SOPHION BIOSCIENCE

Application Report

Characterization of $P2X_3$ receptors using QPatch

Fast ionotropic receptors (LGICs) can be investigated efficiently on QPatch. The $P2X_3$ receptor whole-cell currents were analyzed and the effects of agonist and antagonist were characterized. The EC_{50} and IC_{50} values compared well with literature values

Summary

Ligand-gated ion channels on QPatch and here $P2X_3$ receptor whole-cell current recordings were made with specific focus on:

- Effects of agonists
- Effects of antagonists
- Activation/desensitization
- Concentration-response relationships
- Determination of EC_{50} and IC_{50}
- Perforated patch clamp using amphotericin B

Introduction

P2X receptors are ligand-gated ion channels (LGIC's) with time constants of 10-200 ms for activation and 100-200 ms for desensitization (1). The fast kinetics presents a challenge for patch clamp recordings of physiologically realistic P2X receptor currents, and a fast solution exchange technique is required. This report presents a pharmacological characterization of one of the fastest P2X receptors, the $P2X_3$ receptor, based on perforated patch whole-cell currents from a rat lung epithelial (RLE) cell line stably expressing the human $P2X_3$ receptor. All measurements were recorded with using QPatch.

The $P2X_3$ receptor was targeted with

- Agonists: CTP (cytidine triphosphate) αβ-me-ATP (α,β-methylene adenosine triphosphate)
- Antagonist: TNP-ATP (trinitrophenyl adenosine triphosphate)

Results and discussion

Effects of agonists

Exposure to agonists (CTP or αβ-me-ATP) led to typical transient $P2X_3$ receptor currents characterized by a fast activation and a slower subsequent desensitization (Figure 1). The QPatch current recordings are similar to current recordings obtained with manual patch clamp (inset in Figure 1).

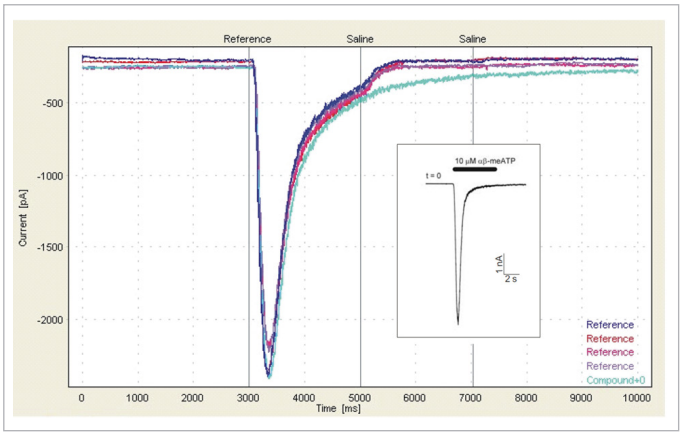

Fig. 1: Whole-cell $P2X_3$ receptor current responses to four successive exposures to 20 µM CTP. Between each agonist application, the cell was washed with saline. The responses were highly reproducible. The peak current was identified in the cursor range between 3000 and 5000 ms. Inset shows a corresponding current recording obtained with manual patch clamp.

Subsequent to each agonist application, a wash (15 µL extracellular Ringer's solution) was performed. The current baseline was stable throughout the experiment period indicating that the agonist was completely washed out between the individual applications.

Concentration-response analyses were based on whole-cell current responses to four agonist concentrations (Figure 2). Three effects of increasing agonist concentrations were demonstrated:

1. Increased amplitudes of the whole-cell $P2X_3$ receptor current

2. Reduced time constants for activation and, in particular desensitization

3. Increased degree of desensitization

The concentration-response relationship for the αβ-me-ATP effect on the peak current amplitudes is shown in Figure 3. The EC_{50} was 1.48 µM. This compares well to literature values (~1 µM, 2). The time to develop peak current was reduced with increasing agonist concentrations. In Figure 2 the peak current was obtained at 400, 370, 210 and 130 ms after ligand application at 500 nM, 1 µM, 2 µM and 10 µM αβ-me-ATP, respectively.

