Can Chloride concentration regulate sodium epithelial channels?

Comparing effects of extracellular chloride concentration on different isoforms of sodium epithelial ion channels (αβγ-ENaC and δβγ-ENaC).

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Introduction

- Epithelial sodium channels (ENaC) mediate absorption of sodium across epithelia, which is essential for body salt and water balance.
- There are two ENaC isoforms which differ in one subunit; δβγ-ENaC or αβγ-ENaC.
- it has been found that one form is usually more sensitive to stimuli than the other.

Aims

- To see if extracellular chloride concentration regulates ENaC activity.
- And whether these isoforms also differ in sensitivity to extracellular chloride concentration.

Results

Data are presented as mean values ± standard deviation (SD). For *Xenopus* δβγ-ENaC, the ratio was 0.686 ± 0.172 (n=4) when ORS was applied twice, and significantly lower (0.289 ± 0.0836; n=6) when low Cl- ORS was applied (Mann-Whitney test, U = 34, n = 4,6, p = 0.0142). For *Xenopus* αβγ-ENaC the ratio was 0.682 ± 0.079 (n=3) when ORS was applied twice, and significantly lower (0.290 ± 0.1875, n=5) when low Cl- ORS was applied (Mann-Whitney test, U = 21, n = 3,5, p = 0.037). When water was injected instead of cDNA, recordings of transmembrane currents showed no change when employing amiloride, ORS or low Cl- ORS.

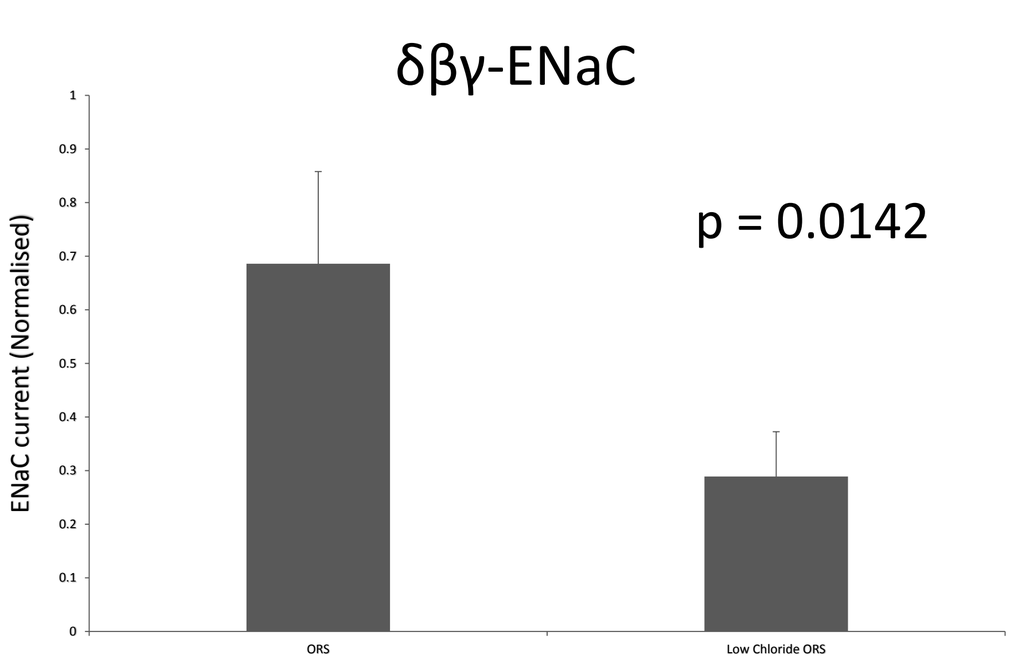

Figure 2: Statistical analysis of δβγ-ENaC data. Depicted are normalised ENaC currents values. This represents the ratio of the first and second current stimulated by low chloride ORS, where a significant decrease was found when low chloride was applies second (Mann-Whitney test, U = 34, n = 4,6, p = 0.0142)

