SOPHION BIOSCIENCE

Application Report

CHO TREx-P2X$_3$ tested on QPatch

The EC_{50} values determined were close to literature values

Summary

CHO-P2X$_3$ cells were used on QPatch and gigaseals and the whole cell configuration were obtained. The EC_{50} values determined by the QPatch Analyzer Software were close to literature values (for Alpha-beta methyl ATP and CTP) and the IC_{50} value for PPADS was within the expected value.

- CHO-P2X$_3$ cells sealed well
- Gigaseal and the whole-cell configuration were obtained
- The EC_{50} values were in line with literature values (for Alpha-beta methyl ATP and CTP)
- The IC_{50} value for PPADS was within the expected value

Introduction

In this report the applications of the CHO TREx-P2X$_3$ cell line (hereafter called CHO P2X$_3$) are described. The purpose was to determine the EC_{50} value for αβ-methyl ATP, and the IC_{50} value of PPADS on the P2X$_3$ receptors. All data presented was obtained with QPatch and analyzed with the QPatch assay software.

Results and discussion

Determination of minimum cycle duration and number of washes

Initial experiments were done to determine the agonist application interval and number of washes to obtain reproducible responses after agonist application (αβ-methyl ATP 1 µM). It was observed that recovery from desensitization was obtained using 6 washes in combination with 360s agonist application interval. (The latter is in QPatch Assay Software terms denoted as minimum cycle duration). Typical current sweeps are shown in Figure 1.

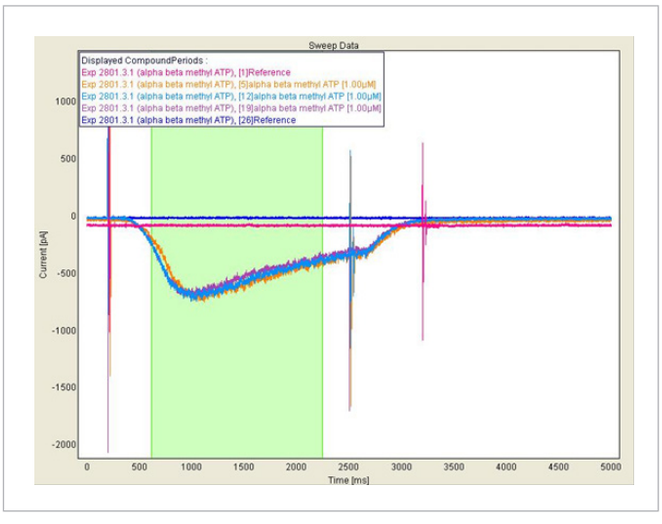

Fig. 1: Recovery from desensitization of P2X$_3$ receptors from the agonist αβ-methyl ATP (1 µM applied three times) obtained with cycle duration of 360s and 6 wash periods. The green bar represents the position of the user-defined cursor for current amplitude measurements.

The peak current was determined for each liquid application from the user-defined cursor and the resulting IT plot is shown in Figure 2.

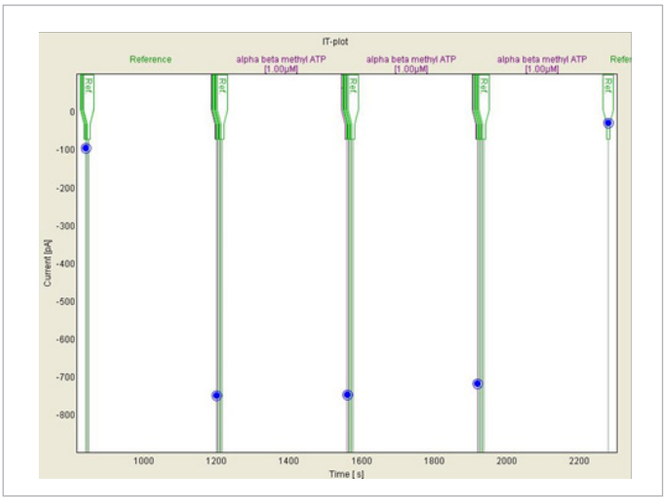

Fig. 2: IT plot from experiment with three repetitive agonist (αβ-methyl ATP 1 µM) applications with minimum cycle duration of 360s and 6 washes after each application. Note that throughout the experiment an EC ringer with glucose and hexokinase was used.

EC_{50} determination of αβ-methyl ATP for $P2X_3$ receptors

αβ-methyl ATP dose-response experiments were done with a minimum cycle duration of 360s and 6 washes after each application. Currents from a typical dose-response experiment are shown in Figure 3.

