

Pharmacology of P2X_{2/3} channels recorded on the SyncroPatch 384PE

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Summary

P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. P2X receptors are found on many cell types including smooth muscle cells, sensory neurones, epithelia, bone and leukocytes (for reviews see Refs 1 - 3). A role for P2X receptors has been suggested in transmission of thermal stimuli⁴, chemosensory signalling⁵, taste^{6,7} and pain⁸. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems (reviewed in Refs 1 - 3). Functional receptors are trimeric⁹, which can be homomeric or heteromeric. The P2X₂ and P2X₃ receptors can function either as homomers or as P2X_{2/3} heteromers. When expressed together, a mixture of P2X₂ and P2X₃ homomers as well as P2X_{2/3} heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. P2X_{2/3} receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs⁸.

Here we present data collected on the SyncroPatch 384PE showing activation and inhibition of P2X_{2/3} currents expressed in CHO cells with rapid and brief application of ligand ('Ligand Puff'). ATP activated P2X_{2/3} receptors in a concentration-dependent manner with an EC₅₀ value similar to those reported in the literature for a mixture of homomeric and heteromeric P2X_{2/3} receptors^{2,3,10,11}. P2X_{2/3} receptors could be repetitively activated by ATP and blocked by suramin with an IC₅₀ in good agreement with the literature (reviewed in refs 2 & 3).

Results

P2X_{2/3} expressed in CHO cells was activated by adding ATP using the Ligand Puff function in PatchControl 384. A single concentration of ATP was applied to each well, this was repeated 3 times and finally a maximum concentration (1 mM) ATP was added to each well. The currents were normalized to the maximum current in each well and the concentration response curve calculated across the whole plate. The data was fitted using a Hill equation revealing an EC₅₀ = 1.1 μM (191 wells), in excellent agreement with the literature^{2,3,10,11} (Figure 1). Only half the plate was used for this experiment giving a success rate for completed experiments of 99% (191 out of a possible 192 wells).

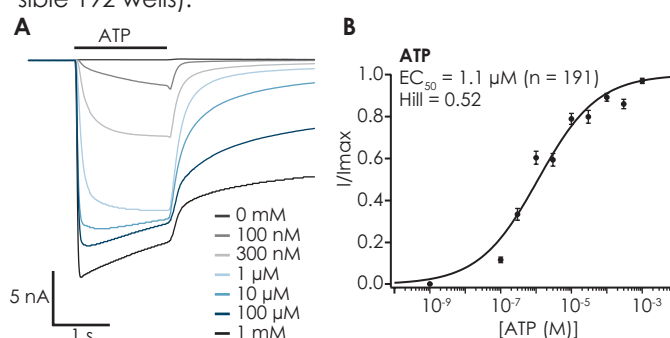


Figure 1: Activation of P2X_{2/3} expressed in CHO cells on the SyncroPatch 384PE by ATP. **A.** A single concentration of ATP was applied to each well and the average traces of all 191 cells are shown. The concentration response curve was calculated across the plate and is shown in Panel **B**. The data was normalized to the highest response for each well. The single concentration of ATP was applied 3 times followed by a maximum concentration of ATP (1 mM) for the normalization. A Hill equation was fit to the curve revealing an EC₅₀ of 1.1 μM (n = 191) in excellent agreement with the literature^{2,3,10,11}.

Application Note

Currents mediated by P2X_{2/3} were also activated by α,β -methylene ATP ($\alpha\beta$ meATP; Figure 2). Since P2X₂ homomeric receptors are insensitive to $\alpha\beta$ meATP¹⁻³, and the kinetics of desensitization are slow rather than rapid, which would be indicative of P2X₃ homomeric receptors¹, we can conclude that the majority to the receptors expressed in these cells are P2X_{2/3} heteromers.

