Pharmacology of $P2X_3$ and $P2X_{2/3}$ receptors in cell lines and hiPSC-derived neurons: An automated patch clamp study

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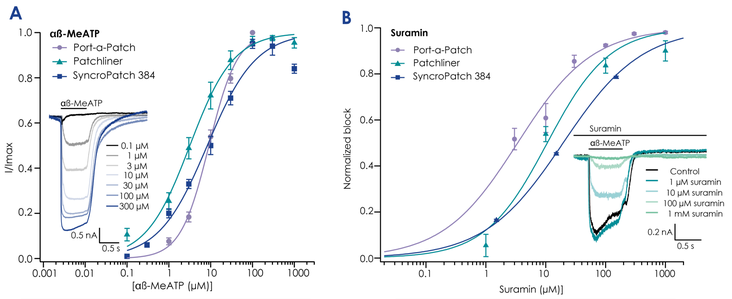
1 Introduction

P2X receptors are ligand-gated ion channels activated by extracellular ATP. They are permeable to small monovalent cations, some having significant divalent or anion permeability. The $P2X_2$ and $P2X_3$ subunits are predominantly expressed in primary sensory neurons and have been proposed to play a role in thermal sensation, taste and pain. They form functional hetero- or homotrimers which are activated by ATP or αβ-methylene ATP (αβ-MeATP). The stoichiometry of $P2X_{2/3}$ heteromers appears to be dependent on the relative abundance of the two subunits. A mixture of $P2X_2$ and $P2X_3$ homomers as well as $P2X_{2/3}$ heteromers are likely to exist, which can be distinguished through their biophysical and pharmacological properties. The receptors open in response to an increase in extracellular ATP which occurs under pathological conditions such as tissue damage. The resulting depolarization leads to propagation of the pain signal. Due to its role in nociception and pain signaling, these receptors are considered to be important targets for pain management.

Here we present data collected on three different automated patch clamp (APC) systems at two different sites showing activation and inhibition of $P2X_{2/3}$ and $P2X_3$ receptors expressed in CHO cells with rapid and brief application of ligand and pre-incubation of inhibitors. In addition, we show P2X-mediated current responses from hiPSC-derived nociceptors (RealDRG™).

2 Cross-platform comparison of pharmacology of $P2X_{2/3}$ and $P2X_3$ channels



Instrument	αβ-MeATP EC_{50} (µM)
Port-a-Patch	9.5 ± 0.5 (n = 7)
Patchliner	3.7 ± 0.9 (n = 6)
SyncroPatch 384	8.3 (n = 190)

Table 1: EC_{50} values for αβ-MeATP ($P2X_{2/3}$).

Instrument	Suramin IC_{50} (µM)
Port-a-Patch	4.7 ± 1.1 (n = 14)
Patchliner	8.7 ± 1.3 (n = 6)
SyncroPatch 384	17.7 ± 0.9 (n = 372)

Table 2: IC_{50} values for suramin ($P2X_{2/3}$).

Instrument	αβ-MeATP EC_{50} (µM)	A-317491 IC_{50} (nM)
Patchliner	1.7 ± 0.4 (n = 5)	85.9 ± 20.7 (n = 9)
SyncroPatch 384	1.9 (n = 373)	92 (n = 336)

Table 3: EC_{50} values for αβ-MeATP and IC_{50} values for A-317491 ($P2X_3$).

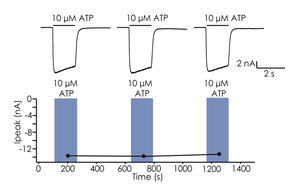


Figure 1: A. Concentration response curves (CRCs) for activation of $P2X_{2/3}$ expressed in CHO cells on the Port-a-Patch, Patchliner and SyncroPatch 384 by αβ-methylene ATP (αβ-MeATP) shown overlaid with example traces for $P2X_{2/3}$ recorded on the Patchliner shown in the inset. The EC_{50}s for αβ-MeATP (**Table 1**) were in good agreement with the literature[1-4]. αβ-MeATP activates $P2X_3$ containing receptors thus activating $P2X_3$ homomeric and $P2X_{2/3}$ heteromeric receptors and not $P2X_2$ homomers[2-4]. Given the slow desensitization of the recordings, activation by αβ-MeATP is consistent with $P2X_{2/3}$ heteromers and not $P2X_3$ homomers (see Ref. 4 and data in **C**). **B.** Inhibition of $P2X_{2/3}$ by suramin. $P2X_{2/3}$ was activated by 30 µM αβ-MeATP (Port-a-Patch and Patchliner) or 10 µM ATP (SyncroPatch 384) and the cells were pre-incubated in suramin for 1 – 3 min prior to co-application with ligand. The IC_{50} values (**Table 2**) were all in good agreement with the literature[2-4]. **C.** Concentration response curves for activation of $P2X_3$ by αβ-MeATP for the Patchliner and SyncroPatch 384 are shown overlaid. The EC_{50} values (**Table 3**) showed little difference between the 2 devices and are in good agreement with the literature[2-4,6]. **D.** $P2X_3$ was inhibited by A-317491 (a $P2X_3$-selective compound) on both the Patchliner and SyncroPatch 384 when activated by 10 µM αβ-MeATP with similar IC_{50} values (**Table 3**) and in good agreement with the literature[5]. Example traces from the Patchliner are shown in the inset. **E.** Stability of $P2X_{2/3}$ recorded on the SyncroPatch 384. Raw traces showing current activation by 10 µM ATP repeated 3 times in the same cell (**upper panel**). Online analysis plot (**bottom panel**) shows stable peak amplitudes versus time, differing by no more than 5% when ATP was applied.

