Identification of epithelial sodium channel (ENaC) in endometrial pipelle biopsy samples

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Introduction and Objectives

Epithelial sodium channel (ENaC) activated by Aldosterone is an important regulator of electrolyte homeostasis, blood volume and blood pressure (1). (Fig.1)

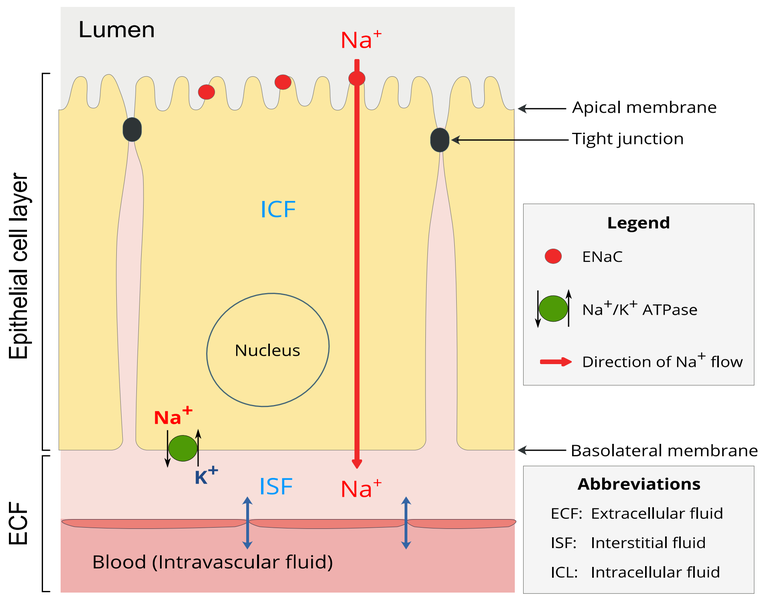
Autosomal recessive pseudohypoaldosteronism (PHA1B) is caused by mutations in genes coding for subunits of the ENaC. Affected individuals suffer from severe, frequent salt-wasting episodes with hyperkalaemia, hyponatremia and metabolic acidosis requiring recurrent hospitalizations (2,3). Mortality rate is high in infancy. Salt wasting episodes decrease with age. However, long salt supplementation is required to prevent salt wasting episodes and allowing them normal life style. In childhood, affected individuals also suffer from recurrent chest infections due to dysfunction of respiratory epithelial cilia.

In older female patients, ciliary dysfunction along the repruductive tract may also result in infertility (4). The fluid milieu along the female reproductive tract has a major role in a complex series of events that follow oocyte ovulation. These include oocyte transport in the fallopian tube, the transport and capacitation of sperm, fertilization, transport of the blastocyst and implantation of the embryo in the uterus. These processes are regulated by the activity of ion channels located on the surface of endometrial epithelia. We have previously shown that in healthy women the alpha ENaC subunit are widely distributed on the ciliary surface of all epithelial cells with motile cilia including the fallopian tubes (4). (Fig.2).

One of our patients aged 32.8 y with PHA1B who failed to conceive naturally and despite nine IVF attempts over six years prompted us to evaluate the expression of ENaC in the repruductive tract. We hypothesized that failure to conceive in women with PHA1B is most probably due to nonsense mutations in one of the ENaC subunits resulting in defective ENaC function in reproductive tract epithelia.

Objectives: To examine the expression of ENaC in endometrial biopsy samples obtained by pipelle suction for diagnostic purposes in healthy women and a patient with PHA1B.

Fig. 1. A schematic view of epithelial sodium channel (ENaC) function in tight epihelia.



Subjects and Methods

The samples were obtained from four normal control subjects and a pseudohypoaldosteronism type 1 patient with an Arg508X mutation in the SCNN1A gene that codes for the ENaC alpha subunit (3).

Endometrial samples were obtained by Pipelle suction. The samples were fixed in formalin and then reacted with anti-ENaC antiserum. After reaction with secondary antibodies and DAPI (to identified nuclei) sample immunofluorescence were visualized by confocal microscopy. The study was approved by the ethics committee at the E. Wolfson Medical Center.

