**Summary**

CHO-P2X₃ cells were used on QPatch and gigaseals and the whole cell configuration were obtained. The EC₅₀ values determined by the QPatch Analyzer Software were close to literature values (for Alpha-beta methyl ATP and CTP) and the IC₅₀ value for PPADS was within the expected value.

- CHO-P2X₃ cells sealed well
- Gigaseal and the whole-cell configuration were obtained
- The EC₅₀ values were in line with literature values (for Alpha-beta methyl ATP and CTP)
- The IC₅₀ value for PPADS was within the expected value

**Introduction**

In this report the applications of the CHO TREx-P2X₃ cell line (hereafter called CHO P2X₃) are described. The purpose was to determine the EC₅₀ value for αβ-methyl ATP, and the IC₅₀ value of PPADS on the P2X₃ 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 P2X₃ 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.](image-url)
**EC₅₀ determination of αβ-methyl ATP for P₂X₃ 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.

The corresponding Hill fit for the agonist is shown in Figure 4, where the EC₅₀ value was determined to 1.4 µM (mean 1.2 µM ± 0.3, n=27).

**IC₅₀ determination of PPADS for P₂X₃ receptors**

For IC₅₀ determination of the antagonist PPADS for P₂X₃ 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 P₂X₃ currents from a dose-response experiment are shown in Figure 5.
The corresponding Hill fit for the antagonist is shown in Figure 6, where the IC\textsubscript{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\textsubscript{50} value for PPADS was determined to 6.0 µM ± 1.8, n=19. There was no significant difference regarding the IC\textsubscript{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).

**EC\textsubscript{50} determination of CTP for P2X\textsubscript{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\textsubscript{50} value was determined to 13.0 µM (mean 11.1 µM ± 3.6, n=9).

![Fig. 6: Hill fit and IC\textsubscript{50} determination PPADS for P2X\textsubscript{3} receptors.](image1)

![Fig. 7: Summary of the IC\textsubscript{50} values for PPADS on P2X\textsubscript{3} receptors obtained with different agonist, αβ-methyl ATP, concentrations. The yellow column represents the cumulated data. The mean IC\textsubscript{50} value for the summarized data was 5.8 µM ± 1.8, n=36, obtained using 4 QPlates.](image2)

![Fig. 8: P2X\textsubscript{3} current sweeps from CTP dose-response experiment. The green bar represents the position of the user defined cursor for current amplitude measurements.](image3)

![Fig. 9: Hill fit and EC\textsubscript{50} determination CTP for P2X\textsubscript{3} receptors.](image4)

**Table 1: EC\textsubscript{50} and IC\textsubscript{50} obtained on P2X\textsubscript{3} receptors.**

<table>
<thead>
<tr>
<th></th>
<th>EC\textsubscript{50} (µM)</th>
<th>IC\textsubscript{50} (µM)</th>
<th>Literature values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>αβ-methyl ATP</td>
<td>1.2 ± 0.3</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>CTP</td>
<td>11.1 ± 3.6</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>PPADS</td>
<td>5.8 ± 1.8</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>
**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.

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**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.

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**References:**