3 Reproducibility and effect of temperature on $P2X_3$ currents

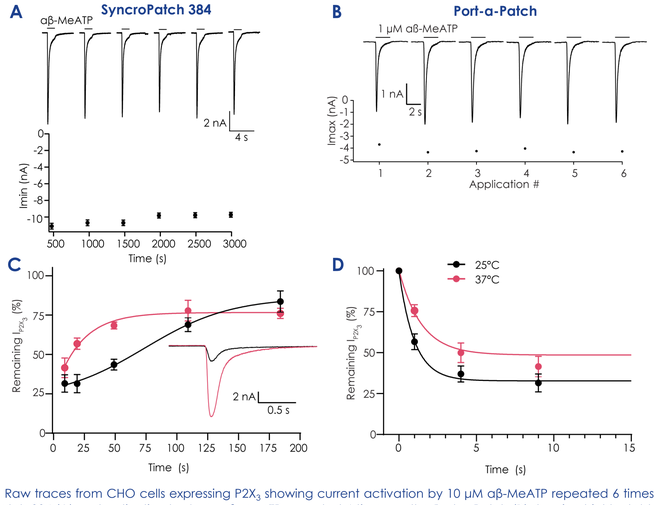


Figure 2: A & B. Raw traces from CHO cells expressing $P2X_3$ showing current activation by 10 µM αβ-MeATP repeated 6 times in the same cell on the SyncroPatch 384 (**A**) and activation by 1 µM αβ-MeATP repeated 6 times on the Port-a-Patch (**B**) showing highly stable responses. $P2X_3$ currents activated by 1 µM αβ-MeATP recorded on the Port-a-Patch were larger in amplitude when heated to physiological temperature compared with room temperature (**C**, **inset**). Heating to physiological temperature resulted in a faster recovery of $P2X_3$ currents when the agonist was applied in longer intervals (**C**) but had negligible effects on tachyphylaxis (**D**).

4 P2X receptors in hiPSC-derived nociceptors - RealDRG™

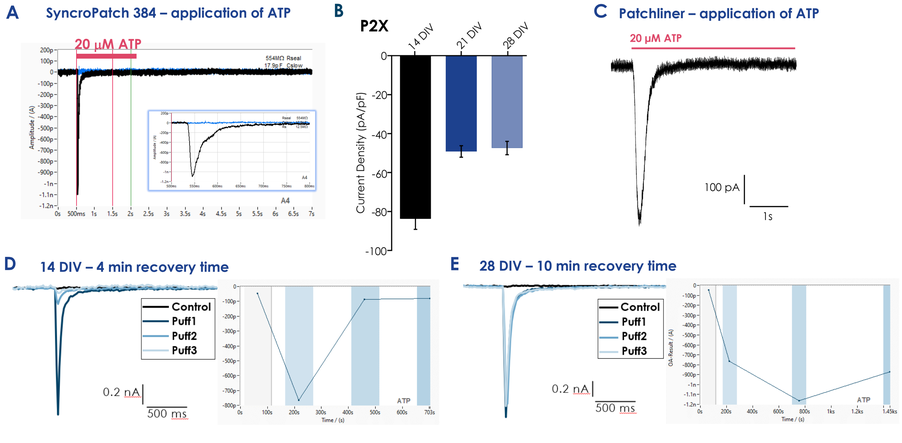


Figure 3: A. P2X-mediated responses from hiPSC-derived nociceptors (RealDRG™) receptors were recorded when activated using 20 µM ATP on the SyncroPatch 384. **B.** Current density of ATP-activated currents decreased during maturation (further experiments are required to confirm this observation). **C.** P2X receptors in RealDRG™ were activated using 20 µM ATP on the Patchliner. **D.** With a recovery time of 4 min between ATP applications, desensitization meant that currents could not be repetitively activated upon a 2nd or 3rd application of ATP. **E** When a recovery time of 10 min was used, P2X could be repetitively activated 3 times within the same cell with a current amplitude similar in amplitude to the 1st application (**D**, **E** Experiments on the SyncroPatch 384).

5 Conclusions

- Ion channel currents mediated by **$P2X_{2/3}$** heteromers expressed in CHO cells were **activated by αβ-MeATP** on the Port-a-Patch, Patchliner and SyncroPatch 384 with similar EC_{50} values and **inhibited by suramin** with expected IC_{50}s.
- **$P2X_3$** homomers were successfully recorded on the **Patchliner** and **SyncroPatch 384**, displaying faster activation and inactivation kinetics compared to the $P2X_{2/3}$ heteromers.
- Currents mediated by $P2X_3$ were highly **reproducible**, activated by αβ-MeATP on the Port-a-Patch, Patchliner and SyncroPatch 384.
- $P2X_3$-mediated currents were **blocked** by **A-317491** on the **Patchliner** and **SyncroPatch 384** with similar IC_{50}s.
- **Increased temperature** caused an increase in amplitude and faster recovery from desensitization.
- P2X-mediated responses were detected in RealDRG™ sensory neurons.

References

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