Application Note

Channel: hASIC1a
Cells: CHO
Tools: SyncroPatch 384i

Pharmacology of human ASIC1a channels on Nanion's SyncroPatch 384i

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Summary

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^+. They are members of the sodium-selective cation channels belonging to the epithelial sodium channel/degenerin (ENaC/DEG) family[2].

To date, six different ASIC subunits (1a, 1b, 2a, 2b, 3 and 4) encoded by four genes have been identified[3]. Three subunits assemble to form homomeric or heteromeric channels. They are found expressed throughout the CNS and PNS and have a proposed role in nociception and pain, and other neurological diseases such as ischaemia and inflammation[4].

ASIC1a is localized in all cellular areas which contribute to synaptic transmission[5]. Further, it is expressed in various regions of the mammalian brain including the hippocampus, cerebral cortex, cerebellum, and amygdala[6-8]. Therefore, ASIC1a is involved in synaptic plasticity, learning and memory and pathophysiology such as psychiatric dysfunction, seizure and neuronal injury[3,8,9].

Here we present high quality data at a high throughput collected on the SyncroPatch 384i showing activation and inhibition of ASIC1a expressed in CHO cells. The pH which elicited a half-maximal response was in good agreement with the literature[3,6,10]. The IC_{50}s for block of the ASIC1a current by amiloride and benzamil, known blockers of ASIC and ENaC channels, were also in good agreement with the literature[2,6,11]. Success rates of over 80% for completed experiments were recorded.

Results

ASICs are activated by extracellular acidosis mediating a cation-conductance. Figure 1 shows success rate (Fig. 1A), raw current trace (Fig. 1B inset) and pH-activation response curve (Fig. 1B) for ASIC1a-mediated current activation by solutions at different pH.

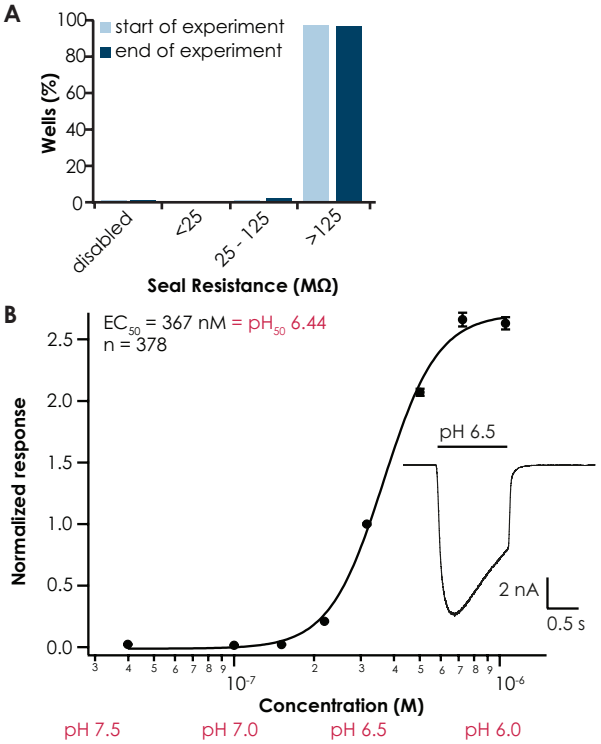

Figure 1: Activation of ASIC1a by decreasing pH. A. Success rate (seal resistance) of wells from one experiment recorded on a 4-hole NPC-384 chip. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B.** pH response curve calculated across the whole plate where currents were normalized to current elicited by pH 6.5 is shown. The curve was fitted with a Hill equation and the $pH_{0.5}$ calculated to be pH 6.44 ± 0.08 (n = 378), in excellent agreement with the literature[3,6,10]. Raw trace of ASIC1a current activation in response to pH 6.5 solution is shown in the inset. Holding potential was -80 mV.

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In the experiment shown in Figure 1, currents were first activated with pH 6.5, to obtain a stable signal and to check current reproducibility. Following this, the test pH was applied and this was repeated twice with washout between each addition. The responses were normalized to the current elicited by pH 6.5 and the pH response curve calculated across the whole plate. The pH which elicited the half-maximal current, $pH_{0.5}$, was pH 6.44 ± 0.08 (n = 378), in excellent agreement with the literature range of pH 6.4 - 6.6[3,6,10]. The success rate for this experiment was 96.4 % determined at the end of experiment.

