B'SYS GmbH

CHO $P2X_2$ patch - clamp assay

Specification Sheet

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1 INTRODUCTION

1.1. P2X Receptors

P2X receptors are a family of cation-permeable ligand gated ion channels that open in response to the binding of extracellular adenosine 5'-triphosphate (ATP). They belong to a larger family of receptors known as the purinergic receptors. The members of this class of ligand gated ion channels differ in their affinity to ATP and blockers and their biophysical properties.

1.2. B'SYS' P2X$_2$

The host of B'SYS' P2X$_2$ are CHO cells. NCBI Reference Protein Sequence NP_733782 (P2X$_2$).

2 MATERIAL AND METHOD

P2X$_2$ currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained (in mM) NaCl 137, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, D-glucose 10, HEPES 10, pH (NaOH) 7.40. The pipette solution consisted of (in mM) KCl 130, $MgCl_2$ 1, MgATP 5, HEPES 10, EGTA 5, pH (KOH) 7.20. For experiments on the QPatch, the same bath solution was used. The pipette solution consisted of (in mM) KCl 130, $CaCl_2$ 2, $MgCl_2$ 4, Na_2-ATP 4, HEPES 10, EGTP 5, pH (KOH) 7.20. After formation of a Gigaohm seal between the patch electrodes and individual cells, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were maintained at room temperature. The holding potential was -80 mV. As soon as a stable seal could be established inward currents were measured upon application of agonist.
After each application bath solution was perfused for at least 30 s

3 RESULTS

3.1. Agonist

B'SYS provides assays with stably expressed P2X$_2$ receptors. The human P2X$_2$ receptors were validated by means of the manual and automated patch-clamp technique.

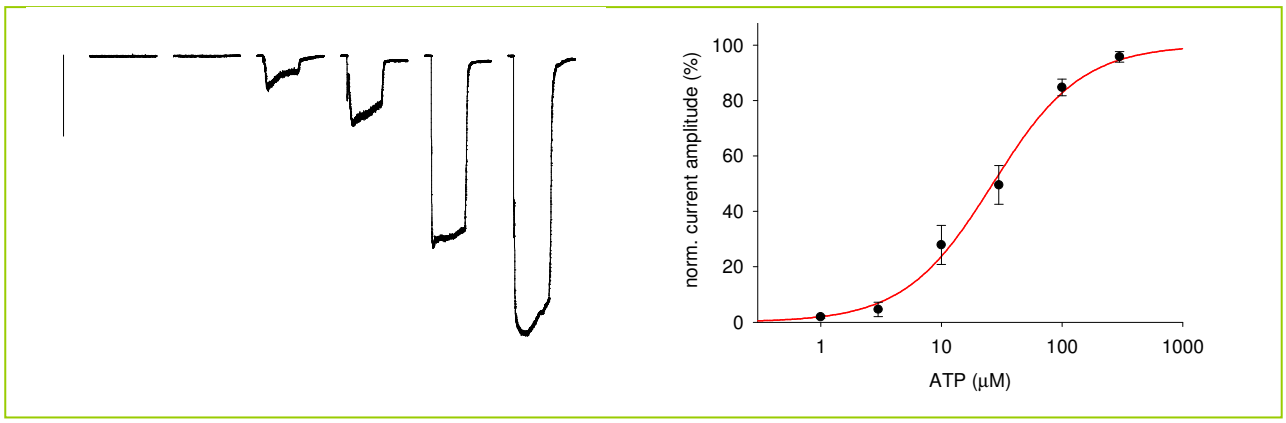

Fig.1: Activation of P2X$_2$ receptor currents upon brief applications of increasing concentrations of ATP to patch-clamped cell The cell membrane was hold at -80 mV. No inward currents were recorded in untransfected cells (data not shown).
left: Representative current recordings of applications of 1.0 µM, 3.0 µM, 10 µM, 30 µM, 100 µM and 300 µM ATP, scale bar: 1.0 nA
right: dose response curve, EC$_{50}$: 26.76 µM (Hill: 1.2)

A sigmoidal dose response curve was fitted to the resulting relative current amplitudes of the ATP concentrations. They resulted in a EC$_{50}$ value of 26.76 µM with a Hill coefficient of 1.2.

