n= 88) at -40 mV.

Results

Current responses of an individual cell expressing the hERG channel is shown in Figure 1. Figure 2 shows the corresponding current-voltage relation.

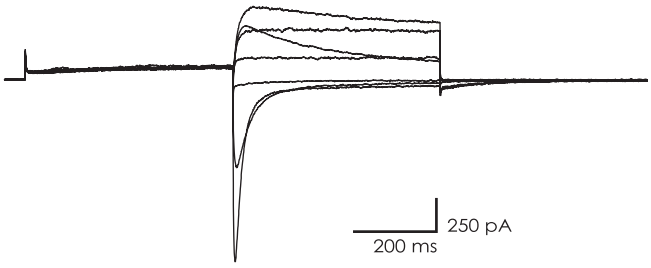

Figure 1:
hERG whole cell current as recorded in CHO cells.

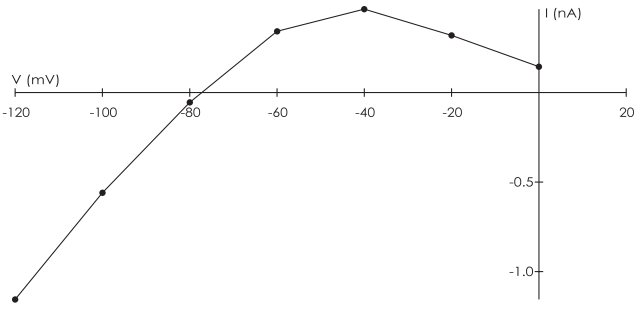

Figure 2:
Current-voltage response of the recording shown in Fig. 1. The holding potential was -80 mV.

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Figure 3 shows representative traces from an individual cell in the absence and presence of increasing concentrations of Astemizole. The inhibitor was applied using a pipettor. The recording was stable and a full concentration response of Astemizole ranging from 0.5 nM to 10 nM was achieved.

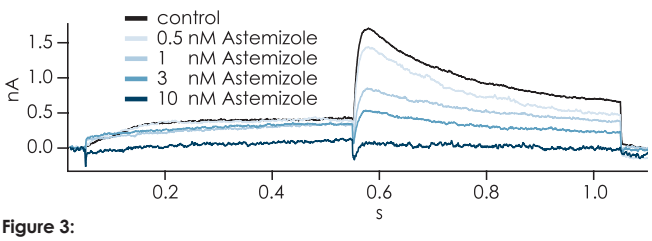

Figure 3:
Full dose response curve to Astemizole acting on the hERG channel. Example traces in the absence and presence of increasing concentrations of Astemizole of one cell.

The IC_{50} value was calculated to a value of 1.27 nM (Fig. 4), which is in good agreement with the literature values, which range between 0.9 nM and 69 nM (e.g. Zhou *et al.* 1999).

Besides Astemizole, also the typical hERG blockers Terfenadine, Cisapride and Flunarizine have been tested (Fig. 4).

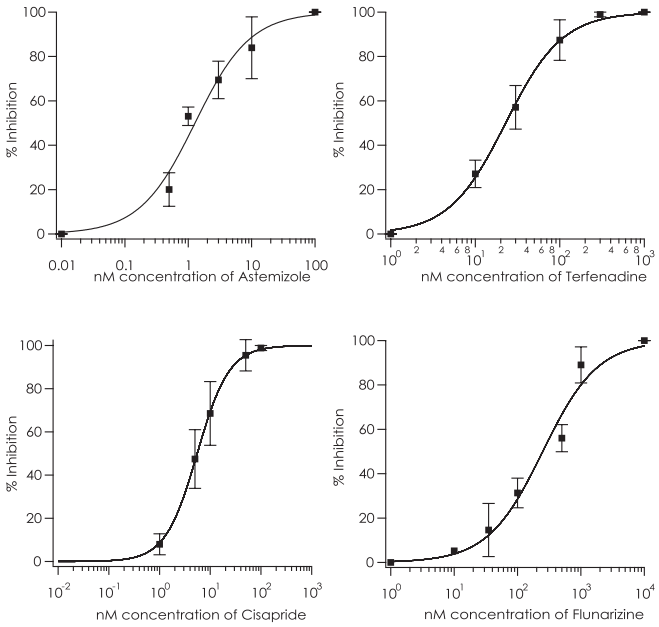

Figure 4:
hERG pharmacology. The figure displays each Hill fit of the accumulated data. Error bars reflect the standard error of the mean. The concentration of the half maximal block is IC_{50} = 1.27 nM (Astemizole), IC_{50} = 11.0 ± 3 nM (Terfenadine, Lit. val. 5-300 nM), IC_{50} = 8.9 nM (Cisapride, Lit. val. 5-60 nM) and IC_{50} = 163.7 nM (Flunarizine, Lit. val. 344 nM).

In conclusion, we have demonstrated stable recordings of the hERG channel on a planar patch clamp system.

References

1. 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: 299-3072.

2. Zhou, Z., Vorperian, V. R., Gong, O., Zhang, S., and January, C. T., 1999. Block of HERG Potassium Channels by the Antihistamine Astemizole and its Metabolites Desmethylastemizole and Norastemizole Journal of Cardiovascular Electrophysiology, Volume 10, Issue 6.

Methods

Cells

CHO 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 for inhibiton experiments were elicited using a voltage step protocol from a holding potential of -80 mV to +40 mV for 500 ms followed by a step to -40 mV for 500 ms and back to the holding potential. The step was repeated every 20 s. Before application of an inhibitor, the current amplitude had to be stable for at least 3 minutes.

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