Application Note

Channel: $P2X_{2/3}$
Cells: 1321N1
Tools: Patchliner®

$P2X_{2/3}$ 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[4], chemosensory signalling[5] and taste[6,7]. To date 7 P2X receptor genes have been cloned and studied in heterologous expression systems (reviewed in Refs 1 - 3). Functional receptors are oligomeric, usually containing 3 or 6 subunits, which can be homomeric or heteromeric. The $P2X_2$ and $P2X_3$ receptors can function either as homomers or as $P2X_2$/$P2X_3$ heteromers. When expressed together, a mixture of $P2X_2$ and $P2X_3$ homomers as well we $P2X_{2/3}$ heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties.

Here we present data collected on a 4- or 8-channel Patchliner® showing the potential use of the Patchliner® to record $P2X_{2/3}$ currents activated by ATP. ATP activated $P2X_{2/3}$ receptors in a concentration-dependent manner with an EC_{50} similar to those reported in the literature for a mixture of homomeric and heteromeric $P2X_2$ and $P2X_{2/3}$ receptors[2,3,8,9]. The currents desensitized slowly confirming that they are mediated by $P2X_2$/$P2X_{2/3}$ receptors rather than $P2X_3$ receptors which desensitize very fast and recover slowly from desensitization[2,3,8,9]. $P2X_2$/$P2X_{2/3}$ receptors could be repetitively activated by ATP and blocked by suramin with an IC_{50} in good agreement with the literature (reviewed in refs 2 & 3).

Results

Current responses of an individual cell expressing $P2X_{2/3}$ receptors to increasing concentrations of ATP are shown in Figure 1. A concentration response curve revealed an EC_{50} for ATP activation of 7.8 ± 1.0 µM (n = 10), in good agreement with the literature for a mixture of $P2X_2$ homomers and $P2X_{2/3}$ heteromers[2,3,8,9]. The slow desensitization is also indicative of $P2X_2$ homomers and $P2X_{2/3}$ heteromers rather than $P2X_3$ homomers as $P2X_3$ receptors which exhibit a much faster desensitization with a slow recovery from desensitization essentially ruling them out for repetitive stimulation[2,3,8,9].

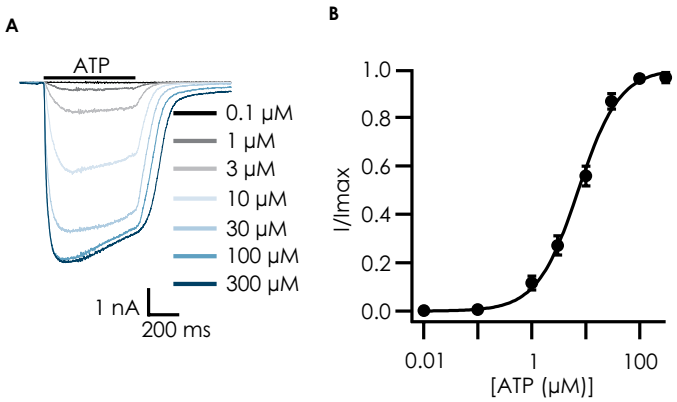

Figure 1:
A Activation of $P2X_{2/3}$ receptors by increasing concentrations of ATP. **B** Concentration response curve for ATP activation, EC_{50} = 7.8 ± 1.0 µM (n = 10).

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Figure 2 shows the repetitive activation of $P2X_{2/3}$ receptors. Currents were activated with a similar peak amplitude when challenged 7 times with 30 µM ATP.

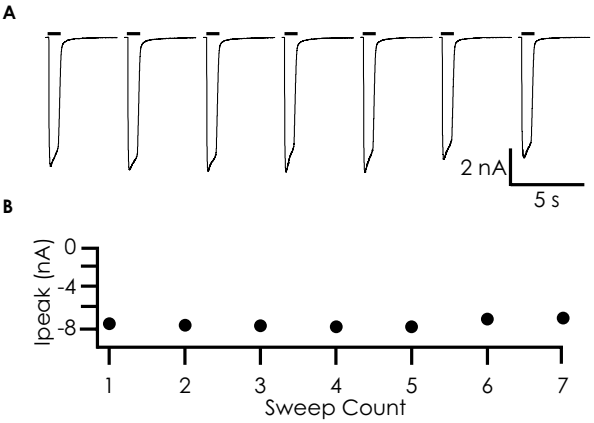

Figure 2:
A $P2X_{2/3}$ receptors could be repetitively activated by 30 µM ATP. **B** Time-course of the experiment. $P2X_{2/3}$ currents of approx. 8 nA were activated 7 times reproducibly in the same cell.

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

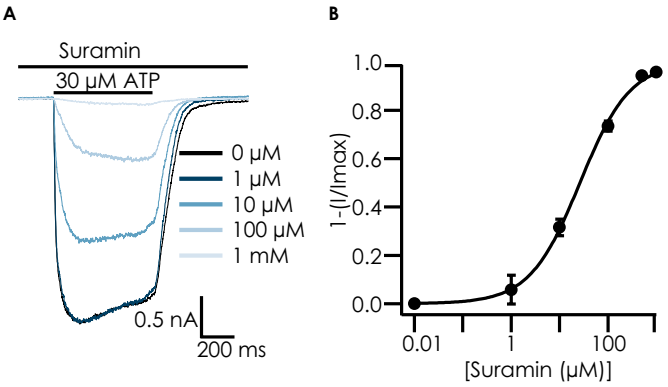

Figure 3:
Block of $P2X_{2/3}$ receptors by suramin. Suramin at increasing concentrations (1 µM - 1 mM) was pre-incubated and then co-applied with 30 µM ATP. Full recovery from block was achieved upon washout (data not shown) **B** Concentration response curve for suramin block, IC_{50} = 28.0 ± 5.3 µM (n = 7).

In summary, $P2X_{2/3}$ receptors stably expressed in 1321N1 cells can be reliably activated by ATP with EC_{50} values and desensitization properties consistent with a mixture of $P2X_2$ homomers and $P2X_{2/3}$ 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 $P2X_{2/3}$ receptors[1-9]. Therefore, the Patchliner® provides a viable, higher throughput alternative to conventional patch clamp for the discovery of active $P2X_{2/3}$ lead compounds.

References

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Methods

Cells

1321N1 cells stably expressing $P2X_2$/$P2X_3$ subunits were used.

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.

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