The reproducibility of ASIC1a-mediated currents is demonstrated by applying pH 6.5 solution 7 times in the same cell. Figure 2 shows raw current traces of a single well (Fig. 2A) and the average current amplitude over time of 64 successfully recorded wells in one experiment (Fig. 2B). ASIC1a mediated currents could be reliably recorded on the SyncroPatch 384i.

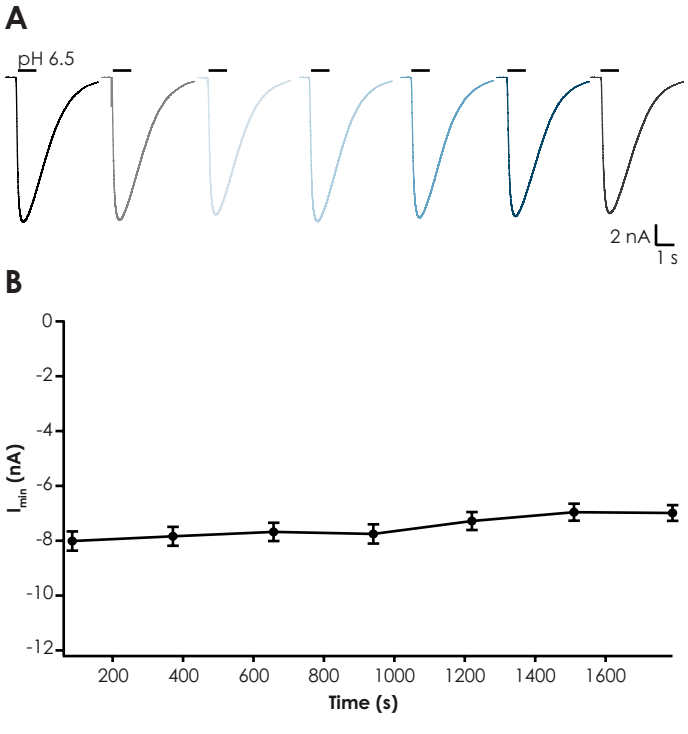

Figure 2: Reproducibility of ASIC1a-mediated currents recorded on the SyncroPatch 384i. The experiment shows current activation in response to 7 consecutive applications of acidified solution in order to evaluate reproducibility of the signal. The experiment was performed on multi hole chips (4x) and the holding potential was -80 mV. **A.** Raw current traces from an example well showing repetitive current activation by pH 6.5 solution. **B.** Online analysis plot of one experiment showing average peak amplitude versus time of 64 wells. The peak amplitudes were very similar with each application of pH 6.5.

Amiloride is a potassium-sparing diuretic used to treat hypertension and congestive heart failure. It is a known blocker of ASICs and ENaC, acting as a pore-blocker of ASICs[6]. Benzamil is an analog of amiloride with higher blocking potency. Figure 3 shows the concentration response curve for amiloride and benzamil block of ASIC1a. ASIC1a-mediated currents were activated using pH 6.5 solution and the concentration response curve for each compound was calculated for each well, 4 concentrations of the compound were added to each well. (see Figure 4). Amiloride and benzamil blocked ASIC1a mediated currents with an IC_{50} of 6.0 ± 0.47 µM (n = 103) and 1.36 ± 0.08 µM (n = 106), respectively, in excellent agreement with the literature[2,6,11]).

