Application Note

Channel: $P2X_3$
Cells: CHO
Tools: Patchliner

Activation and inhibition of $P2X_3$ channels recorded on the Patchliner

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Cells engineered and kindly provided by Axxam S.p.A, Milan.

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], taste[6,7] and pain[8,9]. To date, 7 P2X receptor genes have been cloned and studied in heterologous expression systems (reviewed in Refs 1 - 3). Functional receptors are trimeric[10], which can be homomeric or heteromeric. The $P2X_2$ and $P2X_3$ receptors can function either as homomers or as $P2X_{2/3}$ heteromers. When expressed together, a mixture of $P2X_2$ and $P2X_3$ homomers as well as $P2X_{2/3}$ heteromers are likely to exist, which may be distinguished through their biophysical and pharmacological properties. Both $P2X_3$ homomers and $P2X_{2/3}$ heteromeric receptors have been implicated in nociception and pain signalling and may be important therapeutic targets for analgesic drugs[8]. The $P2X_3$ and $P2X_{2/3}$ receptor antagonist MK-7264 (gefapixant), has recently progressed to Phase III trials for refractory or unexplained chronic cough[11].

Here we present data collected on the Patchliner showing activation and inhibition of $P2X_3$ currents expressed in CHO cells with rapid and brief application of ligand (using the stacked solution approach). αβ-methylene ATP (αβ-MeATP) activated $P2X_3$ receptors in a concentration-dependent manner with an EC_{50} value similar to those in the literature[2,3,12]. $P2X_3$ receptors could be repetitively activated by αβ-MeATP and blocked by A-317491 with an IC_{50} value in good agreement with the literature[13].

Results

$P2X_3$ expressed in CHO cells was activated by adding αβ-MeATP using the stacked solutions addition function of the Patchliner. First αβ-MeATP was applied to the cell and this is quickly washed away by buffer solution also present in the pipette. Multiple applications of αβ-MeATP in increasing concentrations (1-300 µM) were made to each well and the concentration response curve (CRC) for an average of 12 cells is shown in Figure 1. The data was fitted using a Hill equation revealing an EC_{50} = 3.6 ± 0.3 µM (n = 12), for αβ-MeATP in good agreement with the literature[2,3,12] (Figure 1).

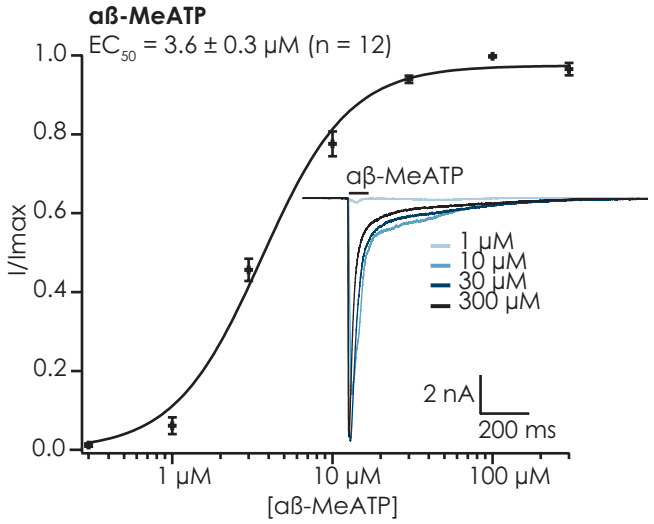

Figure 1: Activation of $P2X_3$ expressed in CHO cells on the Patchliner by αβ-MeATP. Cumulative concentrations of αβ-MeATP were added to each well (1-300 µM) and the data normalized to the highest concentration. The CRC for an average of 12 cells is shown here with the raw data traces from an exemplar cell shown in the inset. The average EC_{50} was 3.6 ± 0.3 µM (n = 12).

