roboocyte®

multichannel systems

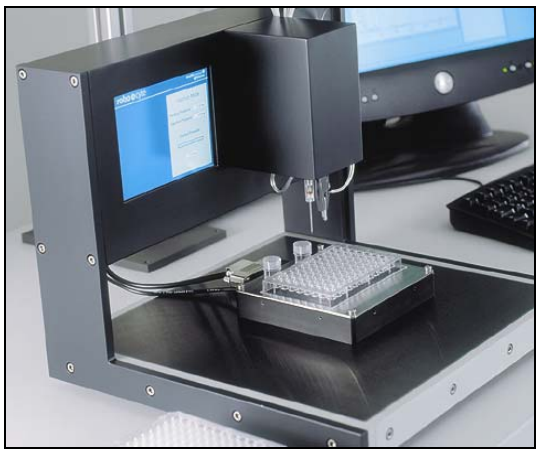
Applications: IKs Current

Introduction

KCNE subunits are membrane glycoproteins. Together with KCNQ1 (Kv7.1 or K_vLQT1), KCNE1 (minK) forms heteromeric potassium channels responsible for the repolarizing IKs (slowly activating cardiac delayed rectifier) currents that influence the action potential duration in cardiomyocytes.
Mutations in these ion channels lead to disorders that may increase the risk of death from cardiac arrhythmia (long QT syndrome or LQTS) and Jervell and Lange-Nielsen syndrome, associated with congenital deafness. These ion channels are also targets for antiarrhythmics. Drug induced LQTS and Torsade de Pointes arrhythmia are a pressing public health issue. Therefore, 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.

Aim

Since *Xenopus* oocytes express endogenously a homologue of KCNQ1, injection of KCNE1 mRNA or cDNA is sufficient for the expression of a functional heteromultimer, which is then incorporated into the oocyte membrane. The aim is to study the biophysical and pharmacological properties of this ion channel with the Two-Electrode Voltage-Clamp method, for example, to test potential IKs blockers, or to study unwanted side effects of drug candidates.
In order to determine the current–voltage (I–V) relationship, potassium outward currents were elicited by consecutive depolarizing pulses for 15 s from –60 mV to +40 mV in 20 mV steps (from a holding potential of -80 mV) in this experiment.

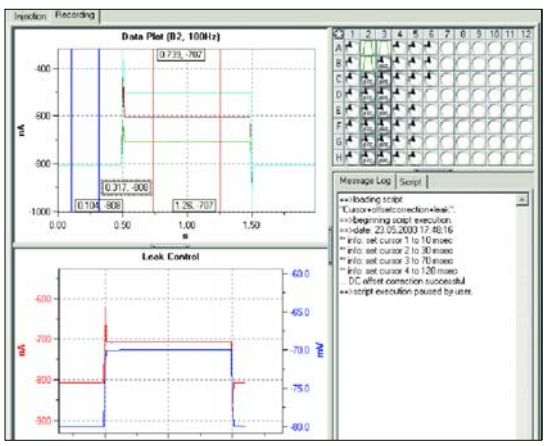


System

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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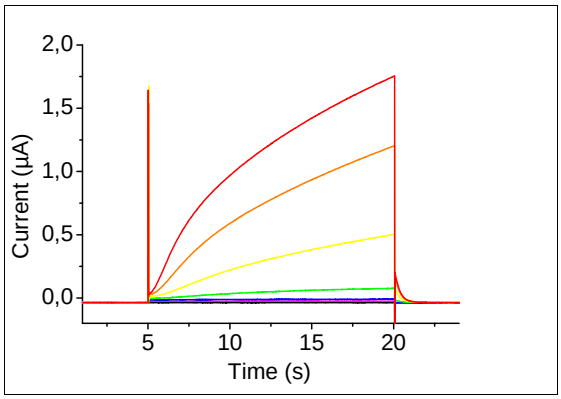
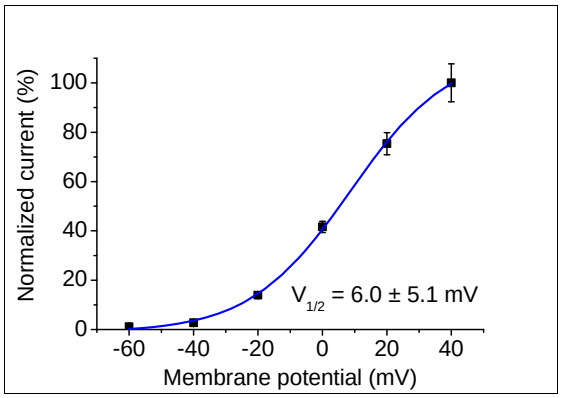


Signals

Injection of human KCNE1 cRNA resulted in the voltage-dependent activation of a slowly activating outward potassium current. The current showed no inactivation during the depolarizing voltage pulse. Potassium currents (> 100 nA) were successfully recorded in about 60 % of the oocytes automatically injected with KCNE1 cRNA.

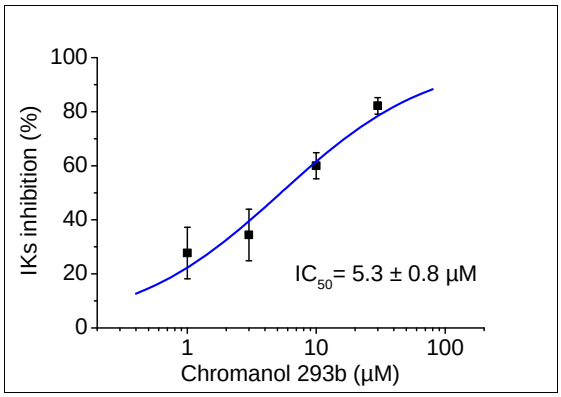
(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). "Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the Xenopus laevis Oocyte Expression System." Assay Drug Dev Technol 2(5): 515-524.)



Current-Voltage Relationship

Normalized mean maximal amplitudes were plotted against the membrane voltage, and the data were fitted with a Boltzmann function. The voltage at which the current was half maximal was 6.0 ± 5.1 mV, compared to 2.4 mV determined with a conventional TEVC setup.

(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). "Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the Xenopus laevis Oocyte Expression System." Assay Drug Dev Technol 2(5): 515-524.)



Inhibition of IKs by Chromanol 293B

The IC_{50} value of 5.3 µM for chromanol 293B blocking of IKs obtained with the Roboocyte was in the same range as IC_{50} values obtained with conventional TEVC recording techniques (6.2 and 6.9 µM).

(Pehl, U., Leisgen, C., Gampe, K. and Günther, E. (2004). "Automated Higher-Throughput Compound Screening on Ion Channel Targets Based on the Xenopus laevis Oocyte Expression System." Assay Drug Dev Technol 2(5): 515-524.)