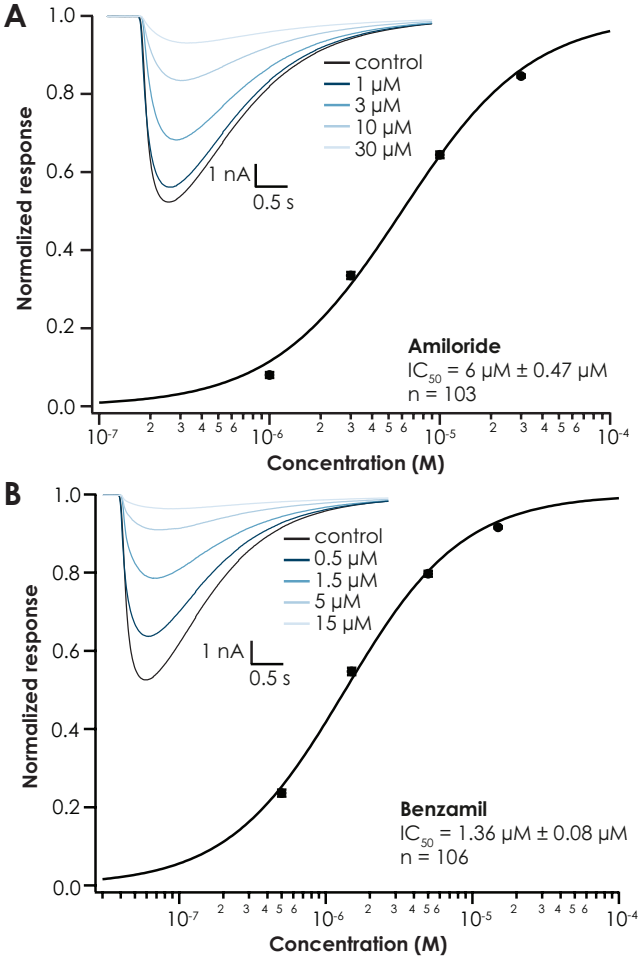

Figure 3: Average concentration response curve for amiloride and benzamil block of ASIC1a. The concentration response curve was constructed from cumulative additions of increasing concentrations of compound in individual wells as shown in Figure 4. The SyncroPatch 384 analysis software (DataControl 384) was used to calculate the average concentration response curve, normalized to maximum activation and fitted with a standard Hill-equation. **A**. The IC_{50} for block of ASIC1a with amiloride was 6.0 ± 0.47 µM (n = 103), and **B**, with benzamil 1.36 µM ± 0.08 µM (n = 106), both in excellent agreement with the literature[2,6,11].

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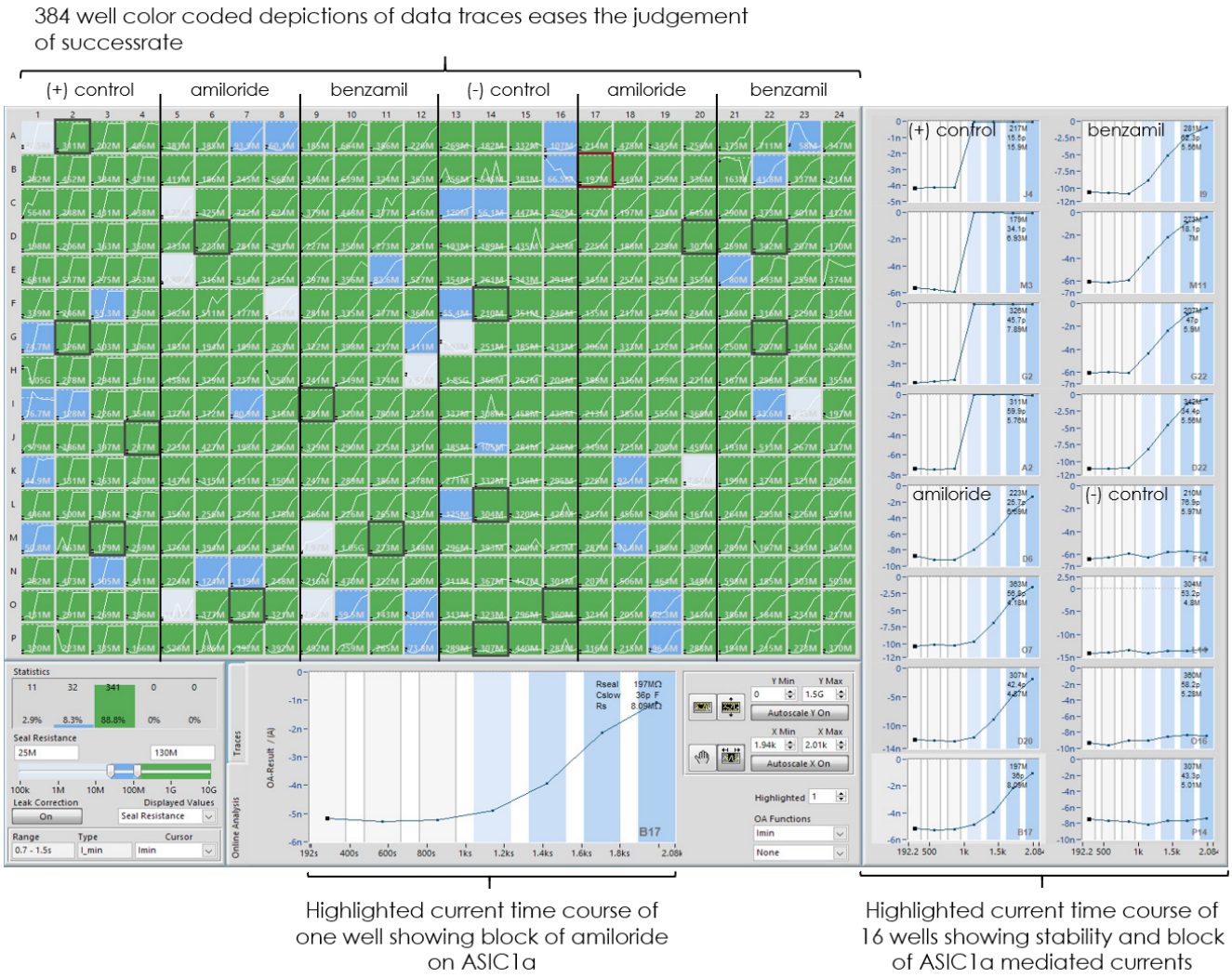

