Development and validation of ASIC1a ligand-gated ion channel drug discovery assays on automated patch clamp platforms.

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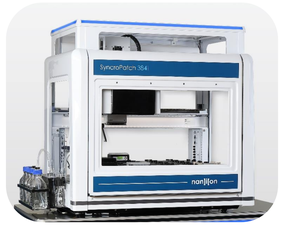
Introduction

Acid-sensing ion channels (ASICs) are proton-gated ion channels which are highly sensitive to extracellular acidosis and are permeable to cations[1], predominantly Na^+. To date, six different ASIC subunits (1a, 1b, 2a, 2b, 3 and 4) encoded by four genes have been identified[2]. Three subunits assemble to form homomeric or heteromeric channels which are expressed throughout the CNS and PNS. Excitatory ASIC channels have a proposed role in nociception and pain, and other neurological diseases such as ischaemia, inflammation and learning and memory[2].

Ligand-gated ion channels with rapid activation and desensitization present specific challenges on automated patch clamp (APC) systems due to low channel expression, the need for rapid application and wash-off of ligands, and loss of responsiveness due to short- and long-term desensitization. In this study we used ASIC1a channels expressed in CHO cells as an exemplar rapid ligand-gated ion channel to develop reliable screening assays on medium (Patchliner) and high throughput (SyncroPatch 384i) APC devices. ASIC1a-mediated current responses were reproducible and could be repetitively activated with similar peak amplitudes at least 7 times in the same cell using techniques which minimize agonist exposure time. ASIC1a was activated by decreasing pH and blocked by benzamil and amiloride. Finally, we demonstrate successful implementation of the classic historical input resistance/conductance-tracking technique to measure ligand-gated current activation on an APC platform.

Patchliner
4 to 8 recording channels



SyncroPatch 384i
384 recording channels

High throughput activation of ASIC1a

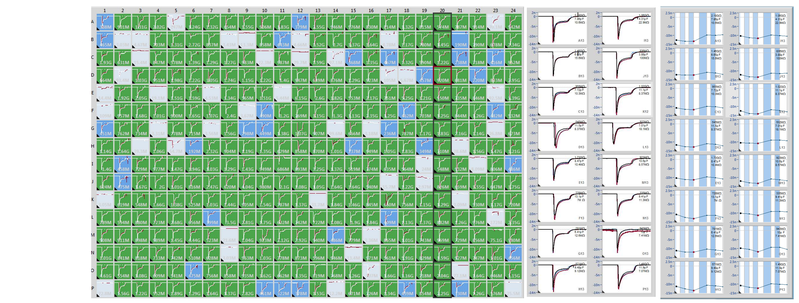


Figure 2: Activation of ASIC1a on the SyncroPatch 384i. A: Screenshot depicts raw data traces of ASIC1a-expressing CHO cells as recorded on one NPC-384 patch clamp chip (single hole per well). Solution at pH 6.5 was applied to the cells (exposure time 1 s) and then washed away by control solution (pH 7.2). **B:** pH response curve for an average of 378 wells revealed a $pH_{0.5}$ = 6.44 in excellent agreement with literature values[2,3].

Repetitive activation of ASIC1a. C: Currents could be reproducibly activated at least 6 times with similar peak amplitudes when repetitively activated with low pH solution (pH 5.5).

ASIC1a conductance tracking

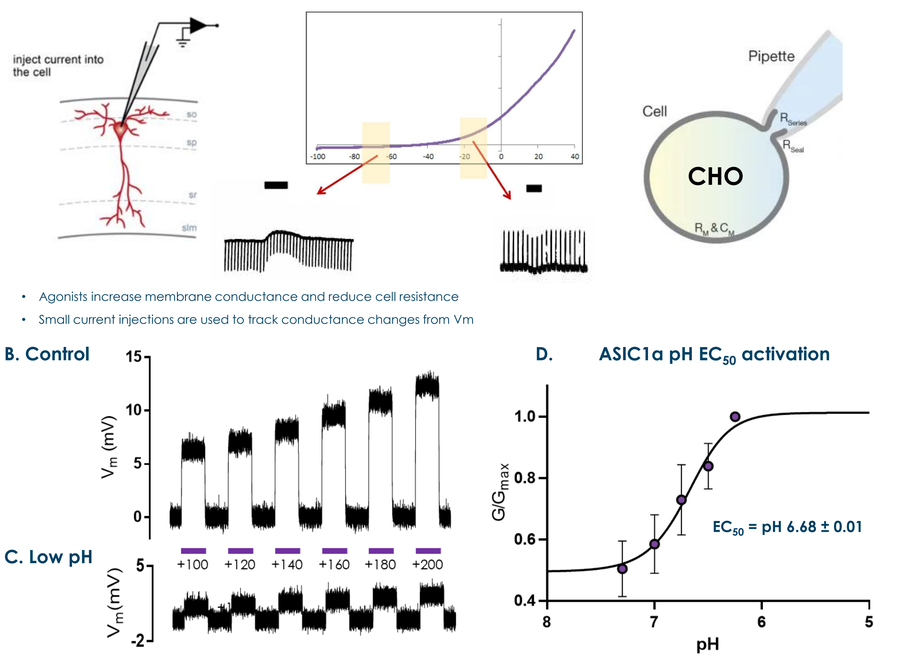


Figure 4: Conductance tracking of ASIC1a activation on the Patchliner. A: Principle of the technique. **B:** Under control conditions (Vm = 0 mV) incremental current injections assay CHO cell input resistance. **C:** During stacked tip application of acidic pH to activate ASIC1a receptors the Vm responses are decreased. **D:** Plotting conductance changes against pH reveals an EC_{50} of 6.68, in good agreement with voltage clamp.

