B'SYS

B'SYS GmbH

P2X Cell Lines

Specification Sheet

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1 BACKGROUND

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 cell lines

Cells were stably transfected and selected for high expression rates. They are suitable for manual and automated patch-clamping, as well for fluorescence based assays like FLEX station ad FLIPR.
The following cell lines are available:

P2X receptor	Host cell	Selection antibiotics
P2X1	CHO	Puromycin
P2X2	CHO	Hygromycin
P2X2/3	CHO	Puromycin
P2X3	CHO	Hygromycin
P2X4	HEK293	Puromycin
P2X7	HEK293	Geniticin

2 VALIDATION

2.1 Electrophysiology

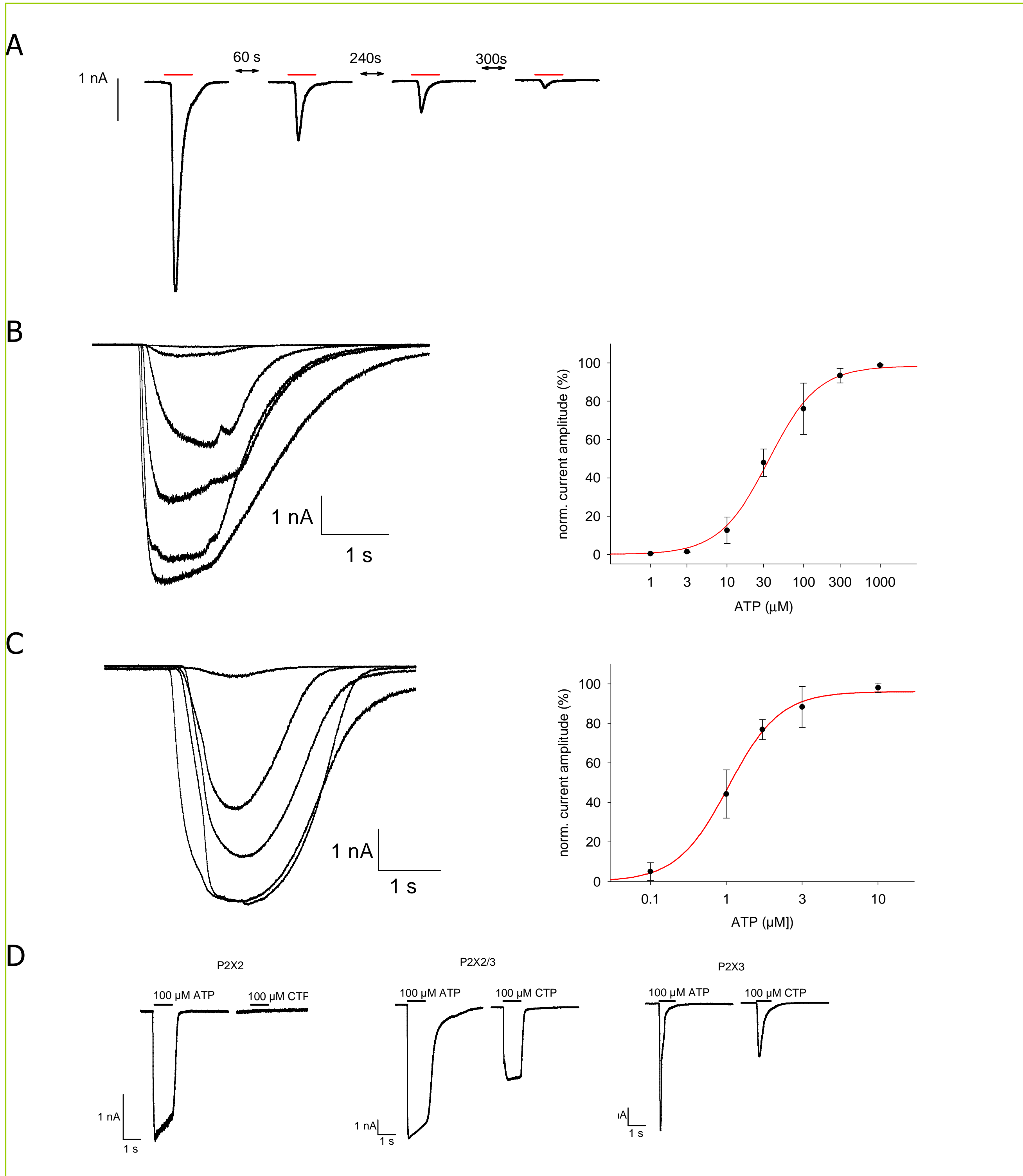
All cell lines were validated using manual patch-clamping and automated patch-clamping (Q-Patch). During validation, the dose dependence and the EC_{50} for at least one agonist were determined. Further during a constant concentration of agonist, increasing concentrations of antagonists were applied and the IC_{50} calculated.