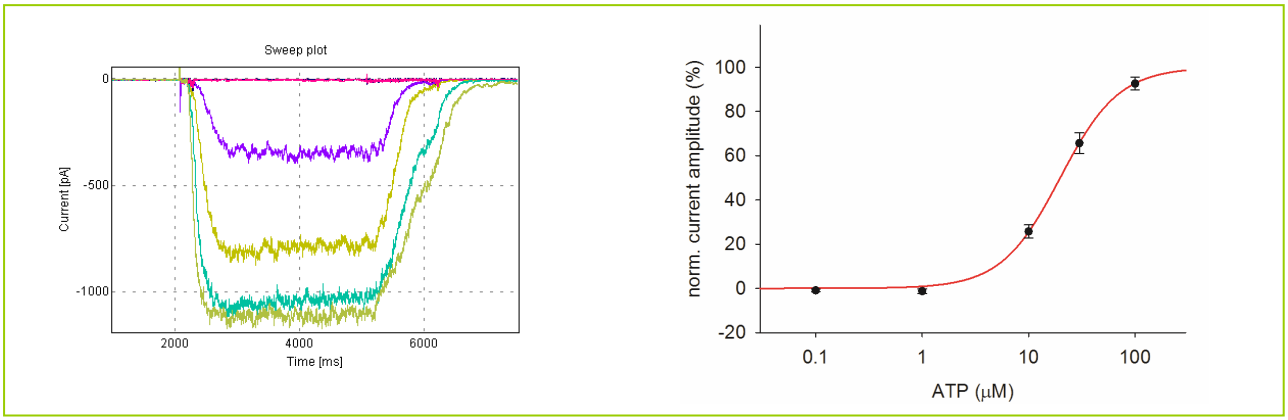

Fig.2: Dose response curve of applications of 0.1 µM, 1.0 µM, 10 µM, 30 µM, 100 µM and 300 µM ATP using automated patch-clamping (Q-Patch) with cells stably expressing P2X$_2$ receptor. EC$_{50}$: 19.81 µM (Hill: 1.6).

For automated patch-clamping (QPatch) a sigmoidal dose response curve was fitted to the resulting relative current amplitudes of the ATP concentrations. They resulted in a EC$_{50}$ value of 19.81 µM with a Hill coefficient of 1.6.

3.2. Effect of the P2X antagonist Suramin

ATP (10 µM, ~EC$_{30}$) was applied repeatedly to ensure constant current amplitudes. Concentrations of 3.0 µM, 10 µM, 30 µM and 100 µM Suramin were co-applied together with ATP (10 µM). The peak current amplitude decreased concentration dependently. The IC$_{50}$ was calculated to be 17.00 µM (Hill: 0.8).