Optimization of ligand addition

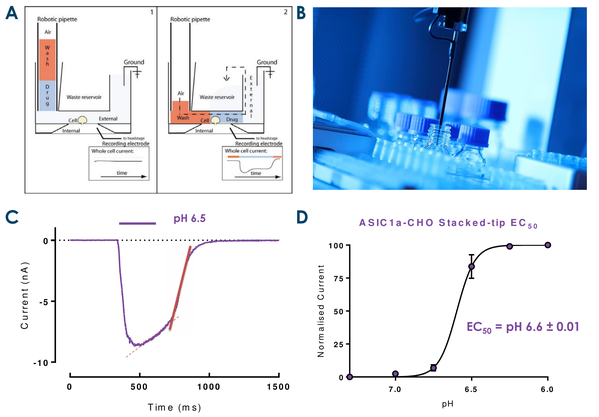
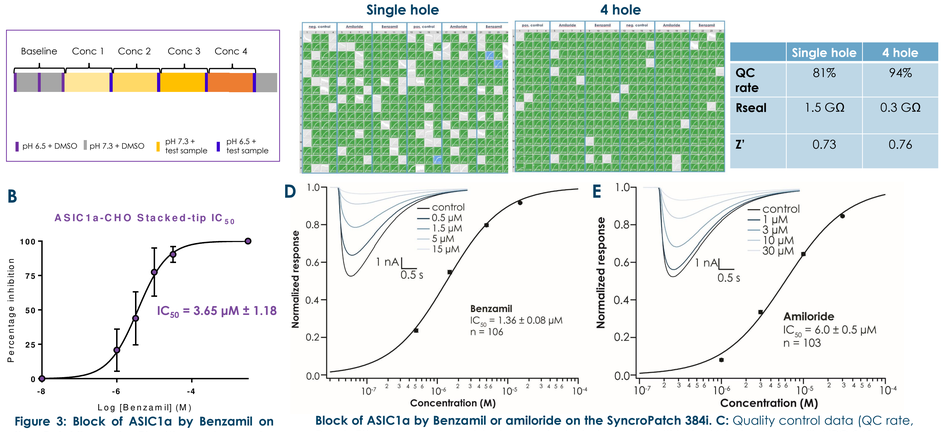


Figure 1 Activation of ASIC1a on the Patchliner. A: Schematic of addition of solution on the Patchliner using the stacked tip approach. In these experiments first wash solution (pH 7.2) and then solution at low pH (6.0 – 7.0) were aspirated into the pipette and then rapidly applied to the cell so that the ASIC1a channels were first activated by low pH and this was quickly washed away by control solution. **B:** Pipette and compound storage area of the Patchliner. **C:** Raw current trace of ASIC1a current during pH 6.5 application showing rapid activation, desensitization and wash-off. **D:** pH response curve reveals a $pH_{0.5}$ 6.6 in excellent agreement with the literature[2,3].

Pharmacology of ASIC1a on Patchliner and SyncroPatch 384i



	Single hole	4 hole
QC rate	81%	94%
Rseal	1.5 GΩ	0.3 GΩ
Z'	0.73	0.76

Figure 3: Block of ASIC1a by Benzamil on the Patchliner. A: Pipetting scheme for application of inhibitor. **B:** Average concentration response curve of Benzamil block of ASIC1a mediated responses. IC_{50} was 3.65 µM in excellent agreement with the literature[4].

Block of ASIC1a by Benzamil or amiloride on the SyncroPatch 384i. C: Quality control data (QC rate, Rseal, Z') were compared between single hole and multi-hole (4 holes per well) NPC-348 chips. **D:** Average concentration response curve for Benzamil block of ASIC1a mediated responses on the SyncroPatch 384i. The IC_{50} was 1.36 µM in excellent agreement with the literature[4] and with the value obtained using the Patchliner (shown in **B**). **E:** Average concentration response curve for amiloride block of ASIC1a mediated responses on the SyncroPatch 384i. The IC_{50} was 1.36 µM in excellent agreement with the literature[4].

Summary

- **ASIC1a** was reliably measured on **automated patch clamp** devices with medium **(Patchliner)** or high **(SyncroPatch 384i)** throughput using the stacked tip approach.
- **ASIC1a** was activated with **$pH_{0.5}$ 6.4** on the **Patchliner** and **$pH_{0.5}$ 6.6** on the **SyncroPatch 384i,** in excellent agreement with the literature[2,3].
- **ASIC1a** could be **repetitively activated** with similar peak amplitudes on the **Patchliner** and **SyncroPatch 384i** enabling pharmacological studies.
- **ASIC1a** was blocked by **benzamil** and **amiloride.** Concentration response curves were constructed with IC_{50} values in good agreement with literature values[4].
- Either **single hole** or **multi-hole** chips can be used. The use of multi-hole chips results in higher success rates for completed experiments **(94% vs. 81%)** and higher Z' values **(0.76 vs 0.73)**.
- **Conductance tracking** can also be used to study ASIC1a channels on the **Patchliner.**

References

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