

## P2X<sub>2/3</sub> receptors recorded on Nanion's Patchliner

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by Evotec.

### Summary

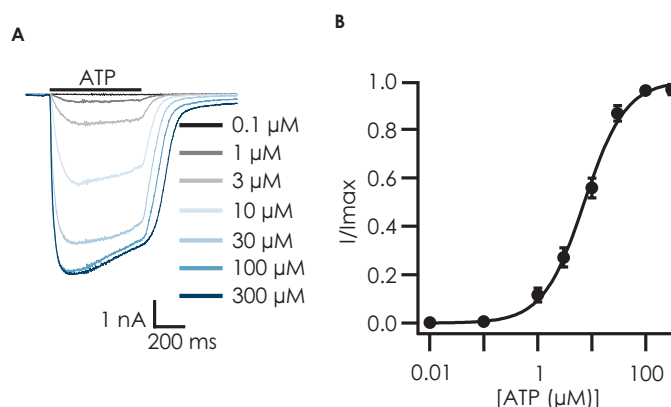
P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. P2X receptors are found on many cell types including smooth muscle cells, sensory neurones, epithelia, bone and leukocytes (for reviews see Refs 1 - 3). A role for P2X receptors has been suggested in transmission of thermal stimuli<sup>4</sup>, chemosensory signalling<sup>5</sup>, taste<sup>6,7</sup> and pain<sup>8</sup>. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems (reviewed in Refs 1 - 3). Functional receptors are trimeric<sup>9</sup>, which can be homomeric or heteromeric. The P2X<sub>2</sub> and P2X<sub>3</sub> receptors can function either as homomers or as P2X<sub>2/3</sub> heteromers. When expressed together, a mixture of P2X<sub>2</sub> and P2X<sub>3</sub> homomers as well as P2X<sub>2/3</sub> heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. P2X<sub>2/3</sub> receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs<sup>8</sup>.

Here we present data collected on a 4- or 8-channel Patchliner showing the potential use of the Patchliner to record P2X<sub>2/3</sub> currents activated by ATP. ATP activated P2X<sub>2/3</sub> receptors in a concentration-dependent manner with an EC<sub>50</sub> similar to those reported in the literature for a mixture of homomeric and heteromeric P2X<sub>2/3</sub> receptors<sup>2,3,10,11</sup>. The currents desensitized slowly confirming that they are mediated by P2X<sub>2</sub> and P2X<sub>2/3</sub> receptors rather than P2X<sub>3</sub> receptors. P2X<sub>2/3</sub> receptors could be repetitively activated by ATP and blocked by suramin with an IC<sub>50</sub> in good agreement with the literature (reviewed in refs 2 & 3).

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### Results

Current responses of an individual cell expressing P2X<sub>2/3</sub> receptors to increasing concentrations of ATP are shown in Figure 1. A concentration response curve revealed an EC<sub>50</sub> for ATP activation of  $7.8 \pm 1.0 \mu\text{M}$  ( $n = 10$ ), in good agreement with the literature for a mixture of P2X<sub>2</sub> homomers and P2X<sub>2/3</sub> heteromers<sup>2,3,10,11</sup>. The slow desensitization is also indicative of P2X<sub>2</sub> homomers and P2X<sub>2/3</sub> heteromers rather than P2X<sub>3</sub> homomers as P2X<sub>3</sub> receptors exhibit much faster desensitization with a slow recovery from desensitization<sup>2,3,10,11</sup>.

