roboocyte®

multichannel systems

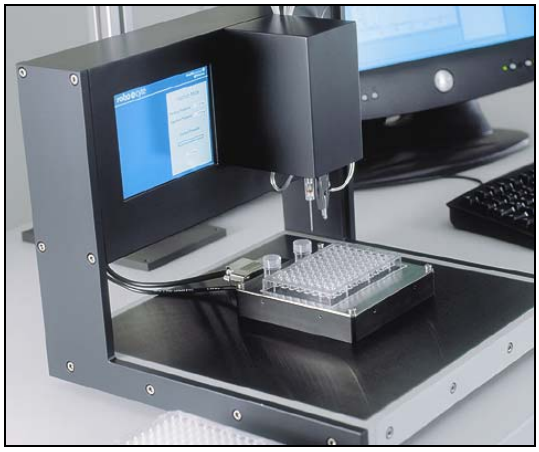
Applications: P2X Receptors

Introduction

P2X receptors are nucleotide-gated cation channels composed of homomeric or heteromeric assemblies of three subunits. Seven different types ($P2X_{1-7}$) are known so far.
P2X receptors are widely expressed in the central nervous system and in the body periphery and play important physiological and pathophysiological roles (muscle contraction, modulation of the cardiovascular and respiratory system, inflammation and cell death, fast synaptic transmission, neuronal excitability, and many more).

Aim

$P2X_1$ and $P2X_2$ receptors are expressed after cDNA or mRNA injection into *Xenopus* oocytes, and the channel protein is integrated into the oocyte membrane.
The aim is to analyze the pharmacological properties of this ion channel with the Two-Electrode Voltage-Clamp method. The oocytes are exposed to test compounds to show potential effects on the ion channel activity. In-between compound applications, ATP control responses were recorded in order to monitor run-down or run-up effects and to check for the complete washout of the respective compound.
The following experiments demonstrate the different kinetic properties of the two channels after application of ATP, and the NF279 mediated inhibition of the $P2X_2$ current. NF279 is a suramin analogue and acts as a P2X-selective antagonist. (Suramin is a polysulfonated urea derivative and has been widely used both to treat infections and as a chemotherapeutic drug.)

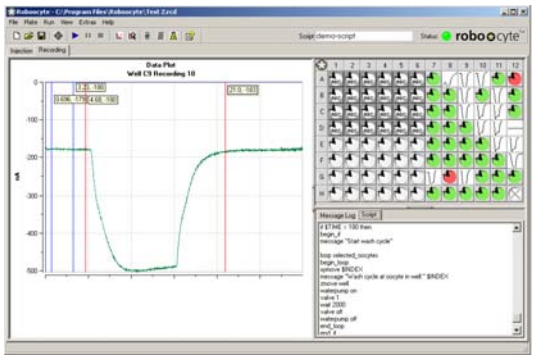


System

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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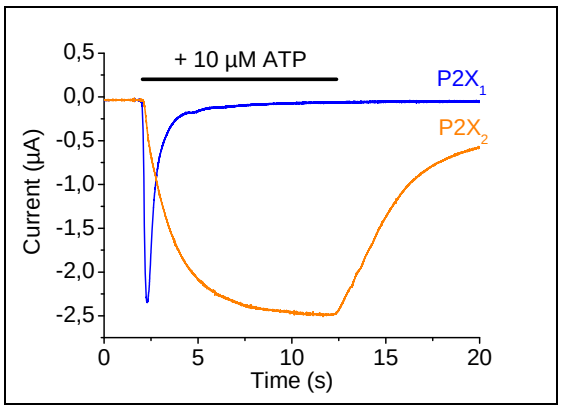
Applications: P2X Purinoceptors

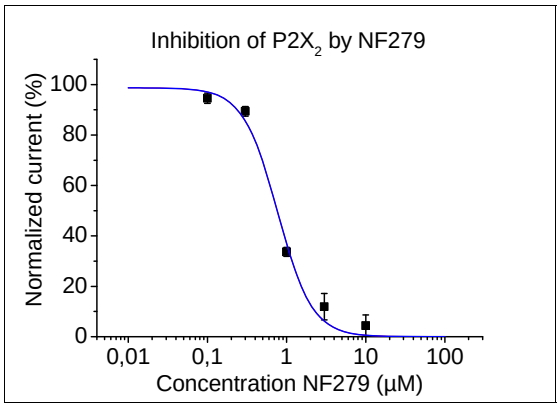
Multi Channel Systems
MCS GmbH
Aspenhaustrasse 21
72770 Reutlingen
Germany
Fon +49 – 7121 – 90 92 50
Fax +49 – 7121 – 90 92 511



Signals

The figure shows typical $P2X_1$ (fast response to ATP, strong desensitization) and $P2X_2$ receptor currents (almost no desensitization).
In order to analyze $P2X_1$-receptor currents, a fast and reproducible solution exchange is necessary. These experiments demonstrate that the Roboocyte supports a continuous solution flow and a rapid compound application.

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



Inhibition of $P2X_2$ Receptor Currents by NF279

For the analysis of NF279-mediated inhibition of $P2X_2$ receptor currents, the receptors were activated five times with 10 µM ATP resulting in reproducible current responses. After this equilibration period, respective NF279 concentrations were applied together with ATP. Between the NF279 applications, ATP control responses were recorded in order to monitor run-down or run-up effects and to check for the complete washout of the antagonist. All experiments were performed at a holding potential of -40 mV. An IC_{50} value of 0.77 µM was determined that is in perfect agreement with published data (0.76 µM).

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