Application Note

Channel: hASIC3
Cells: HEK293
Tools: Patchliner®

Characterization of hASIC3 (HEK) on Nanion´s Patchliner®

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Cells kindly provided by Millipore.

Summary

Acid-sensing ion channels (ASICs) are ligand-gated ion channels activated by protons[1] and are members of the sodium-selective cation channels belonging to the epithelial sodium channel/degenerin (ENaC/DEG) family. They are highly sensitive to extracellular acidosis.

In rodents, where they are mainly expressed in neurons of the peripheral nervous system, ASIC3 plays an important role as sensor of non-adaptive pain which is correlated to tissue acidosis. However, the role of the human ASIC3 channel has not yet been elucidated. In contrast to other ASIC ion channels, ASIC3 shows a sustained window current upon external acidification.

Here we present data recorded on an 8-channel Patchliner. Current responses upon external acidification as well as amiloride block of acidosis-induced currents of hASIC3-expressing HEK293 cells are shown.

Results

Current responses of an individual cell expressing acid-sensing ion channel hASIC3 exposed to acidifying external solution are shown in Figure 1. Short application of acidified external solution (pH 6.8 – 5.5) resulted in sustained increasing inward currents. Acidosis from pH 6.8 to pH 5.5 leads to over 2-fold increase of inward currents (Fig. 1B).

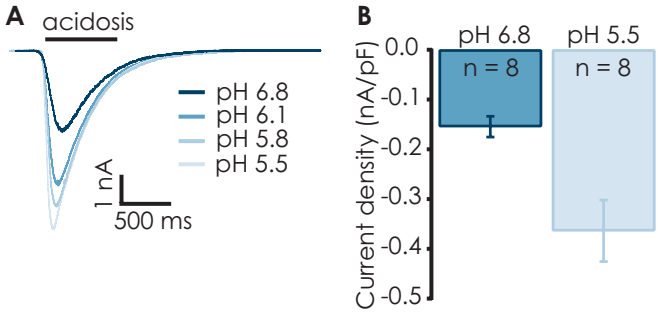

Figure 1:
A Current responses of an individual cell expressing ASIC3 held at -70 mV upon wash in of solution with decreasing pH as indicated. **B** Acidification from pH 6.8 to pH 5.5 leads to over a 2-fold increase in inward currents in hASIC3-expressing HEK293 cells.

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In Figure 2 the effect of the known DEG/ENaC inhibitor amiloride on hASIC3 expressing HEK293 cells is shown. Inward currents induced by acidic external solution (pH 5.5) are suppressed by application of 1 µM – 1 mM amiloride revealing an IC_{50} of 26.0 ± 3.3 µM (n = 14) as shown in Figure 3, in good agreement with values reported in the literature[1-8].

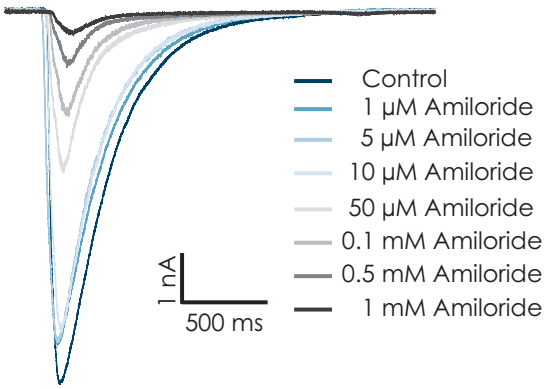

Figure 2:
A Current responses of an individual cell expressing ASIC3 to pH 5.5 solution (holding potential -70 mV) and block by increasing concentrations of amiloride (concentrations as indicated).

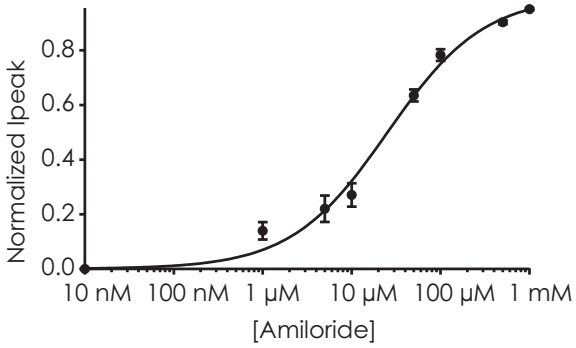

Figure 3:
Concentration response curve for amiloride of hASIC3-expressing HEK293 cells collected on the Patchliner. IC_{50} = 26.0 ± 3.3 µM (n = 14).

In summary hASIC3 ion channels stably expressed in HEK293 cells show reliable acidosis-induced inward currents that can be blocked by amiloride.

Therefore, the Patchliner provides a viable, higher throughput alternative to conventional patch clamp for investigation of active hASIC3 lead compounds.

References

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Methods

Cells

HEK293 cells stably expressing hASIC3 were supplied by Millipore.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner. Cells were held at a holding potential of -70 mV. To achieve short exposure times, solutions were stacked in the robotic pipettor. First, 80 µl wash solution was aspirated followed by 40 µl of the agonist-containing solution and then applied to the cell at a speed of 20 µl/s.

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