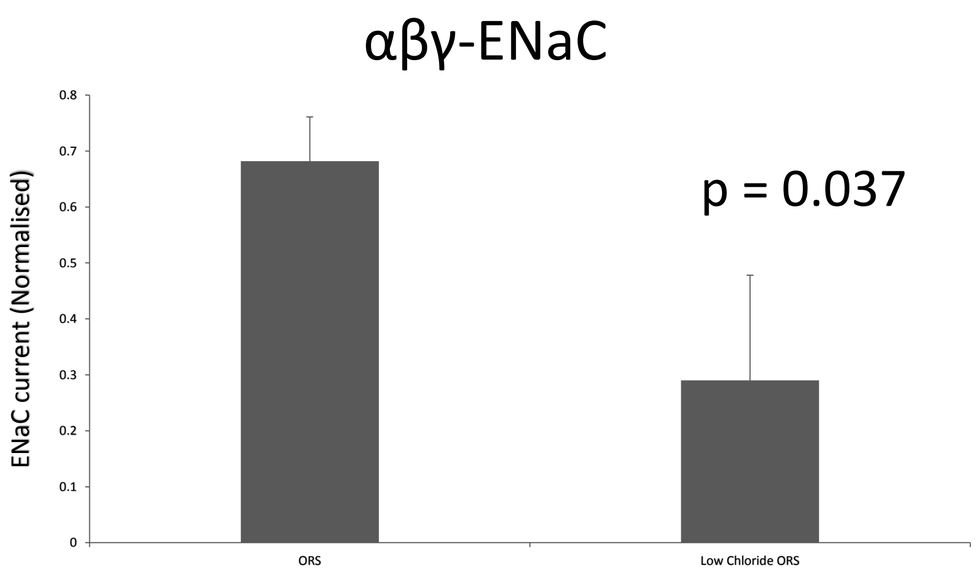

Figure 3: Statistical analysis of αβγ-ENaC data. Depicted are normalised ENaC currents values. This represents the ratio of the first and second current stimulated by low chloride ORS, where a significant decrease was found when low chloride was applies second (Mann-Whitney test, U= 21, n = 3,5, p = 0.037)

Methods

We used *Xenopus laevis* oocytes as an expression system

1. Ovary lobe from X. laevis was dissected and oocytes were isolated and sorted.
2. Oocytes were injected with 32.2nl of *Xenopus* αβγ-ENaC cRNA, δβγ-ENaC cRNA or water (as a control).
3. ENaC activity was measured by microelectrode electrophysiology using the Two-Electrode Voltage-Clamp (TEVC) technique.
4. Transmembrane currents were measured when employing oocyte ringer's solution (ORS) or low Cl^- ORS with or without amiloride (100 µM) which blocks both ENaC isoforms. Allowing amiloride-sensitive currents to be determined
5. Transmembrane currents were recorded when oocytes were exposed twice to ORS or to ORS and then low Cl^- ORS, running amiloride between the two exposures. Normalized ENaC current values representing the ratio of the first and second current were then calculated.
6. ORS; 90mM NaCl, 1mM KCl, 2mM $CaCl_2$, 5mM HEPES, pH 7.4) or low Cl^- ORS (1mM KCl, 2mM $CaCl_2$, 5mM HEPES, 45mM Na_2SO_4, 45mM Mannitol)

Table 1: The concentrations of compounds making up either ORS or low Chloride ORS

	ORS (mM)	Low Chloride ORS (mM)
NaCl	90	-
Na_2SO_4	-	45
Mannitol	-	45
KCl	1	1
$CaCl_2$	2	2
HEPES	5	5

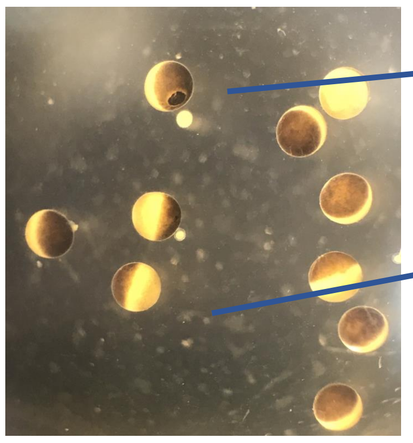

Figure 1: *X. Laevis* oocytes before sorting

Conclusions

These findings demonstrate that a low extracellular Cl- concentration significantly decreases the activity of both *Xenopus* αβγ-ENaC and δβγ-ENaC.

ENaC isoforms do not respond differently to extracellular chloride.

Extracellular chloride represent a novel mechanism regulating ENaC, which is crucial in controlling electrolyte homeostasis in vertebrates.