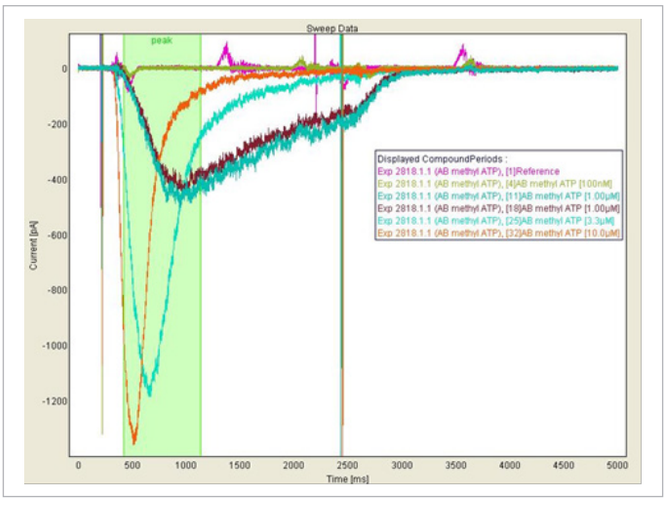

Fig. 3: $P2X_3$ current sweeps from αβ-methyl ATP dose-response experiment. The green bar represents the position of the user defined cursor for current amplitude measurements.

The corresponding Hill fit for the agonist is shown in Figure 4, where the EC_{50} value was determined to 1.4 µM (mean 1.2 µM ± 0.3, n=27).

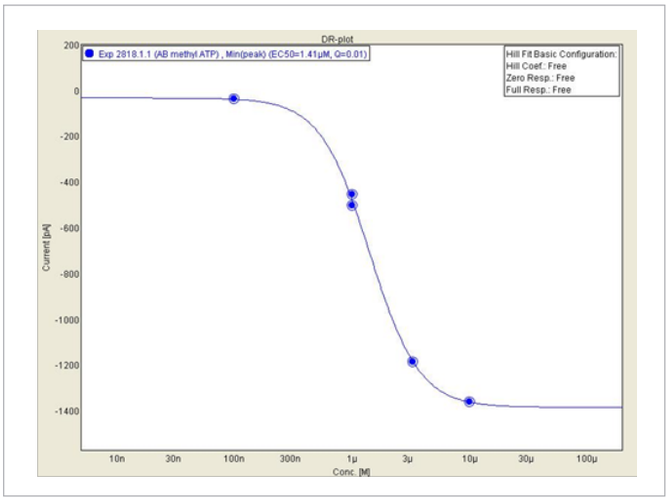

Fig. 4: Hill fit and EC_{50} determination of αβ-methyl ATP for $P2X_3$ receptors. 1 µM agonist was added twice to show the reproducibility of the data.

IC_{50} determination of PPADS for $P2X_3$ receptors

For IC_{50} determination of the antagonist PPADS for $P2X_3$ receptors, experiments were done with a minimum time between applications of 360s and 6 washes after each application. The PPADS compounds at concentrations of 0.1, 1, 3, 10 and 30 µM were applied sequentially using αβ-methyl ATP (3.3 µM) as the agonist. PPADS was preincubated for 360s prior to adding the PPADS and the αβ-methyl ATP agonist. Typical $P2X_3$ currents from a dose-response experiment are shown in Figure 5.

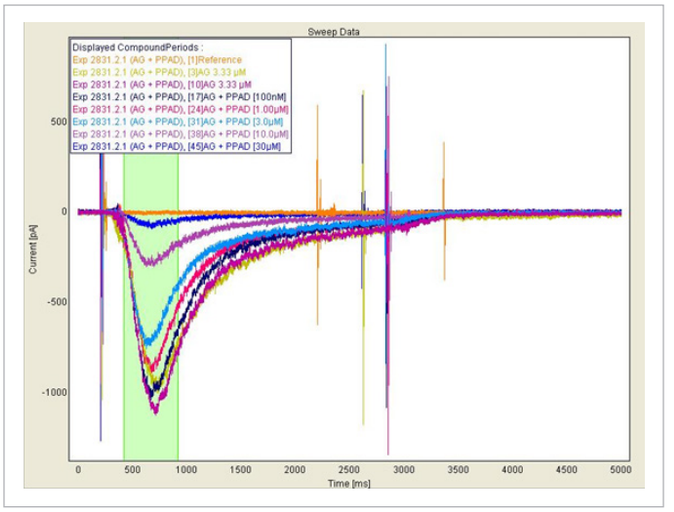

Fig. 5: $P2X_3$ current sweeps from PPADS dose-response experiment. In this experiment the agonist (αβ-methyl ATP) concentration was 3.3 µM. The green bar represents the position of the user defined cursor for current amplitude measurements.

The corresponding Hill fit for the antagonist is shown in Figure 6, where the IC_{50} value was determined to 5.7 µM (mean 5.5 µM ± 1.8, n=17 @ 3.3µM αβ-methyl ATP).