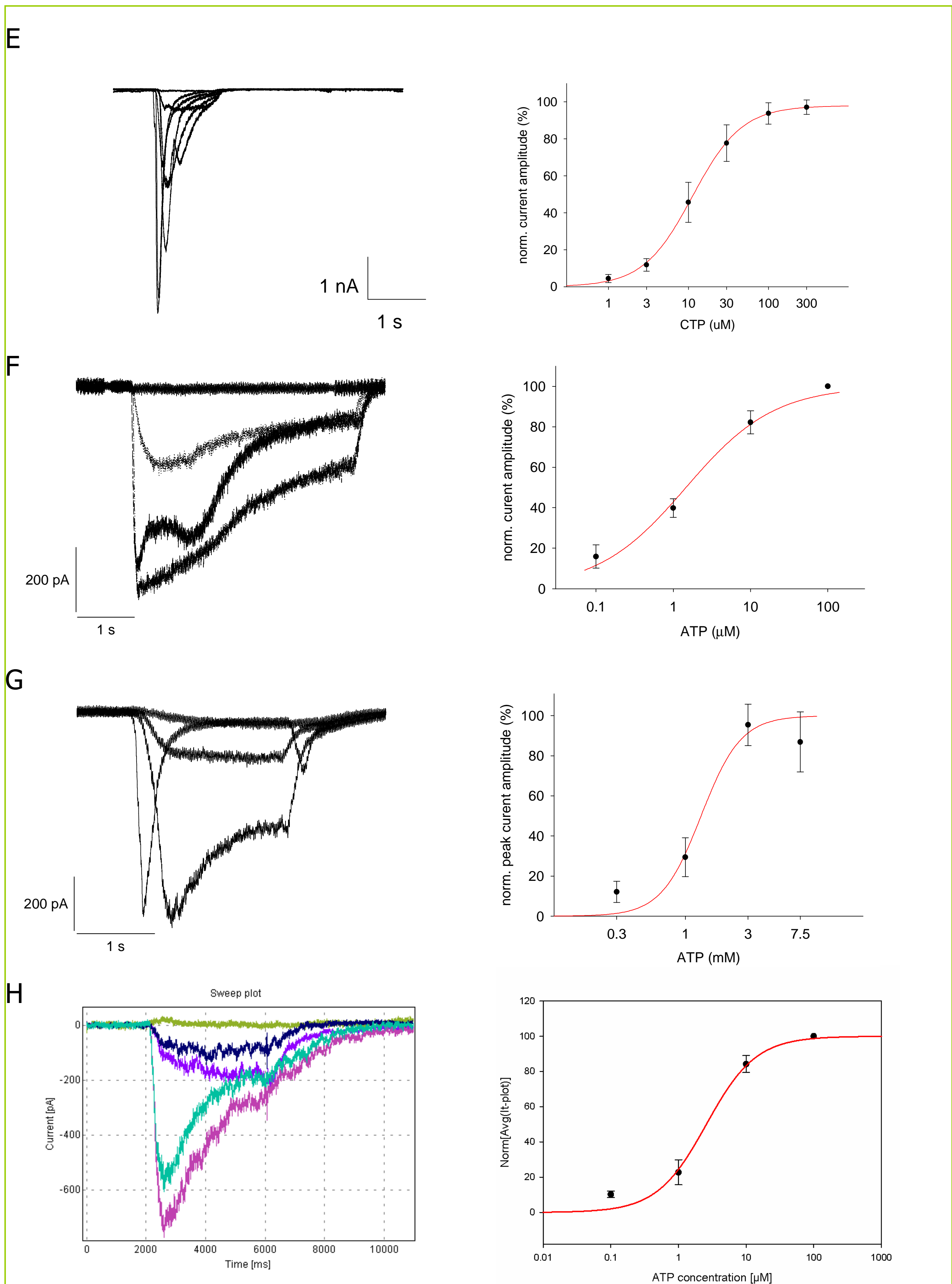
The extracellular solution was composed of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM Glucose, pH 7.4 (NaOH). The pipette solution contained: 130 mM KCl, 1 mM $MgCl_2$, 5 mM MgATP, 10 mM HEPES, 5 mM EGTA, pH 7.2 (KOH). Cells were clamped to -80 mV for the duration of the complete experiment. To accomplish fast solution exchanges, a 8 to 1 manifold was placed in close neighborhood of the tested cell.

