

Applications: Cardiac Sodium Channel

Introduction

The family of voltage-gated sodium channels initiates action potentials in all types of excitable cells. The human gene SCN5A encodes for the alpha subunit of the cardiac voltage-gated sodium channel (Nav1.5).

Local anesthetic molecules such as lidocaine block Na⁺ channels and have been used therapeutically to manage cardiac arrhythmias.

It has recently been found that non-specific binding of drugs to the SCN5A channel can alter normal cardiac rhythm. For this reason, the pharmaceutical industry is starting to include SCN5A in their cardiotoxicity screening panels.

Aim

SCN5A cDNA was expressed in *Xenopus laevis* oocytes, and the channel protein was 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.

20 test pulses were repeated 17 times in a row. The test compounds were applied after the sixth repeat. The compound was washed in for 20 s and then left in the well. Each compound was tested on several oocytes as replicates. An exact timing of the pulses is guaranteed by the system for highly reproducible results.



System

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

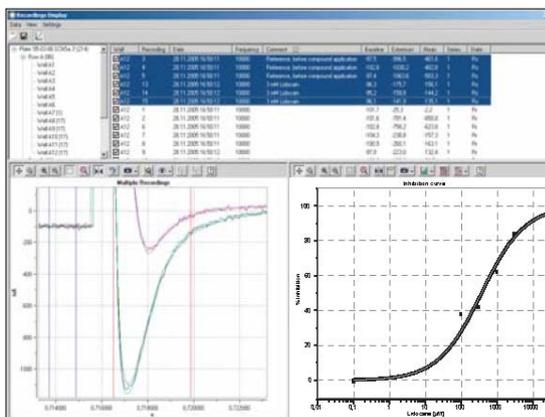
The novel digital amplifier has been optimized for TEVC (Two-Electrode Voltage-Clamp) experiments. Voltage steps can be freely designed to your needs. Resulting currents are recorded with the Roboocyte program.

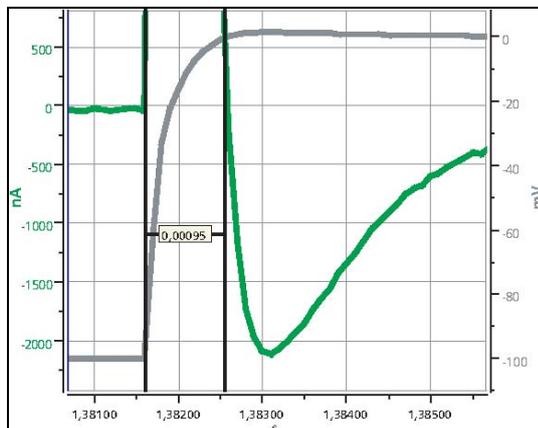
You can choose between a 16-channel perfusion system or a liquid handling station that holds up to 400 compounds. Recording protocols can be run fully automatically without supervision, even over night. Provided that oocytes are of good quality, hundreds of compounds can be tested on a single well plate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



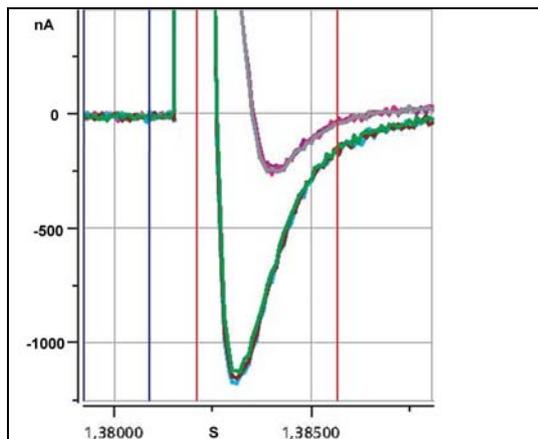


Voltage Clamp Performance and Recorded Signals

The figure shows a typical response of the SCN5A channel after a voltage step from the holding potential of -100 mV to 0 mV.

The setpoint voltage of 0 mV (grey line) is fully reached before the cell starts to respond (green trace). The rise time (10 to 90 %) of the amplifier is approximately 500 μ s.

(Data kindly provided by NMI Reutlingen, Germany)

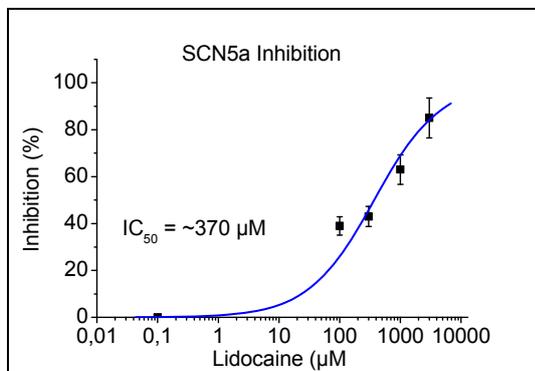


Effect of Lidocaine on SCN5a

An overlay of responses to the first stimulation pulse from repeats 3-5 (before compound application) and 13-15 (after compound application) is shown.

3 mM lidocaine reduces the Na current by 85 %. The response traces are very reproducible. Blue bars mark the baseline, red bars the region of interest for the automated data analysis with the Roboocyte.

(Data kindly provided by NMI Reutlingen, Germany)



Dose-Response Relationship

The Roboocyte program automatically outputs fitted sigmoidal dose-response curves and the corresponding EC/IC_{50} values.

The measured IC_{50} value of 370 μ M for the SCN5a channel is comparable to published data (150 μ M) and shows the reliability of the system and the amplifier.

(Data kindly provided by NMI Reutlingen, Germany)