Figure 4: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of online analysis of ASIC1a-expressing CHO cells as recorded on one NPC-384 (multihole, 4x) patch clamp chip. Positive Control (pH 7.4), amiloride, benzamil or negative control (0.3 % DMSO) in increasing concentrations was applied to 4 columns for each compound . The data of the 384 well plate representation in the upper left part are color-coded for easy assessment of data. Depending on the seal resistance, pictures are green (Rmemb > 130 MΩ), blue (Rmemb = 25 - 130 MΩ), light blue or grey (Rmemb < 25 MΩ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes over time of ASIC1a channels following activation by pH 6.5 for three times (grey region) and inhibition by compound at four increasing concentrations indicated (increasing shades of blue regions).

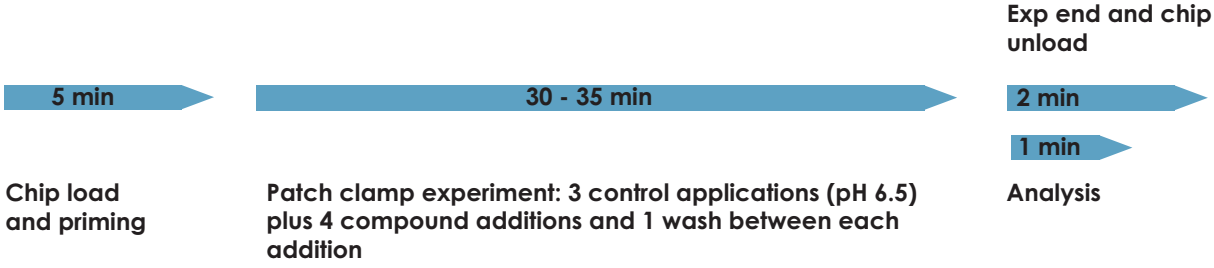

Figure 5: Time line of an experiment on the SyncroPatch 384. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a 4 point cumulative concentration response curve on ASIC1a-mediated currents including 3 reference additions and one wash between each addition took approximately 35-40 min.

Figure 4 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can easily toggle between raw traces and online analysis. In the example shown, online analysis is chosen and the graphs show control responses to pH 6.5 (grey region) and current responses in the presence of amiloride or benzamil at increasing concentrations (increasing shades of blue region). An individual well can be highlighted to monitor progression of the experiment and is shown enlarged at the bottom of the screen.

In conclusion, ASIC1a expressed in CHO cells can be recorded on the SyncroPatch 384 with high success rates for completed experiments (typically >80%). The time line of each experiment was about 35 - 40 minutes (start – end) and included 3 control applications with pH solution followed by pre-incubation with blocker and activation of currents in the presence of 4 consecutive concentrations of blocker and one wash with buffer between each addition (Figure 5). The IC_{50} for amiloride and benzamil block of ASIC1a-mediated currents calculated using the SyncroPatch 384's analysis software, DataControl 384, was in excellent agreement with the literature[2,6,11].

The SyncroPatch 384i is a high throughput and highly reliable automated patch clamp device for recording ASIC1a currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each well and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

hASIC1a expressing CHO cells are kindly provided by Charles River.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol. Channel expression was induced by incubation in 1 µg/ml tetracycline 3-6 hours prior to experiments.

Electrophysiology

Perforated patch clamp recordings were conducted on the SyncroPatch 384i or the predecessor model, the SyncroPatch 384PE, according to Nanion's standard procedure using an internal solution containing 25 µM escin. Cells were held at a constant holding potential of -80 mV. Cells were activated 3 times with pH 6.5 to monitor reproducibility of the signal and to elicit maximum peak amplitude. Compounds were pre-incubated in pH 7.4 for 3 minutes. Responses after compound addition were normalized to the response evoked by pH 6.5. Cumulative compound concentration curves were constructed and fitted with Hill equation.

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