Results

The analysis showed strong ENaC immunofluorescence along the luminal border (apical membrane) of the epithelial cells in pipelle samples from four healthy subjects (Fig.3). In contrast, none of the samples from the PHA patient showed ENaC immunofluorescence. In contrast to endometrium where ENaC is localized in the apical membrane of the epithelial cells (Fig 4). In keratinocytes ENaC is expressed in cytoplasmic pools. Thus, we examined ENaC immunofluorescence in plucked hair follicles from normal subjects and the PHA patient. As expected, ENaC immunofluorescence was detected in the cytoplasm of keratinocytes of both normal and PHA samples (Fig.5). Our results for the first time showed that ENaC expression in the endometrium can be detected by confocal microscopy of immunofluorescently labeled samples obtained from women using Pipelle suction (Fig. 3). ENaC immunofluorescence observed in the Pipelle samples from all three healthy women is highly similar to the images we obtained with endometrial tissue sections (Fig. 2) (4).

Discussion

In contrast to samples from healthy women, the endometrial samples obtained from the PHA1B patient showed that the expression of ENaC on the endometrial luminal surface is drastically reduced and hardly detectable (Fig. 4). we hypothesized that the truncated alpha ENaC subunit with an Arg508X mutation (that could not be transported to the cell surface should be detectable in the cytoplasm. Indeed, in hair follicles from the PHA1B patient, ENaC immunofluorescence was abundant in the cytoplasm of the keratinocytes except for the outermost single layer of cells (Fig. 5). These results are consistent with our hypothesis that the Arg508X mutation interrupts the transport of the ENaC subunit to the cell surface, but does not prevent its localization and accumulation in the cytoplasm of keratinocytes. As noted in the Introduction, the renin-angiotensin-aldosterone axis plays a major role during pregnancy. PHA1B is characterized by major changes in the levels of these hormones throughout patient's lifetime]. Yet, based on the results reported here, we suggest that the major cause of the failure of embryo implantation in the PHA patient is severely reduced expression of ENaC in the reproductive tract. Apparently, ENaC has to be counted among ion channels that play an important role in endometrial receptivity. The report on another systemic PHA1B patient who conceived but gave birth to an infant with severe intrauterine growth retardation(7) is also indicative of the importance of ENaC function throughout pregnancy including maintenance of placental function.

Fig. 2. Apical localization of α-ENaC in human endometrial glands.
3D image showing uniform distribution of ENaC along the apical walls of the gland. Human endometrium sections were stained with anti-α-ENaC antiserum and secondary antibodies labeled with Cy3 (green). The nuclei were stained with DAPI.

