Cystic fibrosis transmembrane conductance regulator in the endolymphatic sac of the rat.

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Abstract

Aims and Introduction: In the endolymphatic fluid in the endolymphatic sac (ES), Na^+ and Cl^- are dominant ions, and both are thought to be important for volume regulation. Na^+,K^+-ATPase at the basolateral membrane of ES epithelial cells provides the driving force for Na^+ absorption. The Na^+ flow is generally coupled with Cl^- flow to neutralize the charge movement, thereby guaranteeing ionic neutrality. However, no chloride channels have been identified in the ES. Cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma membrane cAMP-regulated Cl^- channel. The CFTR also acts as a regulator by exerting modulatory influence over the epithelial sodium channel (ENaC). In the ES, ENaC was identified in human and guinea pigs and shown to be localized at the apical membrane. The aim of this study was to examine the expression of CFTR in ES epithelia, which may play roles in the regulation of endolymph in the ES.

Methods: Four-week-old female Sprague–Dawley rats were used. Specific mRNA from ES epithelia was prepared using laser capture microdissection (LCM) and examined using RT-PCR. Localization of CFTR and ENaC in the endolymphatic sac was examined using immunohistochemistry.

Results and Conclusions: RT-PCR form the ES samples detected the expression of mRNA of the CFTR. Immunohistochemical analysis showed the expression of the CFTR on apical side of the ES epithelia and co-localization with the ENaC. These results suggest a pathway for Cl^-, possibly through interaction with the ENaC, which may regulate the endolymph in the ES.

Materials and Methods

Animals and tissue preparations (approved by the Animal Care and Use Committees of Kagawa University)

4w-old, SD rats were anesthetized and decapitated to obtain the ES, kidney, colon and pancreas. After fixation and decalcification, the samples were cryo-sectioned. Laser capture microdissection (LCM) was used to collect the ES epithelia.

RT-PCR

Total RNA was extracted from LCM sample and reverse-transcribed into cDNA. cDNA was amplified by 30 cycles of PCR.

Immunohistochemical analysis

After post-fixation and blocking, the sections were stained by immuno-fluorescence method with specific antibodies for CFTR and ENaC. Secondary antibodies; Alexa Fluor-546 donkey anti-goat IgG or Alexa Fluor-488 goat anti-rabbit IgG. 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid stain was also used. The colon and kidney sections were used for positive controls.

Results and discussion

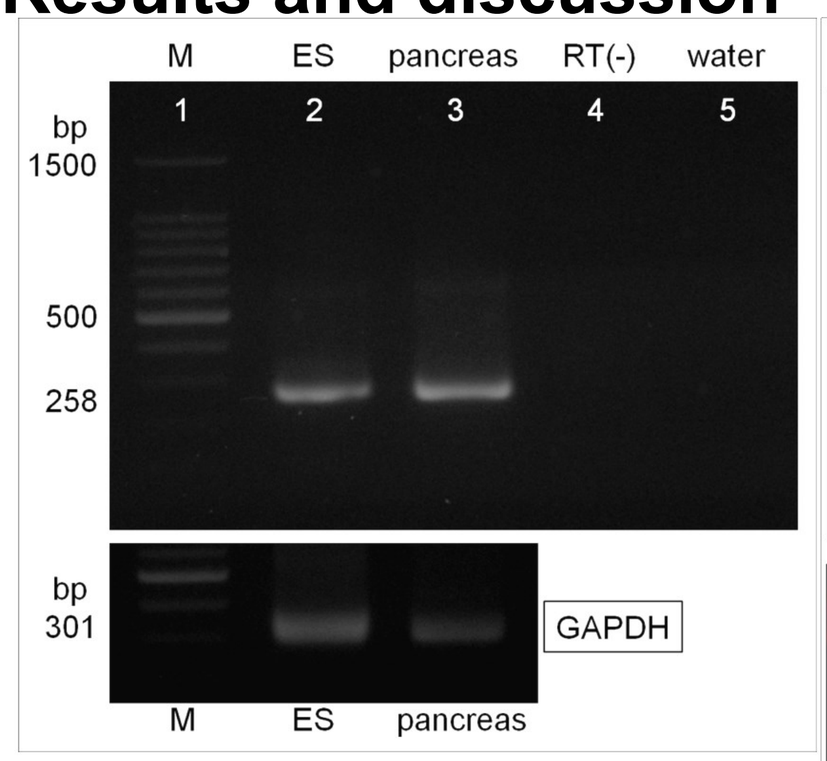

Fig. 1 Agarose gel electrophoresis of PCR products.
The band for CFTR from the ES was detected (258 bp; Lane2). The band for CFTR from the pancreas is shown as a positive control (Lane3).
Lane4; RT-PCR without reverse transcriptase.
Lane5; PCR template replaced with pure water.
Lower picture; GAPDH detected from the ES and pancreas.
M; size marker.