Application Note

For pharmacology experiments, a stable and reproducible peak amplitude is mandatory. We repetitively activated $P2X_3$ five times with 30 µM αβ-MeATP (Figure 2). Peak amplitude was highly reproducible, varying by no more than 12% from the first application, demonstrating the suitability of the Patchliner for pharmacology experiments.

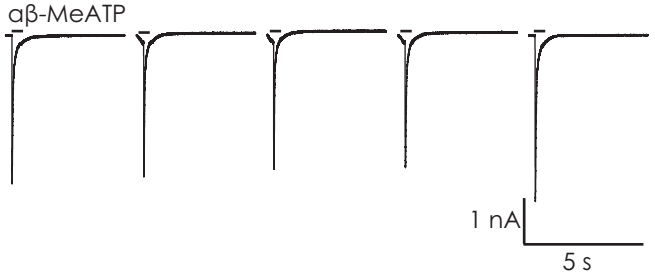

Figure 2: Stability of $P2X_3$ recorded on the Patchliner. Raw current traces from an example cell showing current activation by application of 30 µM αβ-MeATP repeated 5 times in the same well.

We used the $P2X_3$ selective inhibitor A-317491 to block $P2X_3$-mediated responses. αβ-MeATP was applied 3 times to check for stability followed by pre-incubation with A-317491 and then co-application of A-317491 with αβ-MeATP. Full CRCs were performed on each cell. The CRC for an average of 9 cells is shown in Figure 3. The IC_{50} for A317491 was 85.9 ± 20.7 nM (n = 9) in excellent agreement with the literature[13].

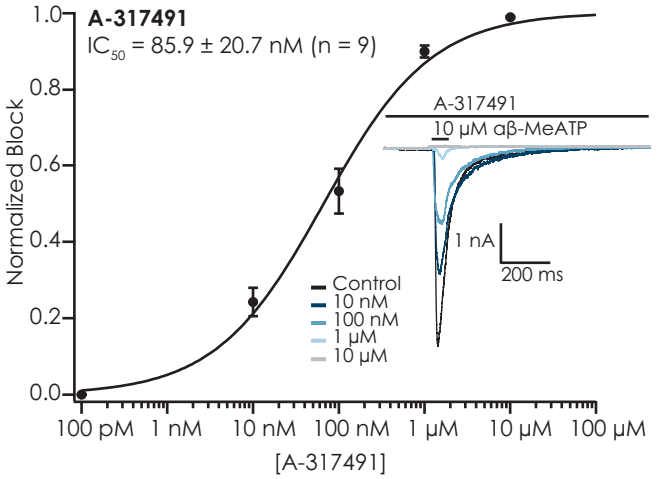

Figure 3: Inhibition of $P2X_3$-mediated currents by A-317491. A-317491 was pre-incubated for 3 - 5 mins followed by co-application with 10 µM αβ-MeATP. Full concentration response curves were performed on each cell and the average concentration response curve for 9 cells is shown. The average IC_{50} was 85.9 ± 20.7 nM (n = 9).

In conclusion, the Patchliner can be used to reliably record $P2X_3$-mediated responses with activation and inhibition pharmacology in good agreement with the literature. Given the putative role of $P2X_3$-containing receptors in inflammatory and neuropathic pain[9], the Patchliner could be used to identify $P2X_3$-specific inhibitors as potential novel pain therapeutics and to treat other conditions such as chronic cough.

References

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Methods

Cells

CHO cells expressing $P2X_3$ were engineered and 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 Patchliner. Cells were held at -70 mV for the duration of the experiment. For αβ-MeATP concentration response curves, 3U/ml hexokinase was used with all concentrations of αβ-MeATP. For pharmacology experiments, cells were pre-incubated in A-317491 for 2 min and then compound was co-applied with 10 µM αβ-MeATP. $P2X_3$ was activated using the stacked solutions approach. Agonist volume: 5 µl, wash volume: 150 µl, application speed 114 µl/s. Cells were then washed twice with external solution before re-application of agonist.

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