The following EC_{50} were determined:

Cell Line	Agonist	Method	EC_{50}
CHO P2X1	ATP	Manual patch-clamp	Not determinable, very slow recovery of receptor desensitization
CHO P2X2	ATP	Manual patch-clamp	36 ± 4 µM
CHO P2X2/3	ATP	Manual patch-clamp	1.2 ± 0.1 µM
CHO P2X3	CTP	Manual patch-clamp	11.3 ± 0.4 µM

HEK P2X4	ATP	Manual patch-clamp	1.46 µM
	ATP	Automated patch-clamp (Q-Patch)	2.59 µM
HEK P2X7	ATP	Manual patch-clamp	1320 µM
	ATP	Automated patch-clamp (Q-Patch)	282.76 µM




E
1 nA
1 s
norm. current amplitude (%)
100
80
60
40
20
0
1
3
10
30
100
300
CTP (uM)
F
200 pA
1 s
norm. curent amplitude (%)
100
80
60
40
20
0
0.1
1
10
100
ATP (µM)
G
200 pA
1 s
norm. peak curent amplitude (%)
100
80
60
40
20
0
0.3
1
3
7.5
ATP (mM)
H
Sweep plot
0
-200
-400
-600
Current [pA]
0
2000
4000
6000
8000
10000
Time [ms]
Norm[Avg(lt-plot)]
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100
80
60
40
20
0
0.01
0.1
1
10
100
1000
ATP concentration [µM]

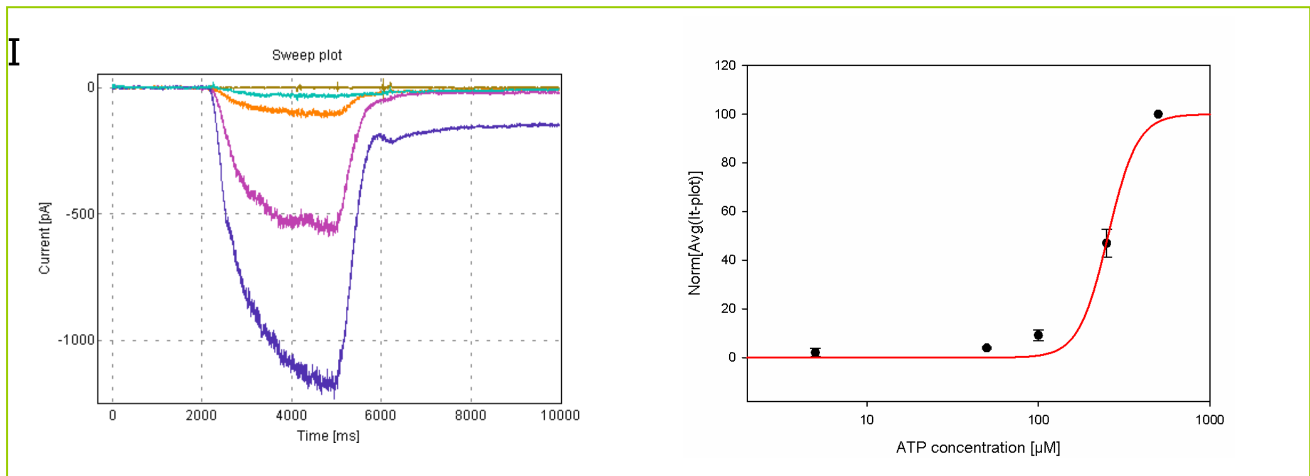

Fig.1: (Precious Page) A) P2X1 Current run-down / desensitisation / slow recovery from desensitisation does not allow to measure ATP dose response curves. B) ATP dose response curve for P2X2. C) ATP dose response curve for P2X2/3. D) Comparison of the effects of 100 µM ATP and 100 µM CTP on P2X2, P2X2/3 and P2X3 receptors, E) CTP dose response curve for P2X3, F) manual patch-clamping of $P2X_4$, G) manual patch-clamping of $P2X_7$, H) automated patch-clamping of $P2X_4$,): automated patch-clamping of $P2X_7$