Similar results were determined using the agonist, αβ-methyl ATP at a concentration of 1 µM. In these experiments the IC_{50} value for PPADS was determined to 6.0 µM ± 1.8, n=19. There was no significant difference regarding the IC_{50} value obtained for PPADS with respect to the concentration of the agonist – Figure 7. This confirms that PPADS does not act as a competitive inhibitor as it is also described in the literature (1).

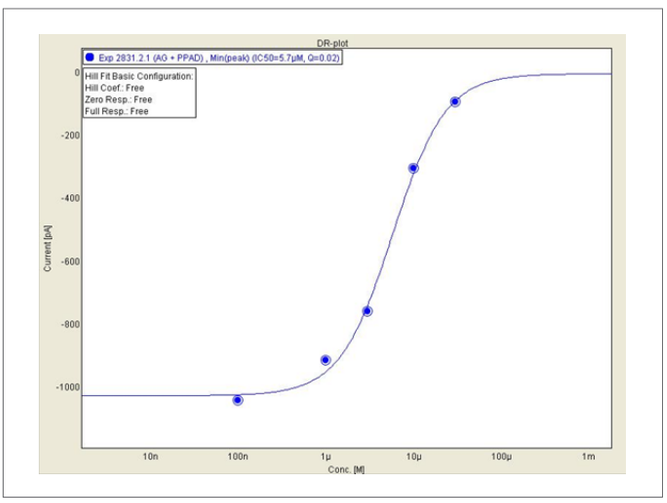

Fig. 6: Hill fit and IC_{50} determination PPADS for $P2X_3$ receptors.

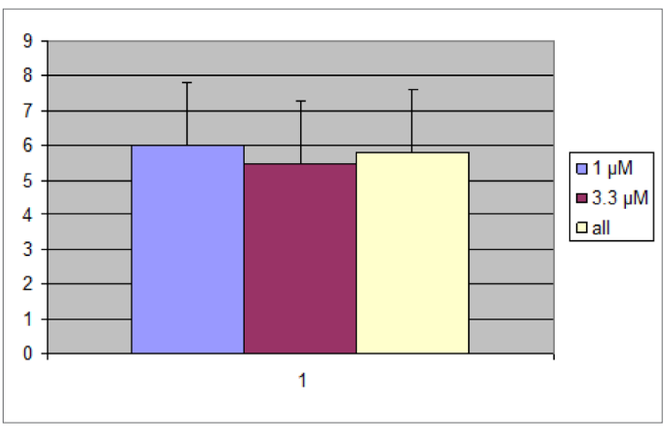

Fig. 7: Summary of the IC_{50} values for PPADS on $P2X_3$ receptors obtained with different agonist, αβ-methyl ATP, concentrations. The yellow column represents the cumulated data. The mean IC_{50} value for the summarized data was 5.8 µM ± 1.8, n=36, obtained using 4 QPlates.

EC_{50} determination of CTP for $P2X_3$ receptors

CTP dose-response experiments were done with a minimum cycle duration of 180s and 6 washes after each application. Currents from a typical dose-response experiment are shown in Figure 8.

The corresponding Hill fit for the agonist is shown in Figure 9, where the IC_{50} value was determined to 13.0 µM (mean 11.1 µM ± 3.6, n=9).

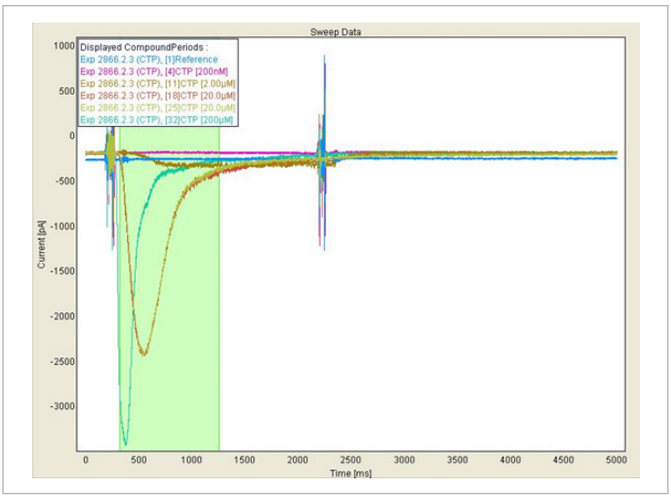

Fig. 8: $P2X_3$ current sweeps from CTP dose-response experiment. The green bar represents the position of the user defined cursor for current amplitude measurements.