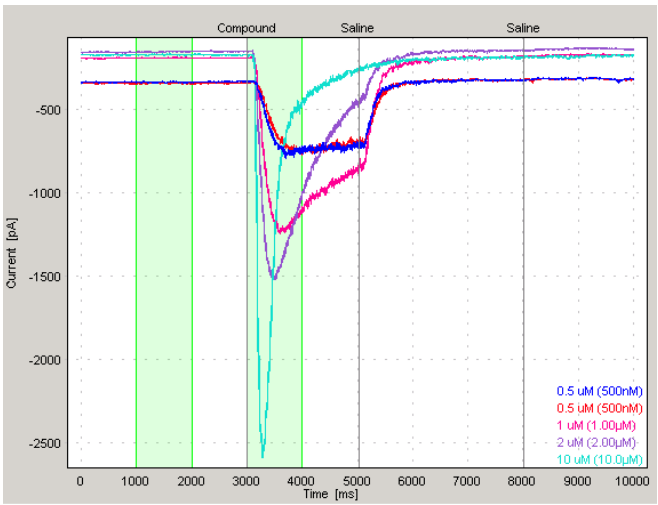

Fig. 2: Effects of four increasing concentrations of agonist (αβ-me-ATP) on whole-cell $P2X_3$ receptor currents. Cursors at 3000 and 4000 ms define the time interval in which peak current was determined. Cursors at 1000 and 2000 ms define the interval in which the leak current was measured.

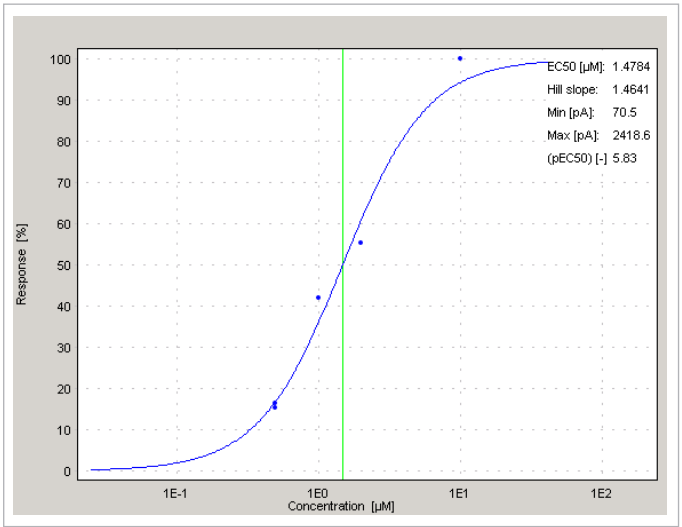

Fig. 3: Concentration-response relationship for the effect of αβ-me-ATP on peak $P2X_3$ receptor currents based on the data shown in Figure 2. Current values were corrected for leak.

Effects of antagonists

The effects of the competitive antagonist TNP-ATP were investigated using four antagonist concentrations (Figure 4). The agonist employed was CTP (20 µM). A marked concentration-dependent reduction of the peak $P2X_3$ receptor current amplitude was observed. Time constants for activation/desensitization were not significantly affected.