For P2X2, P2X3 and their heteromer, TNF was used as reference antagonist. This compound was selected due to its different affinities to P2X2 and P2X3 receptors:

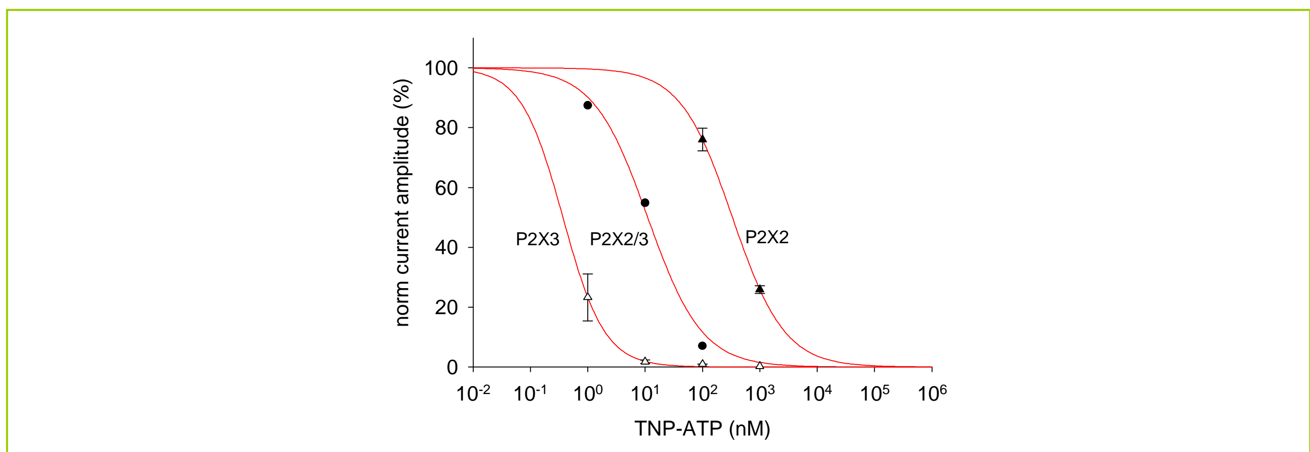

Fig.2: TNP-ATP block of P2X channels. Dose response curves for P2X2, P2X2/3 and P2X3

Cell Line	**Method**	**IC_{50}**
HEK $P2X_2$	Manual patch-clamp	334 nM
HEK $P2X_{2/3}$	Manual patch-clamp	11 nM
HEK $P2X_3$	Manual patch-clamp	0.4 nM

For P2X4 and P2X7 Suramine was tested as antagonist:

The following IC_{50} for Suramine were determined (currents were evoked by ATP, concentrations given in Table):

Cell Line	Method	IC_{50} / ATP concentration
HEK $P2X_4$	Manual patch-clamp	385.15 µM / 2.00 µM
HEK $P2X_7$	Manual patch-clamp	100.95 µM / 100 µM

