roboocyte®

multichannel systems

Applications: Potassium Channel Kv4.3

Introduction

The voltage-gated potassium channel family (Kv) can be divided into 12 subfamilies, termed Kv1-12. These channels mediate K^+-selective outward currents and can be characterized by their different affinities to peptide toxins isolated from animal venoms. The Kv4 subfamily has three members, designated Kv4.1, Kv4.2 and Kv4.3.

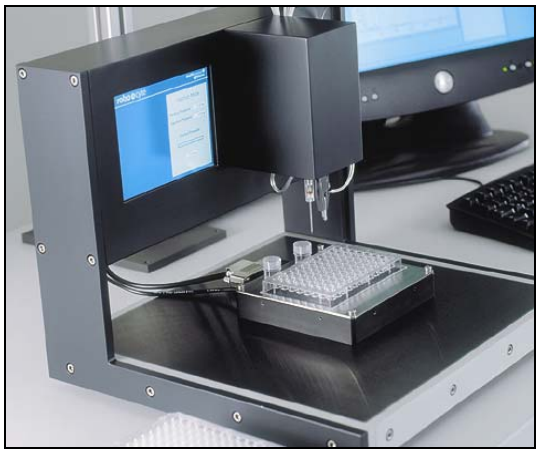
Kv4.3 channels belong to the rapidly inactivating potassium channel group. These ion channels are important molecular components of transient K^+ currents in the human brain and heart, and they are involved in setting the frequency of neuronal firing and heart pacing. Altered Kv4.3 channel expression has been demonstrated under pathological conditions like heart failure indicating their critical role in heart function.

Aim

hKv4.3 mRNA is expressed in *Xenopus laevis* oocytes, and the channel protein is incorporated into the oocyte membrane.

The aim is to analyze the biophysical and pharmacological properties of this ion channel with the Two-Electrode Voltage-Clamp method. A voltage step series is run from -80 mV to +40 mV with an increment of 20 mV to analyze the activation characteristics of the ion channel.

The oocytes are exposed to test compounds to show potential effects on the ion channel activity.

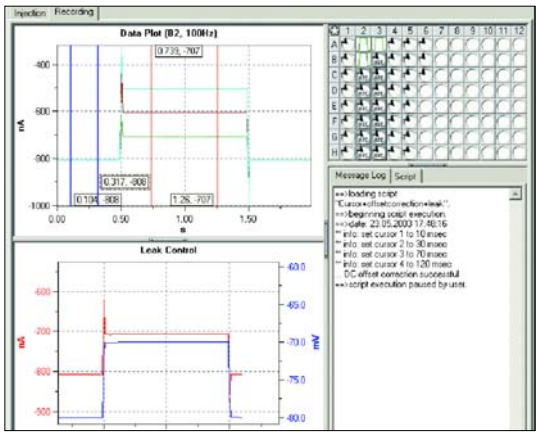


System

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.

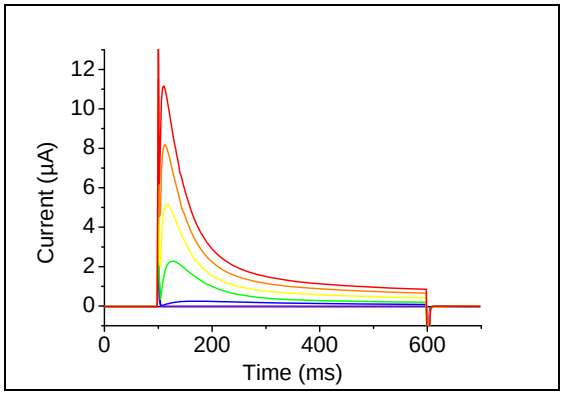
Amplifier and perfusion parameters, recording times, viability and stability checks, P/n leak subtraction, and your own custom checks are set up in separate recording protocols, one for each application. You load the appropriate protocol and start the session with a single mouse-click.

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.

roboocyte®

Applications: Potassium Channel Kv4.3

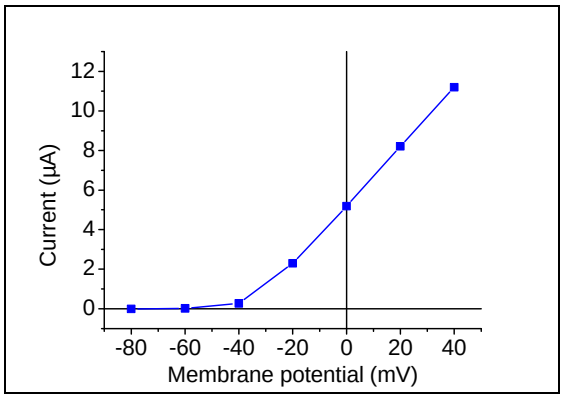
Multi Channel Systems
MCS GmbH
Aspenhaustrasse 21
72770 Reutlingen
Germany
Fon +49 – 7121 – 90 92 50
Fax +49 – 7121 – 90 92 511



Signals

The expression of hKv4.3 mRNA in *Xenopus laevis* oocytes resulted in a rapidly activating and inactivating K^+ current, as shown in the graph.
The holding potential was –80 mV. Kv4.3 currents were elicited by 500 ms voltage step pulses in 20 mV increments, ranging from –60 mV to +40 mV.

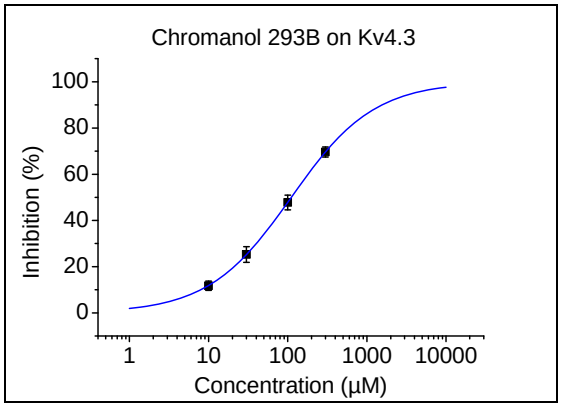
(Data kindly provided by IonGate Bioscience GmbH, Frankfurt / Main, Germany, www.iongate.de)



Current-Voltage Relationship

The current-voltage relationship that was analyzed with the Roboocyte is in agreement with published data. The threshold for activation of the current was between –50 and –40 mV. The current increases monotonically in size at more positive voltages.

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



Inhibition of hKv4.3 by Chromanol 293B

Data obtained with the Roboocyte were fitted with the Hill equation. The resulting dose-inhibition curve shows the effect of the Chromanol derivate 293B, which has been previously shown to be a specific antagonist of hKv4.3 currents.
An IC_{50} value of 110.7 ± 4.6 µM was calculated, which is in good agreement with published data for Kv4.3 expressed in CHO cells (60 ±1.5 µM).

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