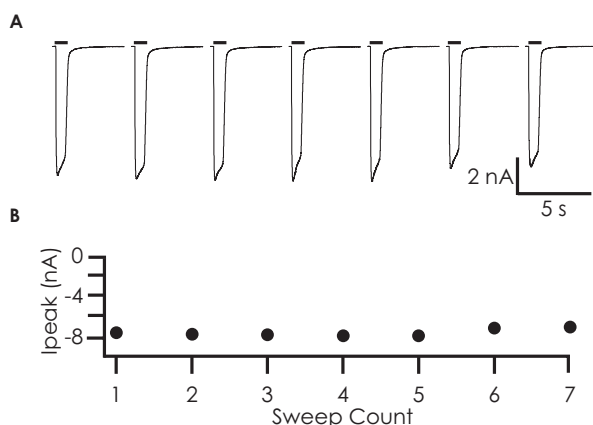


**Figure 1:**

**A** Activation of P2X<sub>2/3</sub> receptors by increasing concentrations of ATP. **B** Concentration response curve for ATP activation, EC<sub>50</sub> =  $7.8 \pm 1.0 \mu\text{M}$  ( $n = 10$ ).

# Application Note

Figure 2 shows the repetitive activation of P2X<sub>2/3</sub> receptors. Currents were activated with a similar peak amplitude when challenged 7 times with 30 μM ATP.



**Figure 2:** **A** P2X<sub>2/3</sub> receptors could be repetitively activated by 30 μM ATP. **B** Time-course of the experiment. P2X<sub>2/3</sub> currents of approx. 8 nA were activated 7 times reproducibly in the same cell.

A full concentration response curve to suramin was performed (Fig. 3). Suramin blocked the ATP activated current with an IC<sub>50</sub> of 28.0 ± 5.3 μM (n = 7) in good agreement with literature values (reviewed in refs 2 & 3).

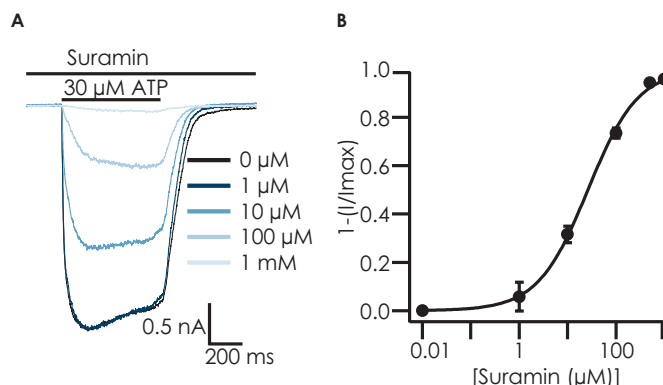
## References

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## Methods

### Cells

1321N1 cells stably expressing P2X<sub>2</sub>/P2X<sub>3</sub> subunits, kindly provided by Evotec, were used.



**Figure 3:** Block of P2X<sub>2/3</sub> receptors by suramin. Suramin at increasing concentrations (1 μM - 1 mM) was pre-incubated and then co-applied with 30 μM ATP. Full recovery from block was achieved upon washout (data not shown) **B** Concentration response curve for suramin block, IC<sub>50</sub> = 28.0 ± 5.3 μM (n = 7).

In summary, P2X<sub>2/3</sub> receptors stably expressed in 1321N1 cells can be reliably activated by ATP with EC<sub>50</sub> values and desensitization properties consistent with a mixture of P2X<sub>2</sub> homomers and P2X<sub>2/3</sub> heteromers. The ATP activated currents could also be blocked by suramin. The data shown here agrees well with published literature using conventional patch clamp electrophysiology to study P2X<sub>2/3</sub> receptors<sup>1-11</sup>. Therefore, the Patchliner provides a viable, higher throughput alternative to conventional patch clamp for the discovery of active P2X<sub>2/3</sub> lead compounds.

### 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 Patchliner. Cells were held at a holding potential of -70 mV. To achieve short exposure times, solutions were stacked in the robotic pipettor. First, wash solution (155 μl) was aspirated followed by aspiration of the agonist-containing solution (40 μl) and then applied to the cell at a speed of 57 μl/s. Wash solutions contained the antagonist in the suramin experiments. The cells were pre-incubated in suramin before co-application with 30 μM ATP.