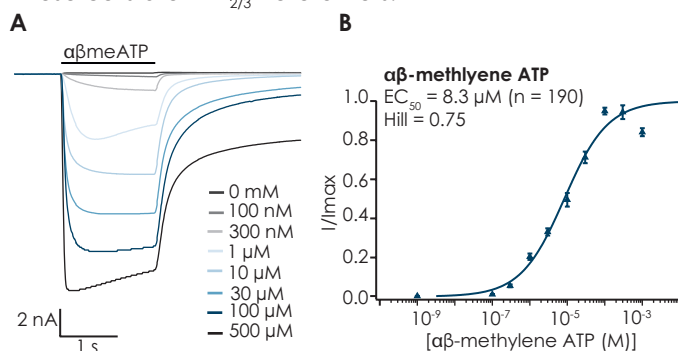


Figure 2: Activation of P2X_{2/3} expressed in CHO cells on the SyncroPatch 384PE by $\alpha\beta$ -methylene ATP. A. A single concentration of ATP was applied to each cell and the average traces of all 190 cells are shown. The concentration response curve was calculated across the plate and is shown in Panel B. The data was normalized to the highest response for each well. The single concentration of $\alpha\beta$ meATP was applied 3 times followed by a maximum concentration of $\alpha\beta$ meATP (500 μ M) for the normalization. A Hill equation was fit to the curve revealing an EC₅₀ of 8.3 μ M (n = 190) in good agreement with the literature¹⁻³.

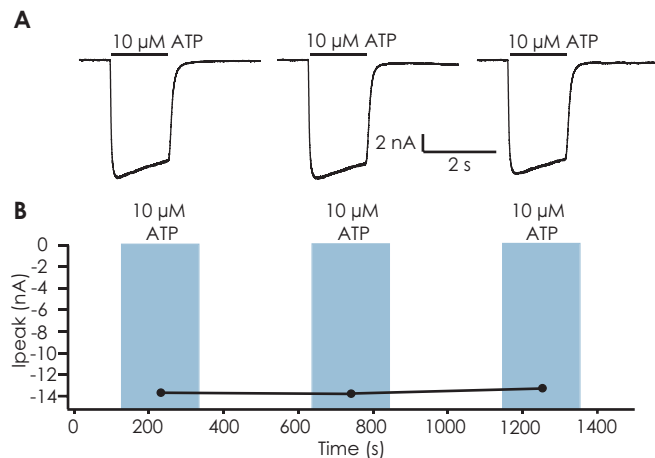


Figure 3: Stability of P2X_{2/3} recorded on the SyncroPatch 384PE. A. Raw current traces from an example cell showing current activation by application of 10 μ M ATP repeated 3 times in the same cell. B. Online analysis plot showing peak amplitude versus time for the same cell shown in A. The peak amplitudes were very similar with each application of ATP, differing by no more than 5%.

For pharmacology experiments, a stable and reproducible peak amplitude is critical (Figure 3). For each of the experiments, 3 control applications of ATP were made before incubation in the compound (suramin). The control applications of 10 μ M ATP showed a reproducible peak amplitude, where peak amplitude varied with each application of no more than 5%. This proves the suitability of the SyncroPatch 384PE for pharmacology experiments.

For pharmacology experiments, suramin was pre-incubated and then co-applied with ATP. Three concentrations of suramin were applied to each well allowing full concentration response curves for each well to be constructed (Figure 4). This gave an IC₅₀ = 17.7 $\pm$ 0.9 μ M (n = 372), in excellent agreement with the literature^{2,3}. The success rate for completed experiments was 97% and the cells were stable for over 30 minutes. A single point concentration response curve, where a single concentration of suramin was applied to each well and the concentration response curve constructed over the whole plate, was also performed. The IC₅₀ for suramin was similar (29 μ M, n = 342) using this approach compared with the cumulative concentration response approach.