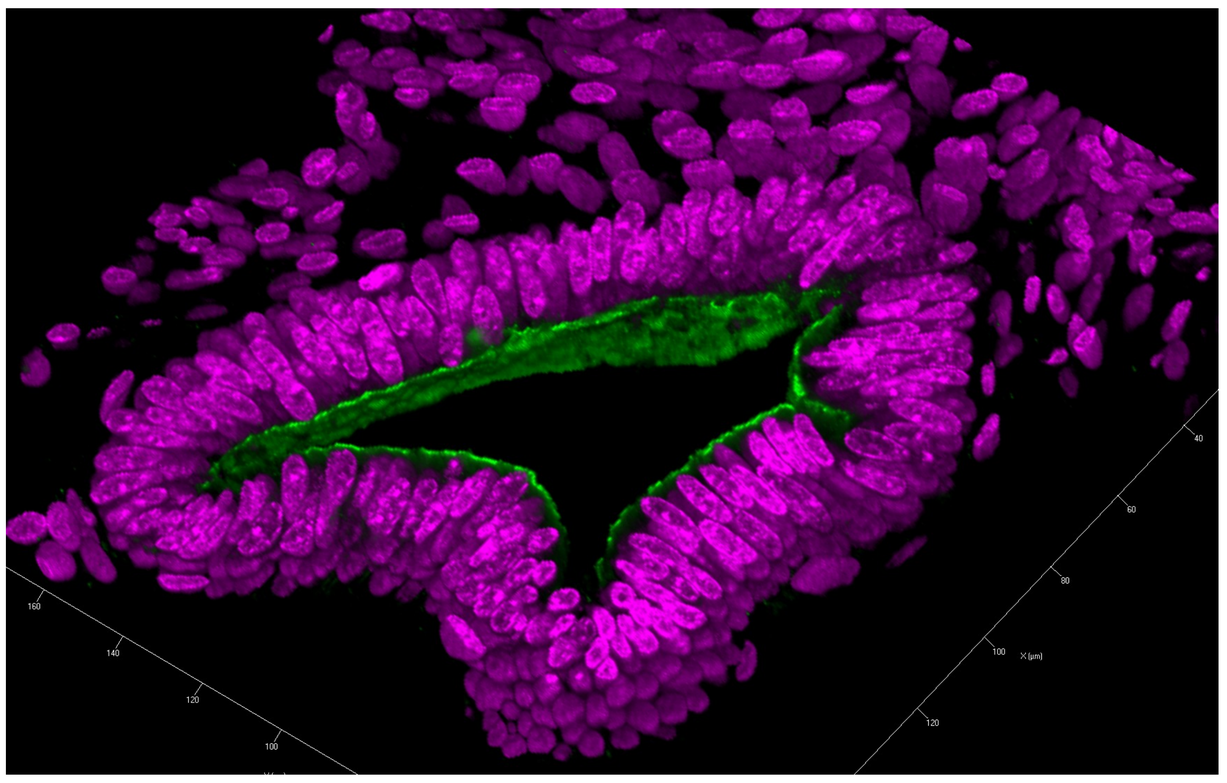


Fig. 3. Confocal microscopic imaging of immunofluorescence of endometrial Pipelle samples from a healthy subject.
The samples were reacted with anti-ENaC antiserum followed by secondary antibodies, and DAPI.
A and D) DAPI immunofluorescence of nuclei.
B and E) ENaC immunofluorescence.
C and F) Merged image showing that ENaC immunofluorescence is located on the apical side of the epithelial cells.

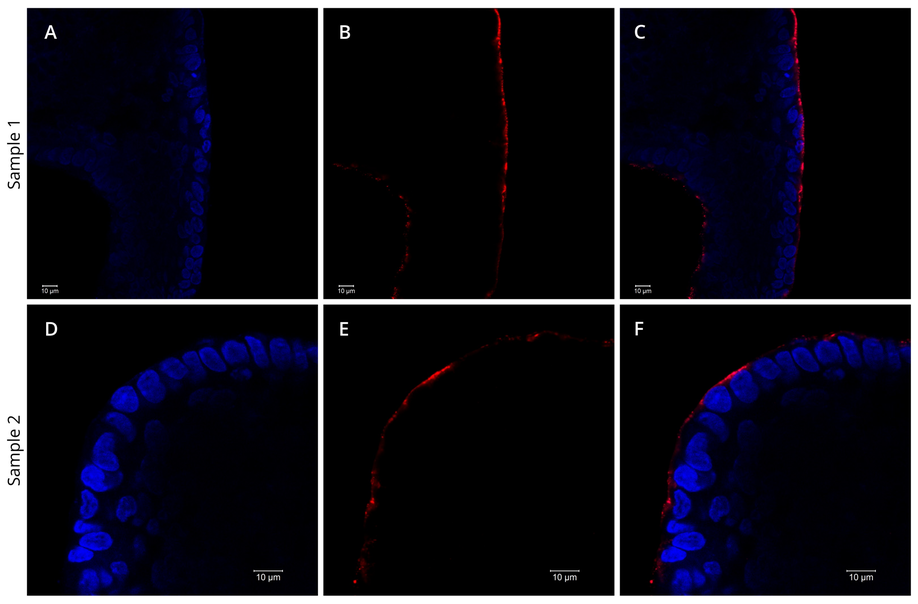


Fig. 4. Confocal microscopic imaging of immunofluorescence of endometrial Pipelle samples from the patient with PHA1B.
The samples were reacted with anti-ENaC antiserum followed by secondary antibodies, and DAPI as described in Methods.
A and D) DAPI immunofluorescence of nuclei.
B and E) ENaC immunofluorescence.
C and F) Merged image.

