roboocyte®

multichannel systems

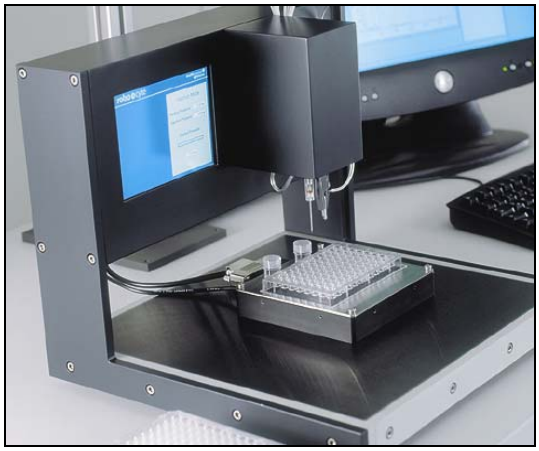
Applications: hERG Current

Introduction

Potassium channels critically contribute to cardiac repolarization, that is, to the final phase of the action potential that returns the cell to its resting state. The human *ether-a-go-go related gene* (hERG) encodes the pore forming subunits of the potassium channel that mediates rapidly activating delayed rectifier K^+ currents (IKr). Drugs that block potassium channels can lead to a prolongation of the action potential.

Aim

Drug induced Long QT Syndrome (LQTS) and Torsade de Pointes arrhythmia are a pressing public health issue. Inhibition of hERG is considered a significant risk factor for cardiac safety. In the last few years, a number of drugs have been withdrawn from the market due to adverse cardiac side effects leading to LQTS. As a consequence, the pharmaceutical industry tends to screen for unwanted side effects of drug candidates on the cardiac action potential already in the earlier drug profiling stage. An automated electrophysiological screening with the Roboocyte can be used to characterize the effects of pharmaceutical compounds on hERG ionic currents.

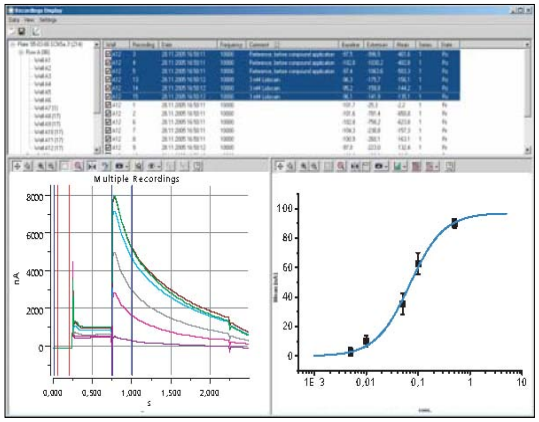


System

Oocytes are injected, recorded, transported, and stored conveniently in standard 96 well plates. mRNA or cDNA is injected fully automatically with the Roboocyte.

The novel digital amplifier has been optimized for TEVC (Two-Electrode Voltage-Clamp) experiments. Voltage steps can be freely designed to your needs. Resulting currents are recorded with the Roboocyte program.

You can choose between a 16-channel perfusion system or a liquid handling station that holds up to 400 compounds. Recording protocols can be run fully automatically without supervision, even over night. Provided that oocytes are of good quality, hundreds of compounds can be tested on a single well plate with 96 oocytes.



Software

The Roboocyte system is fully software controlled.

Amplifier and perfusion parameters, recording times, viability and stability checks, P/n leak subtraction, and your own custom checks are set up in separate recording protocols, one for each application. You load the appropriate protocol and start the session with a single mouse-click.

The extremum, the mean, and the region under the curve are extracted from a predefined region of interest with baseline subtraction, and current-voltage and dose-response curves are plotted fully automatically as well. All results are filed into a database. You can sort the results, print report sheets, and export the extracted results, the graphs, or the raw data to your custom program.

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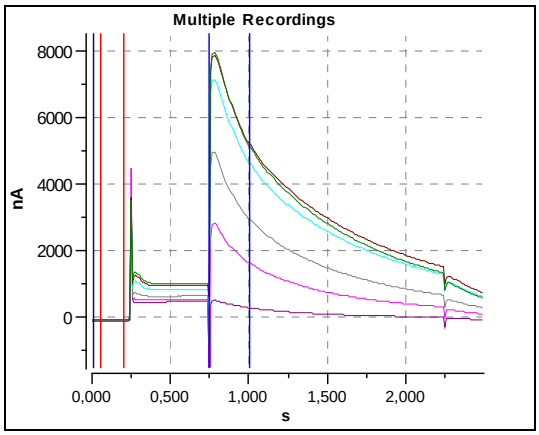
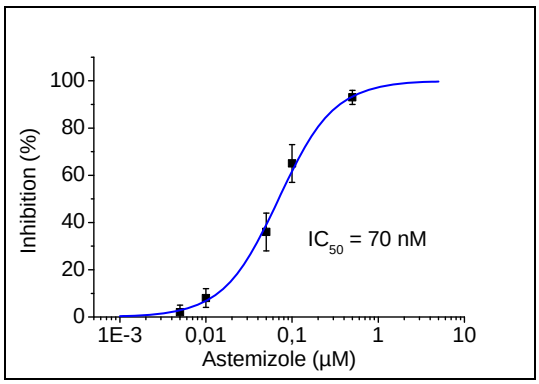


Recording of hERG Currents

The screen shot from the Roboocyte Analysis window shows an overlay of hERG induced currents. The heterologously expressed hERG channel was activated by a 500 ms depolarizing step to 0 mV from a holding potential of –90 mV, and a steady state current was observed. Since the rate constant for recovery from inactivation is faster than the deactivation rate constant, a step back to –85 mV elicits a large tail current, as there are many channels that have not proceeded from the opened to the closed state. The hERG channels were blocked by increasing concentrations of Astemizole at 5, 10, 50, 100, and 500 nM.

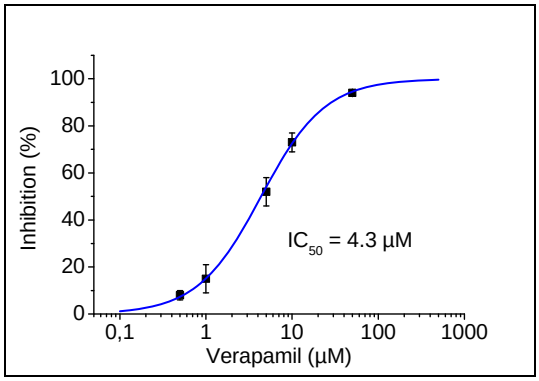
(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)



Inhibition of hERG Tail Currents by Astemizole

Astemizole is an antihistamine that provides relief from symptoms of allergies. The drug has been withdrawn from the U.S. market due to cardiac safety problems. Astemizole blocks the IKr current by inhibition of underlying the hERG K^+ channels. The measured IC_{50} value of 0.069 µM is virtually identical to published data.

(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)



Inhibition of hERG Tail Currents by Verapamil

The phenylalkylamine verapamil is used in the treatment of cardiovascular diseases such as angina pectoris, hypertension, and supraventricular tachyarrhythmias. The IC_{50} value of 4.3 µM measured with the Roboocyte is comparable to published data (3.8 µM) measured with a conventional setup.

(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)