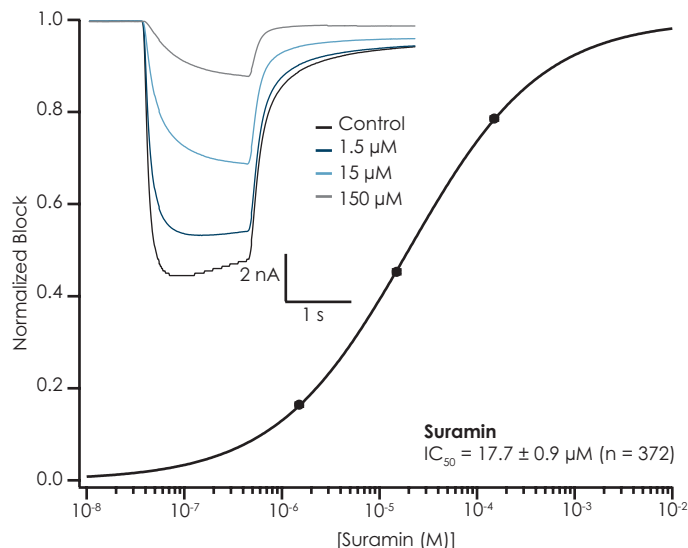
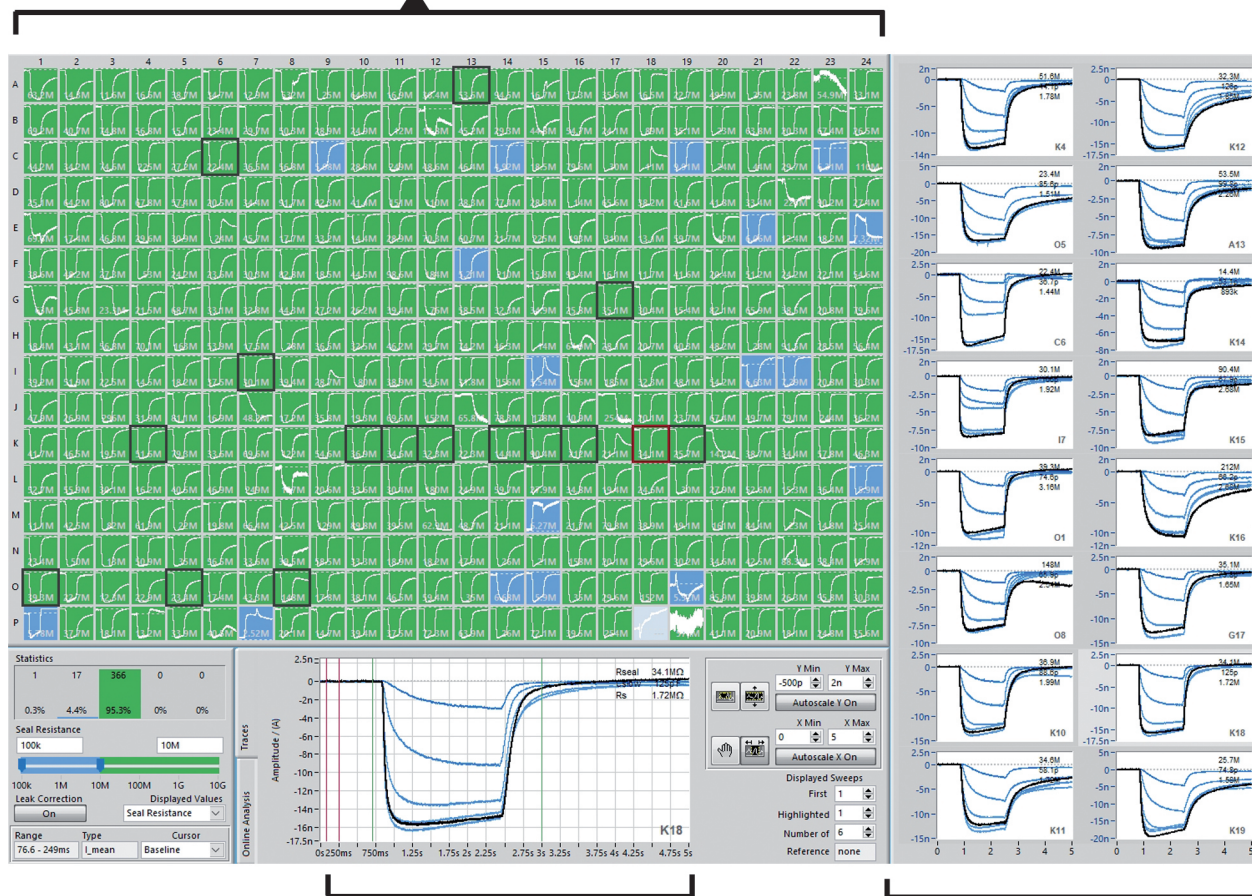


Figure 4: Inhibition of P2X_{2/3} by suramin. Suramin was pre-incubated at each concentration for 3 - 5 mins followed by co-application with 10 μ M ATP. All 3 concentrations of suramin were applied to each well and a cumulative concentration response curve was constructed for n = 372 wells out of a possible 384. Success rate was 97% for completed experiments.

Application Note

384 color coded depictions of data traces eases judgement of success rate



Highlighted raw traces of 1 cell showing effect of suramin on P2X_{2/3}-mediated currents

Highlighted raw traces of 16 cells showing block of P2X_{2/3} by suramin

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot depicts raw data traces of P2X_{2/3}-expressing CHO cells as recorded on one NPC-384 patch clamp chip. A cumulative concentration response curve for suramin was performed on each well. Three control applications of 10 μ M ATP were made followed by 3 increasing concentrations of suramin (1.5, 15 and 150 μ M) on each well. Suramin was pre-incubated 3 - 5 mins followed by co-application of ATP. Multi-hole chips were used where 4 holes were present per well. The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green ($R_{memb} > 10 M\Omega$) or blue ($R_{memb} < 10 M\Omega$). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show raw data traces of P2X_{2/3} channels following activation first by ATP followed by co-application with suramin.