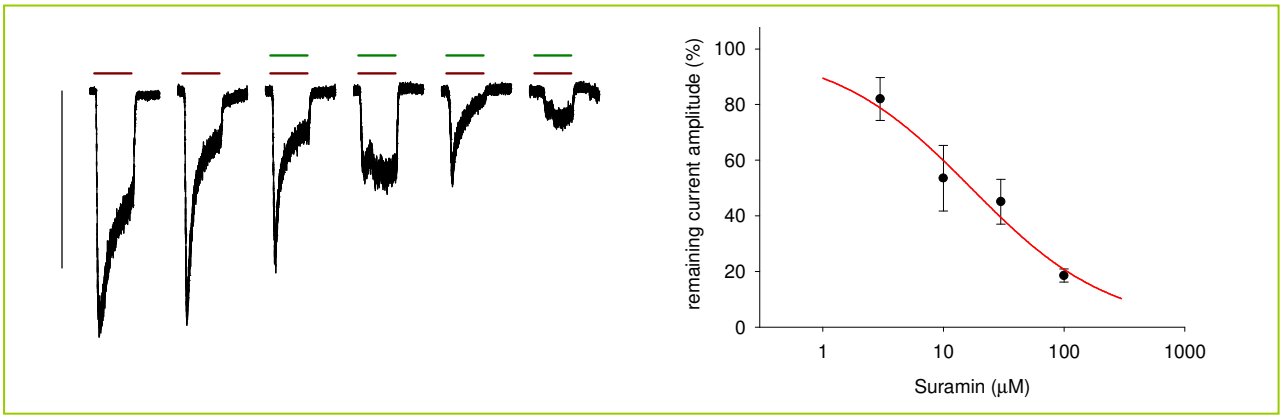

Fig.3: Activation of P2X$_2$ receptor currents upon brief applications of ATP (10 µM, dark red bar) and co-application of concentrations of 3.0 µM, 10 µM, 30 µM and 100 µM Suramin (green bar) to a patch-clamped cell. The cell membrane was hold at -80 mV.
left: Representative current recordings, scale bar: 0.5 nA; right: dose response curve, IC$_{50}$: 17.00 µM (Hill: 0.8)

For experiments with automated patch-clampingon the QPatch, concentrations of 1 µM, 3 µM, 10 µM, 30 µM and 100 µM Suramin were co-applied together with ATP (300 µM).

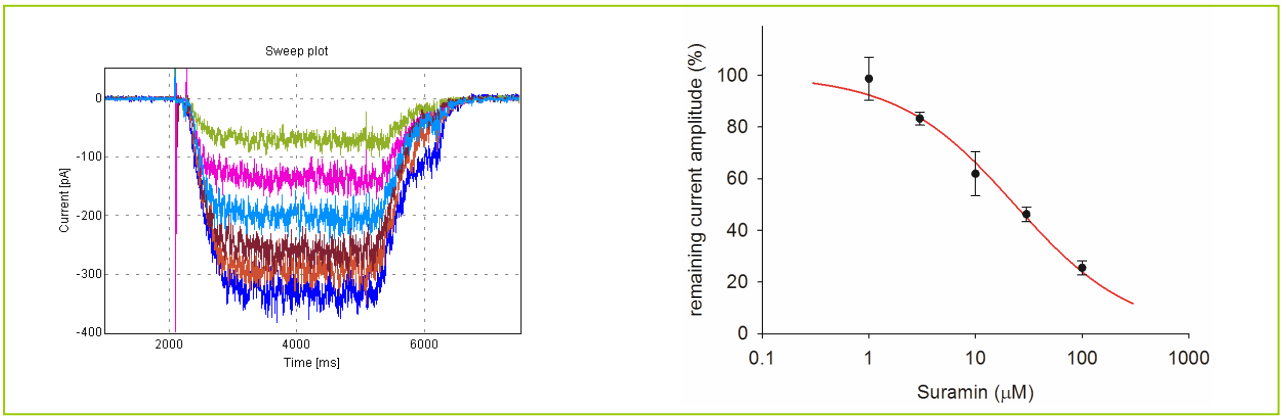

Fig.4: Activation of P2X$_2$ receptor currents on the QPatch upon brief applications of ATP (300 µM, dark blue sweep) and co-application of concentrations of 1.0 µM, 3.0 µM, 10 µM, 30 µM and 100 µM Suramin to a patch-clamped cell. The cell membrane was hold at -80 mV.
left: Representative current recordings; right: dose response curve, IC$_{50}$: 23.26 µM (Hill: 0.8)

4 ASSAY FLEXIBILITY

B'SYS offers its clients a high degree of flexibility. Client designed stimulation or application protocols can be transferred and validated within a short time.
Pre-wash and extended wash-out are possible options.
We offer to develop and optimize your assay using manual or automated patch-clamp (Q-Patch).

5 CONTACT INFORMATION

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