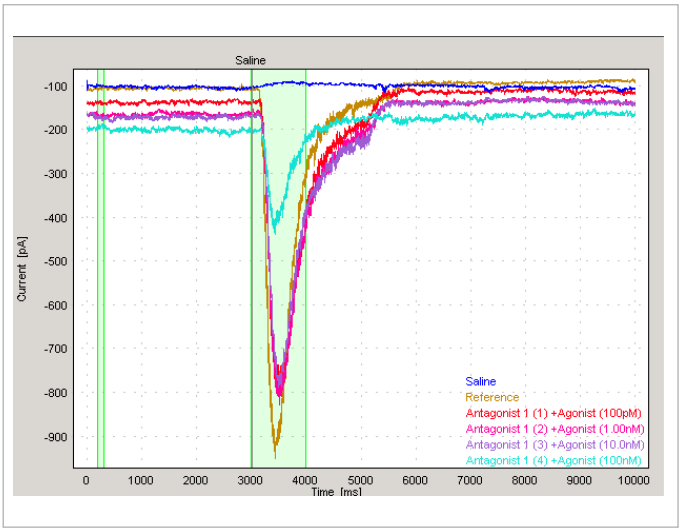

Fig. 4: Effects of the antagonist TNP-ATP on whole-cell $P2X_3$ receptor currents. First, the currents were recorded in response to pure saline. Next, recordings were done in response to the agonist (CTP) only ('reference'). Finally, the four increasing concentrations of TNP-ATP were applied. Concentrations in parentheses are antagonist concentrations.

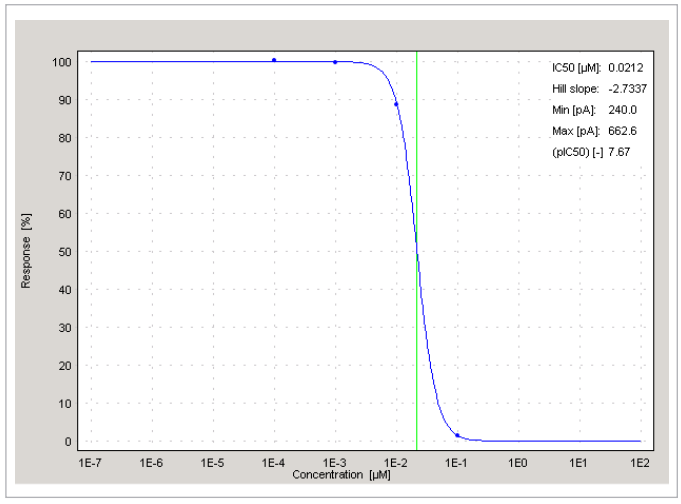

Fig. 5: Concentration-response relationship for the inhibitory effect of TPN-ATP on peak $P2X_3$ receptor currents based on the results shown in Figure 4. Current values were corrected for leak currents.

The TNP-ATP concentration-response relationship for peak $P2X_3$ receptor whole-cell currents was determined using the QPatch Assay Software (Figure 5). The IC_{50} was 21 nM which compares to literature values (~1 nM, 2).

Conclusions

This report demonstrates that fast ionotropic receptors, i.e. LGICs with fast activation/desensitization kinetics that require a rapid solution exchange system, can be investigated efficiently with QPatch technology. Thus, $P2X_3$ receptor whole-cell currents were recorded and analyzed with QPatch, and the effects of two agonists and one antagonist were characterized. The EC_{50} and IC_{50} values obtained compared well with values published in the literature. Also, the effect of compounds on activation/desensitization time constants and the extent of desensitization was determined using QPatch technology.

Methods

Cells: RLE cells expressing $P2X_3$ were grown in a cell culturing medium consisting of Williams' Medium E (supplemented with GlutaMAX), 10% fetal bovine serum (heat-inactivated) and geneticin G-418. The cells were harvested in HEK SFM medium.

Agonists and antagonists were applied in 5 µL aliquots. $P2X_3$ whole-cell currents were measured in perforated patch configuration. Patch perforation was accomplished using amphotericin B.

Compounds: CTP was applied in concentrations from 0.5 to 200 µM. αβ-me-ATP was applied in concentrations from 0.5 to 10 µM. TNP-ATP was applied in concentrations from 10 pM to 100 nM. The agonists were in most cases applied for 2000 ms. The antagonist was applied 120 s prior to activation by the agonist.

References:

1. Conley, EC (1996). The ion channel facts book. Extracellular ligand-gated channels. Academic Press.
2. 2. North RA (2002). Molecular Physiology of P2X Receptors. Physiol.Rev. 82:1013-1067.

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