

Applications: Na/K-ATPase Transporter

Introduction

The Na/K-ATPase is a ubiquitous and critically important membrane protein that transports 2 K⁺ ions into and 3 Na⁺ ions out of the cell against the electrochemical gradient by using the energy of the hydrolysis of 1 ATP molecule per transport cycle.

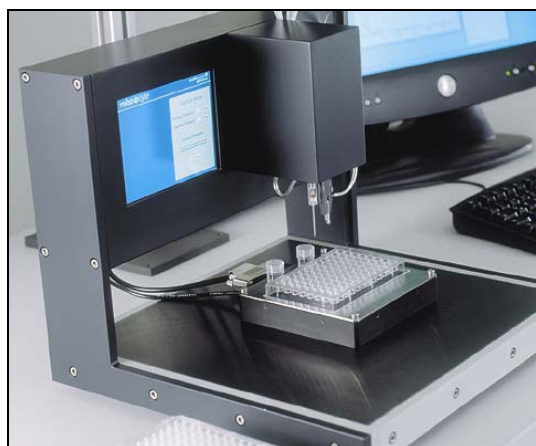
The transporter serves many functions including creating and maintaining the transmembrane Na⁺ and K⁺ gradients that contribute to the membrane potential and excitability, driving secondary active transport systems coupled to Na⁺ fluxes, and determining a significant fraction of the cellular metabolic rate via ATP hydrolysis. 30–70 % of the cell's ATP is used for this transporter.

Moreover, the Na/K-ATPase is the pharmacological receptor for cardiac glycosides, which are widely used in the treatment of heart failure because of their positive inotropic effect, and is possibly also the physiological receptor for endogenous ouabain-like compounds.

Aim

Four different Na/K-ATPase isozymes were expressed in *Xenopus laevis* oocytes and investigated with the Roboocyte. *Xenopus laevis* oocytes also express an endogenous Na/K-ATPase. To distinguish between the endogenous and the heterogenous forms, the Na-pump current of injected oocytes was compared with that of non-injected cells.

Compounds can then be tested on oocytes to reveal potential effects on the Na-pump transport activity. In this case, the effect of the inhibitor ouabain on endogenous and human α_2/β_1 Na/K-ATPase was analyzed.



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.

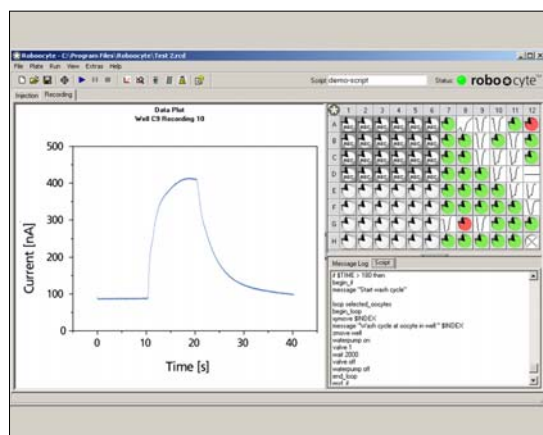
You can choose between a 16-channel perfusion system or a liquid handling station that holds up to 400 compounds. Recording protocols can be run fully automatically without supervision, even over night. Provided that oocytes are of good quality, hundreds of compounds can be tested on a single well plate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

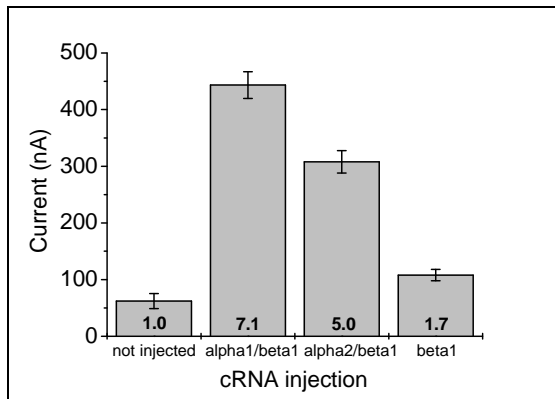
Software

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.

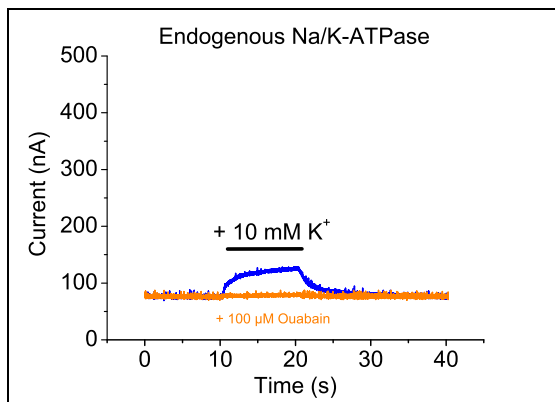




Oocyte Expression

Oocytes injected with cRNA encoding human Na/K-ATPase subunits and preloaded with Na⁺ showed pump currents that were 1.7-fold (*Xenopus laevis* α /human β_1 Na/K-ATPase) to 7.1-fold (human α_1/β_1 Na/K-ATPase) higher than those measured in non-injected oocytes (endogenous *Xenopus laevis* Na/K-ATPase), which is in good agreement with published data. Thus, a discrimination between *Xenopus laevis* and human Na/K-ATPase is possible.

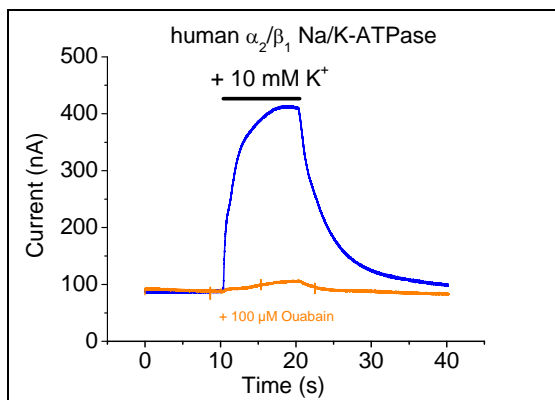
(Data kindly provided by IonGate Biosciences GmbH, Frankfurt / Main, Germany, www.iongate.de)



Inhibition of the Endogenous Na/K-ATPase by Ouabain

The effect of the inhibitor ouabain (100 μ M, exposure time 2 min) on the endogenous Na/K-ATPase is shown. Note, that even the small endogenous currents of only 50 nA can be well resolved with the Roboocyte's digital TEVC amplifier.

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



Inhibition of a Human Na/K-ATPase by Ouabain

This graph shows the effect of ouabain (100 μ M, exposure time 2 min) on the human α_2/β_1 Na/K-ATPase.

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