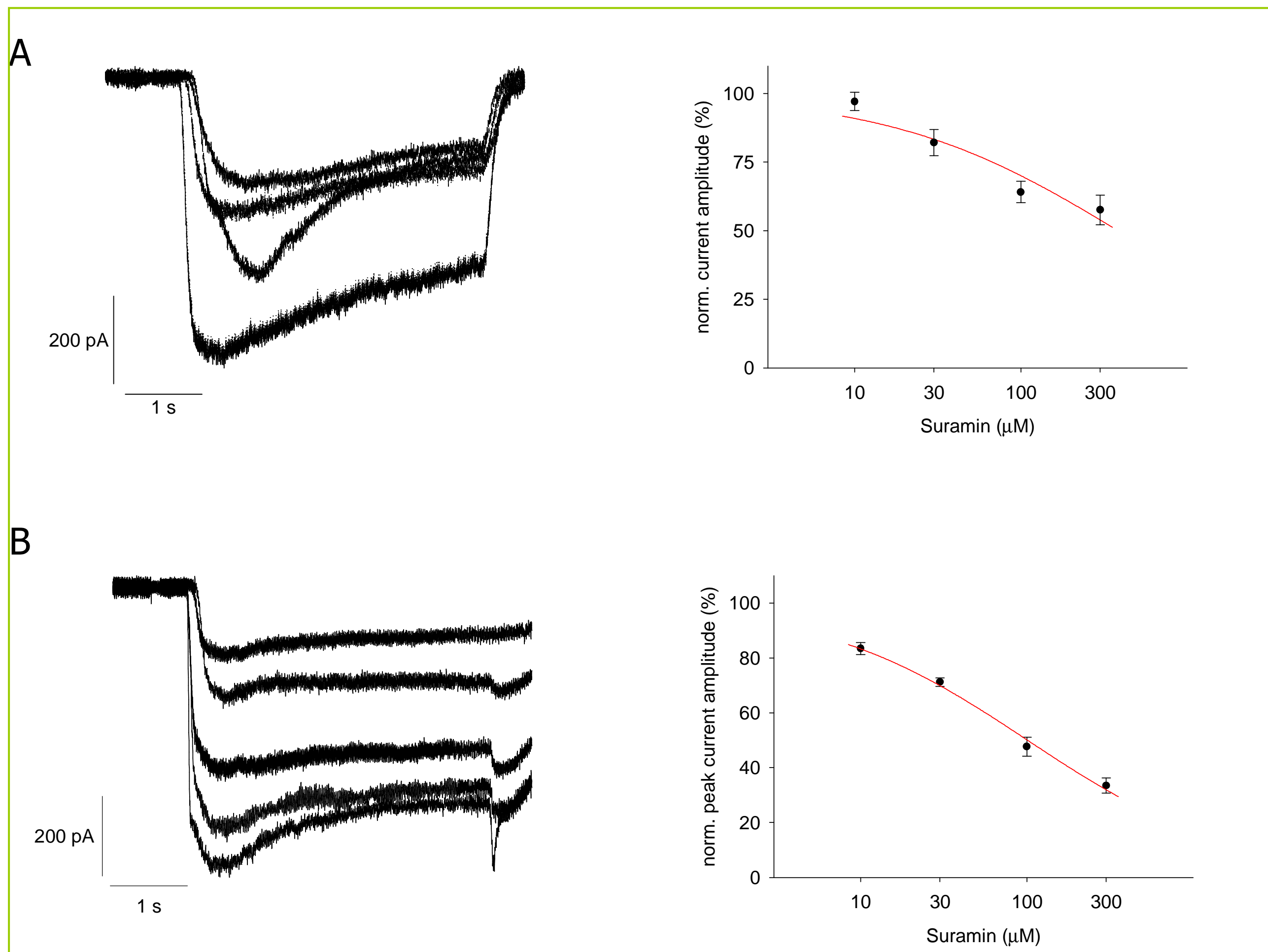

Fig.3: Suramin Block of P2X channels. Left: representative current recordings, left: Dose response curves. A: manual patch-clamping of $P2X_4$ cells, B: manual-patch-clamping of $P2X_7$ cells

2.2 Fluorescence based assays

The Calcium influx in HEK P2X7 cells was analyzed using a FLEXstation in combination of the Calcium Plus Assay Kit (Molecular Devices). 8 x 104 cells were seeded per well of a black 96 well plate with clear bottom. The assay was carried out in Hanks Balanced Salt solution (HBSS, calcium, magnesium, no phenol red) containing 2-2.5 mM Probenicid. Different ATP concentrations were added after recording the baseline fluorescence for 45 s. The time course of the fluorescence was recorded.