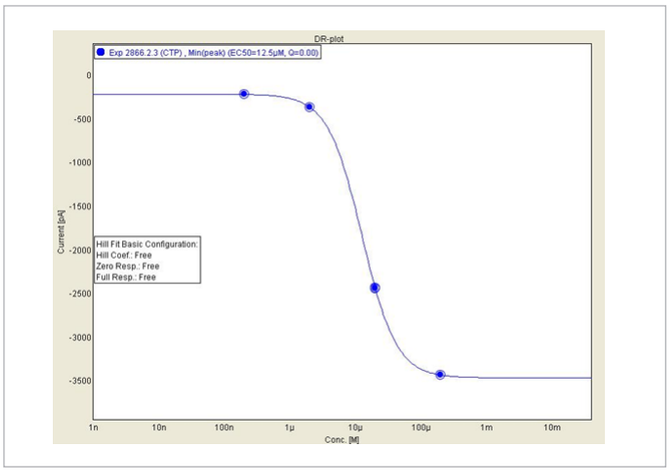

Fig. 9: Hill fit and EC_{50} determination CTP for $P2X_3$ receptors.

Table 1: EC_{50} and IC_{50} obtained on $P2X_3$ receptors.

	EC_{50} (µM)	IC_{50} (µM)	Literature values (µM)
αβ-methyl ATP	1.2 ± 0.3		2.4
CTP	11.1 ± 3.6		17.3
PPADS		5.8 ± 1.8	2.5

QPlate overview

It was observed that high seal resistances in the giga seal mode was easily obtained with CHO $P2X_3$ cells on QPatch, and the membrane resistance was tight also after the whole cell configuration was established. The experiments lasted typically 45 minutes during which the currents were recorded with minimal leak development. Figure 10 shows QPlate statistics from a typical experiment.

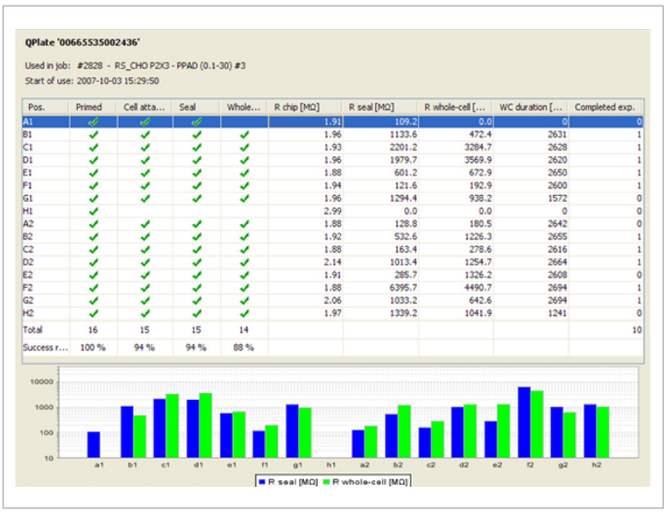

Fig. 10: QPlate statistics from a typical experiment with CHO $P2X_3$ cells. The table lists success or failure for each QPlate position (A1 to H2), priming of the measurement site, cell attaching, formation of seal (R_{seal} > seal criterion), and formation of whole-cell configurations. Overall success rates are listed in the lower line of the table. In addition, the measured electrical resistance at each measurement site (R_{chip}), the cell-attached configuration resistance (R_{seal}), the whole-cell resistance ($R_{whole\text{-}cell}$), the whole-cell duration, and the number of completed experiments are listed.

Conclusion

The CHO-$P2X_3$ cells sealed well on the QPatch; gigaseals and the whole-cell configuration were easily obtained, and the average number of completed dose-response experiments pr. QPlate was 10.5 ± 2.9, n=11 QPlates.

The EC_{50} values determined by the QPatch assay software were close to literature values (for Alpha-beta methyl ATP and CTP).

The IC_{50} value for PPADS was within the expected value.

Methods

Cell culture

CHO TREx-$P2X_3$ cells were grown according to the Sophion SOP.

Hexokinase

Hexokinase catalyzes the phosphorylation of D-hexose sugars at the C6 position utilizing ATP as a phosphate source. Having hexokinase in the saline buffer reduce the amount of ATP leaked from cells in order not to have this natural agonist in the cell suspension.

Ringer's solution

Two EC Ringer's were prepared and the ringer used for cell preparation contained glucose, but not hexokinase, as the cells sealed better in a ringer without hexokinase. The "reference Ringer", which was used for wash periods contained glucose and hexokinase.

References:

1. Li C (2000). Novel mechanism of inhibition by the P2 receptor antagonist PPADS of ATP-activated current in dorsal root ganglion neurons. J Neurophysiol 86(5); 2533-41
2. Pratt EB (2005). Use-dependent inhibition of P2X3 receptors by nanomolar agonist. J Neurosci 25(32); 7359-7365

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