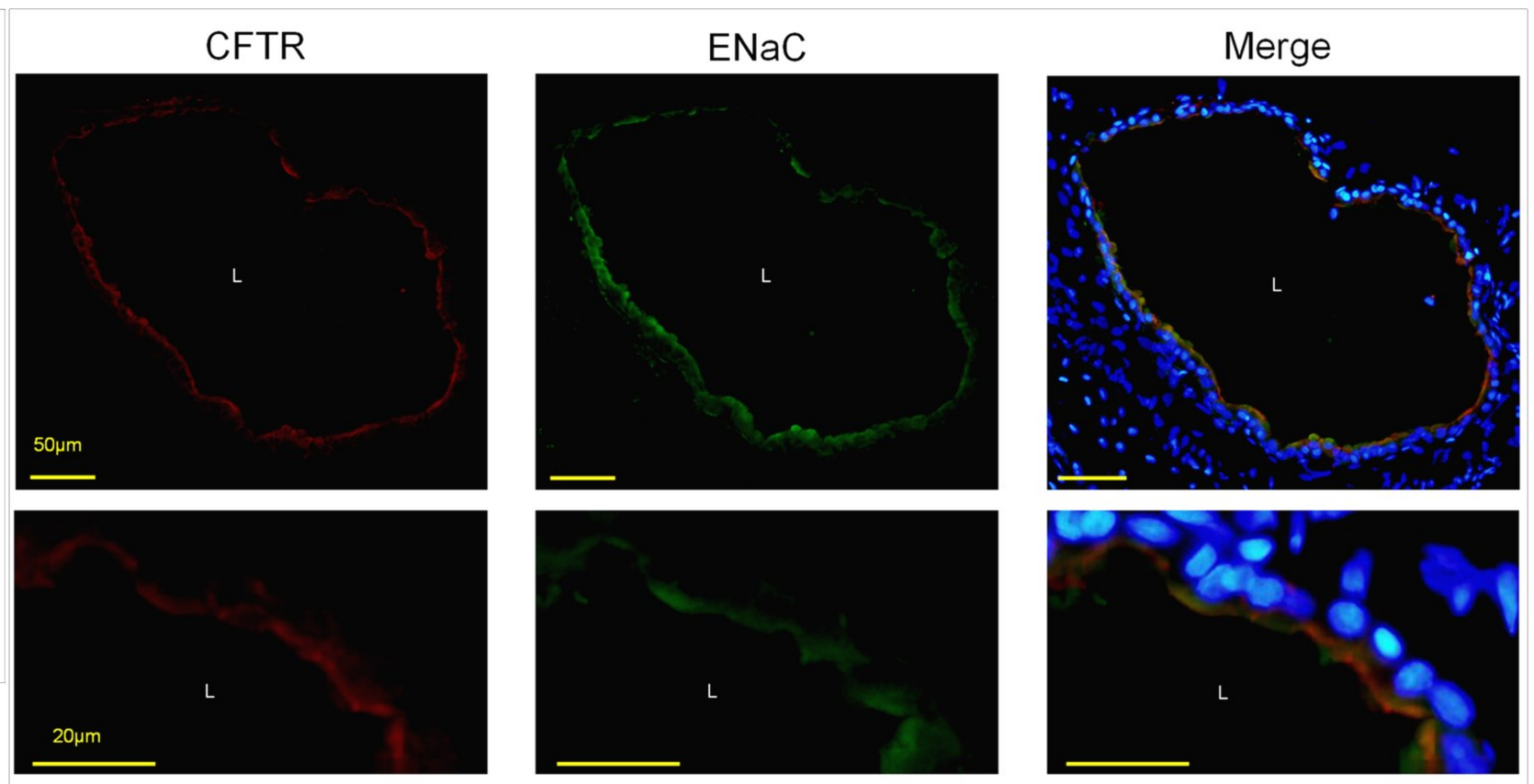

Fig. 2 Immunohistochemical analysis of the intermediate portion of the ES
Projection images obtained by fluorescence microscopy that show the localization of CFTR, ENaC, and both proteins with DAPI represented by merged images. CFTR is stained with specific red on the apical side of epithelia that co-localized with green-stained ENaC. L; lumen of the ES

Na^+,K^+-ATPase at the basolateral membrane provides the driving force for Na^+ absorption. Na^+ flow is generally coupled with Cl^- flow for neutralization of the charge movement, thereby guaranteeing ionic neutrality. ENaC needs Cl^- channels to neutralize the charge movement caused by Na^+ inflow. In the present study, CFTR expression was confirmed in the ES epithelia, which suggested a pathway for Cl^- at the apical membrane.

CFTR and ENaC exhibit tissue-specific functional interactions (Reddy MM et al. 1999,Schwiebert EM et al. 1999). We confirmed that both CFTR and ENaC were expressed in the apical membrane of ES epithelia. We postulate that the CFTR–ENaC interaction that is observed in other tissues occurs in the ES epithelia. Future experiments on CFTR conductance and CFTR–ENaC interactions are needed to understand the ion transport system in the ES.

Fig.3 Hypothetical model of ion transport in the ES epithelial cell