Analyzing bioelectric phenomena during *Drosophila* oogenesis: Quantification of changes in membrane potential and intracellular pH by genetically encoded sensors expressed in epithelial cells

**Introduction**

Changes in the activities of ion transport mechanisms that result in transcellular bioelectric signals are known to play important roles during developmental and regenerative processes [1]. In order to study their functions as well as cellular mechanisms, *Drosophila* oogenesis provides a suitable model system [2].

In previous studies, we used various inhibitors and two fluorescent dyes to investigate the role of specific ion transport mechanisms in the generation of membrane potentials (*V*<sub>mem</sub>) and intracellular pH (*pH*) in *Drosophila* ovarian follicles [2]. The changes in *V*<sub>mem</sub> and *pH* as revealed by the fluorescent indicators DiBAC<sub>4</sub>(3) and 5-CFDA-AM, were now evaluated using the genetically encoded fluorescent *V*<sub>mem</sub> sensor ArcLight [3] and the genetically encoded pH sensor pHluorin [4], respectively.

**Materials and methods**

(A) *V*<sub>mem</sub> was analyzed using the voltage sensor ArcLight, and *pH* was analyzed using the pH sensor pHluorin. Both pHluorin and ArcLight, which consists of a voltage-sensing domain and a pHluorin-GFP variant, were specifically activated in the follicular epithelium by the dUAS-system.

(B) Follicles of S1OB were incubated in R14 medium with either the inhibitor or the respective solvent.

(C) Fluorescence intensities (grey values) in the FC epithelium were measured using ImageJ software.

**Results**

**Changes of bioelectrical properties in the follicular epithelium resulting from the specific inhibition of various ion transport mechanisms**

- **Cl<sup>-</sup>** channels Anthracene-9-carboxylic acid

  - Hyperpolarization
  - Acidification

- **Na<sup>+</sup>/H<sup>+</sup>** exchangers
  - Amiloride
  - Na<sup>+</sup> channels

  - Voltage-dependent Ca<sup>2+</sup> channels Verapamil

  - Hyperpolarization
  - No effect

- **Follicle cell**
  - ATP-sensitive K<sup>+</sup> channels Gabuncilamide
  - V-ATPases Concanamycin A

The results obtained with ArcLight-expressing follicle cells correspond to those observed with DiBAC<sub>4</sub>(3), as shown before. For example, strong effects on *V*<sub>mem</sub> were observed after inhibition of voltage-dependent calcium channels or sodium transporters; both resulted in relatively hyperpolarized cells. Comparable effects were observed using pHluorin or 5-CFDA-AM. For instance, strong effects on *pH* were detected after inhibition of sodium-potassium-chloride cotransporters or ATP-sensitive potassium channels; both resulted in relatively alkalized cells (*p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant).

**Conclusions**

We found that both genetically encoded sensors reliably revealed changes of bioelectrical properties resulting from the specific inhibition of various ion transport mechanisms in the follicular epithelium. Therefore both sensors are useful tools for investigating bioelectrical phenomena and their significance for cellular and developmental processes. In future experiments, we want to analyze the knock down of various ion transport mechanisms in the follicular epithelium using RNAi in comparison with different inhibitors.

**References**