The experimental setup parameters:

Excitation wavelength: 584 nm
Emission wavelength: 525 nm
Emission cut-off: 515 nm

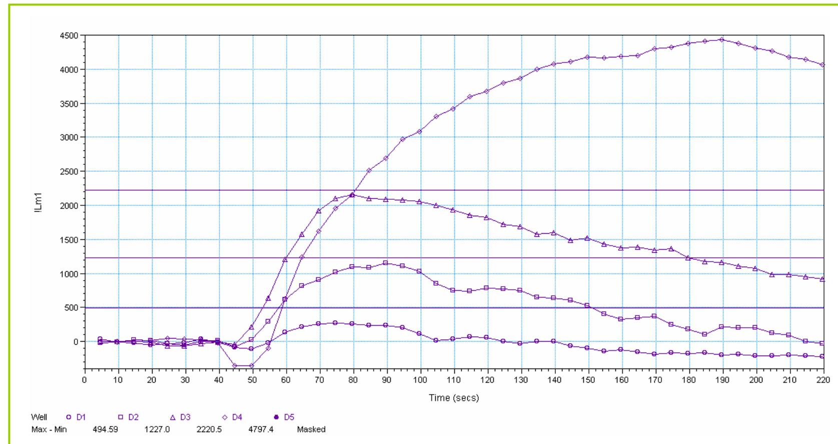

Fig. 4: Overlay of fluorescence signals recorded from wells treated with increasing concentrations of ATP.

3 CELL CULTURE CONDITIONS

3.1 General

HEK P2X cells are incubated at 37°C in a humidified atmosphere with 5% CO2 (rel. humidity > 95%). The cells are continuously maintained and passaged in sterile culture flasks containing DMEM/F12 medium supplemented with 9% foetal bovine serum, 0.9% Penicillin/Streptomycin solution and selection antibiotics. The HEK P2X cells are passaged at a confluence of about 50-80%. For electrophysiological measurements the cells are seeded onto e.g. 35 mm sterile culture dishes containing complete medium.

- All solutions and equipment coming in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.
- Be sure to have frozen cell stocks at hand before starting experiments.
- Cells should be splitted every 4-5 days at 50% - 80% confluency at 1:3 to 1:5 ratio.

3.2 Recommended Complete Medium

- DMEM/F12 with GlutaMAX I or L-Glutamine
- 9% FBS
- 0.9% Penicillin/Streptomycin

3.3 Antibiotics

- HEK P2X clones were selected under antibiotic pressure.
- To cultivate HEK P2X cells, a reduced antibiotic pressure should be used.

Remark: The permanent application of antibiotic pressure has no effect on current density.

3.4 Thawing Cells

- Remove vial of cells from liquid nitrogen and thaw quickly at 37°C.
- Decontaminate outside of vial with 70% ethanol.
- Transfer cells to a T-25 culture flask containing 5 mL complete medium.
- Incubate cells at 37°C for 4-6 h to allow the cells to attach to the bottom of flask.
- Aspirate off the medium and replace with 5 mL complete medium & antibiotics.
- Incubate cells and check them daily until 50% - 80% confluency is reached.

3.5 Splitting Cells

- When cells are 50% - 80% confluent remove complete medium.
- Wash cells once with 1x Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Passage cells into new flask with complete medium and antibiotics at 1:3 to 1:5 ratio.
- Use remaining suspension for counting the cells.

3.6 Freezing Medium

- Mix 0.9 mL fresh complete medium and 0.1 mL DMSO for every 1 mL freezing medium.
- Sterilize freezing medium by means of appropriate micro filter (0.1 µm – 0.2 µm).

3.7 Freezing Cells

- Prepare fresh freezing medium and keep it on ice.
- Cells should have 80% - 90% confluency prior to freezing.
- Remove the complete medium.
- Wash cells once with 1x Trypsin/EDTA.
- Remove Trypsin/EDTA quickly and incubate cells for 3-5 min at room temperature.
- Detach cells, add complete medium and pipet up and down to break clumps of cells.
- Pellet cells with centrifuge and carefully aspirate off medium.
- Resuspend cells at a density of approximately 3.0 x 10^6 cells per mL with fresh freezing medium.
- Aliquot 0.5 mL of cell suspension into each cryovial.
- Overnight incubate cells in a styropor box at –80°C.
- The next morning transfer cryovial in liquid nitrogen tank for long-term storage.

3.8 Stability of HEK P2X cells

HEK P2X cells stably express functionally active P2X channels over 20 passages. Under recommended cell culture conditions no variation in current density was observed over 20 cell splitting cycles.

4 CONTACT INFORMATION

4.1 Contact Address for Technical Support & Ordering Information

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