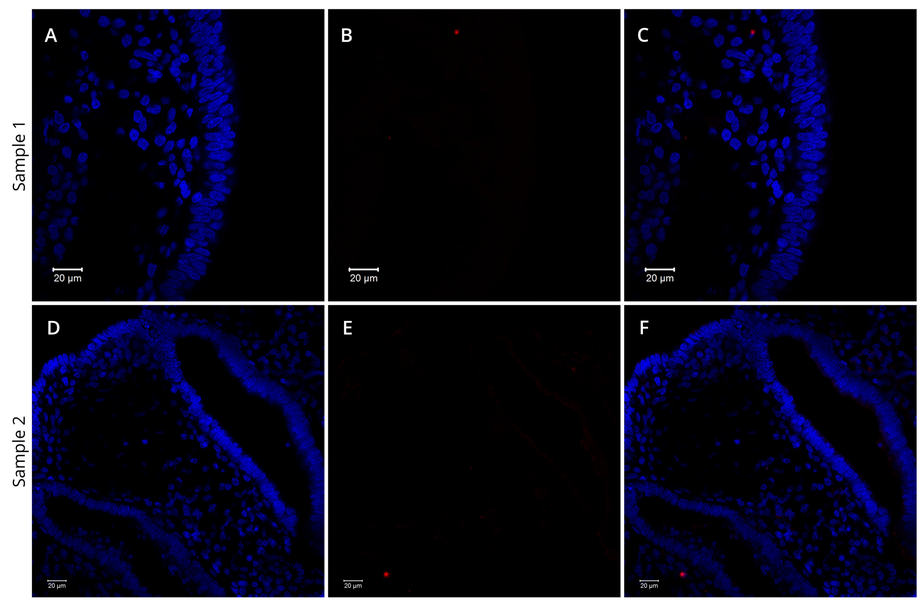
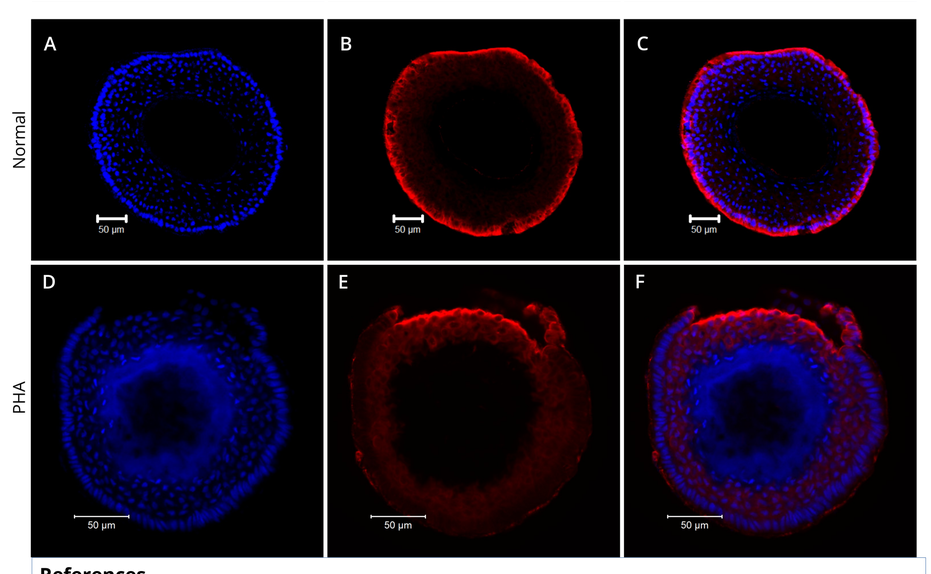


Fig. 5. Confocal microscopic imaging of ENaC immunofluorescence in a cross-section of a hair-follicle from a healthy subject and the PHA1B patient.
A and D) DAPI immunofluorescence of nuclei.
B and E) ENaC immunofluorescence.
C and F) Merged image.



Conclusions:

1.The renin-angiotensin-aldosterone axis plays a major role during pregnancy.

2.The major cause of the failure of embryo implantation in the PHA patient is severely reduced expression of ENaC in the reproductive tract.

3.Pipelle biopsy can be used for the identification of key proteins by immunofluorescence.

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