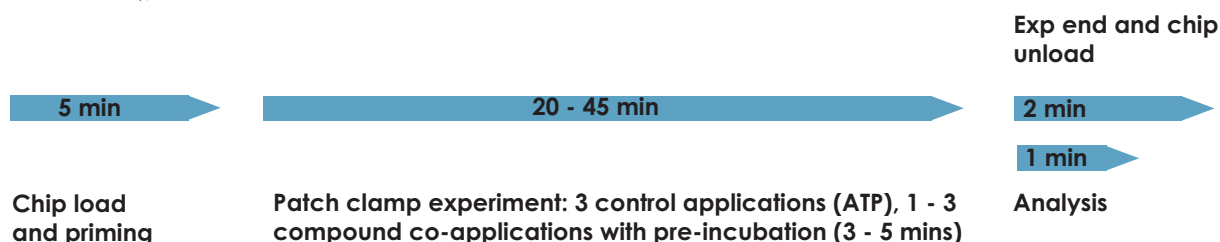


Figure 6: Timeline of an experiment on the SyncroPatch 384PE. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a 1 - 3 point concentration response curve on P2X_{2/3}-mediated currents took approximately 27 - 45 min.

Application Note

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, raw traces are chosen and the graphs show control responses to 10 μ M ATP followed by inhibition by co-application of suramin. The experiment shows that a full concentration response curve can be performed on each well, thus reducing the cost per data point. The cells were stable for over 30 minutes and compound could be pre-incubated for up to 5 minutes per concentration ensuring that full effect of the compound was reached. An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, P2X_{2/3} expressed in CHO cells can be reliably and reproducibly recorded on the SyncroPatch 384PE. The timeline of each experiment was approxi-

mately 30 - 45 minutes (start – end) and included 3 control applications of ATP followed by co-application with 1 - 3 concentrations of an inhibitor. The inhibitor (suramin) was pre-incubated for 3 - 5 mins and then co-applied with ATP. The P2X_{2/3}-mediated response was inhibited by suramin in a concentration-dependent manner as expected with an IC₅₀ within the range found in the literature^{2,3}. The EC₅₀ values for ATP and $\alpha\beta$ meATP and the kinetics of desensitization were consistent with P2X_{2/3} heteromers^{1-3,10,11}.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording P2X_{2/3} receptor-mediated currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each well and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

1. Ralevic & Burnstock., 1998. Pharmacol. Rev. 50: 413-492
2. North & Suprenant., 2000. Annu. Rev. Pharmacol. Toxicol. 40: 563-80
3. Khakh *et al.*, 2001. Pharmacol. Rev. 53: 107-118
4. Shimizu *et al.*, 2005. Pain. 116: 96-108
5. Prasad *et al.*, 2001. J. Physiol. 537.3: 667-677
6. Finger *et al.*, 2005. Science. 310: 1495-1499
7. Eddy *et al.*, 2009. Chem. Senses. 34: 789-797
8. Chizh & Illes., 2000. Pharmacol. Rev. 53(4): 553-568
9. Kawate *et al.*, 2011. J. Gen. Physiol. 137(6) 579–590
10. Ueno *et al.*, 1998. Br.J. Pharmacol. 124: 1484-1490
11. Liu *et al.*, 2001. JPET. 296: 1043-1050

Methods

Cells

CHO cells expressing P2X_{2/3} kindly provided by Axxam S.p.A., Milan; (<https://axxam.com/>).

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384PE using multi-hole (4 holes per well) chips. Cells were held at -80 mV for the duration of the experiment. For ATP concentration response curves, 3U/ml hexokinase was used with higher concentrations of ATP. For pharmacology experiments, cells were pre-incubated in suramin for 3 - 5 min and then compound was co-applied with 10 μ M ATP. P2X_{2/3} was activated using the 'Ligand Puff' command in PatchControl 384. Agonist volume: 3 μ M, wash volume: 7 μ M, application speed 40 μ l/s. Cells were then washed twice with external solution before re-application of agonist.