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Distribution and Regulation of ENaC Subunit and CFTR mRNA Expression in Murine Female Reproductive Tract

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Abstract. The present study investigated the regional distribution and cyclic changes in the mRNA expression of epithelial Na^+ channel (ENaC) subunit and cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated Cl^- channel, in adult female mouse reproductive tract. In situ hybridization revealed that in contrast to the abundant expression of CFTR, ENaC (α, β, γ) mRNA signal was not detected throughout the estrus cycle in the ovary and oviduct. Messenger RNA for all ENaC subunits was abundantly detected in the cervical and vaginal epithelia throughout the estrus cycle but for CFTR, mRNA was found only at proestrus. In the uterine epithelium, αENaC mRNA was detected at diestrus but not found at any other stage, while CFTR mRNA was only detected at early estrus but not other stages. Semi-quantitative RT-PCR detected mRNA for all ENaC subunits in the uterus throughout the cycle with maximal expression at diestrus and CFTR mRNA was only found in the early stages of the cycle. The involvement of ENaC and CFTR in Na^+ absorption and Cl^- secretion was demonstrated in cultured endometrial epithelia using the short-circuit current technique and found to be influenced by ovarian hormones. Taken together, these data indicate a main secretory role of the ovary and oviduct and a predominantly absorptive role of the cervix and vagina. The present results also suggest an ability of the uterus to secrete and absorb at different stages of the estrus cycle. Variations in the fluid profiles may be dictated by the regional and cyclic variations in expression of ENaC and CFTR and are likely to contribute to various reproductive events in different regions of the female reproductive tract.

Key words: CFTR — ENaC — Estrus cycle — Female reproductive tract — In situ hybridization

Introduction

The fluid environment in the female reproductive tract is considered of physiological significance for a number of reproductive events such as sperm transport and capacitation, sperm-egg interaction and fertilization, embryo transport, development and implantation. Deviations from the normal volume or compositions of the reproductive tract fluid are probably responsible for the reduced rate of fertilization seen in women with pathological conditions such as cystic fibrosis (Kopito, Kosasky & Shwachman, 1973) or hydrosalpinges (Mukherjee et al., 1996). Although cyclic changes in the fluid composition and volume have long been observed (Long & Evans, 1922; Beller & Schumacher, 1979), the origins and dynamics of the female reproductive tract fluid are incompletely understood and the molecular mechanisms underlying the fluid formation and regulation remain largely unknown.

Fluid movements across epithelia are secondary to the movement of solutes, particularly ions. Na^+ and Cl^- conductances in the apical membrane of epithelia are essential for electrolyte and fluid absorption and secretion, respectively. Active Na^+ absorption drives Cl^- counter ion and fluid out of the lumen into the blood, while active Cl^- secretion

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drives both Na^+ and fluid from the plasma into the lumen. The epithelia lining the female genital tract have been found to be able to absorb and secrete electrolytes. However, the ion channels responsible for transporting electrolytes across the female genital tract remain largely unknown. Recent electrophysiological investigations into the ion-transporting mechanisms in the female reproductive tract have indicated an amiloride-sensitive active Na^+-absorptive process and a secondary active Cl^--secretion mechanism in both the oviduct and endometrial epithelia in a number of species, including humans (Downing et al., 1997; Matthews et al., 1998; Deachapunya & O'Grady, 1998; Fong & Chan, 1998). Evidence also suggests that epithelial sodium channels (ENaC) and cystic fibrosis transmembrane conductance regulators (CFTR) are possibly involved in mediating the absorptive and secretory activities, respectively, of the endometrium (Deachapunya & O'Grady, 1998; Chan et al., 2000a; 2000b).

CFTR is a cAMP-regulated Cl^- channel, mutations in which are found to be responsible for the disease cystic fibrosis (CF), which affects most of the exocrine glands and tissues including the reproductive tracts (Quinton, 1999). Reduced fertility rate has been observed in women with CF (Kopito et al., 1973) although the relationship between defective CFTR and pathological consequences in the female reproductive tract remains obscure. Recent studies on cultured mouse endometrial epithelia have implicated CFTR in mediating a number of neurohormonal-regulated secretory responses (Fong & Chan, 1998; Chan et al., 1997a, 1999), further supporting an important role of CFTR in female reproduction.

ENaC, which consists of 3 homologous subunits (α, β and γ), is recognized by the high affinity to diuretic blocker amiloride and is expressed in a wide variety of epithelia including sweat glands, lung, distal colon, kidney and urinary bladder (Garty, 1994). Previous studies have shown that uterine fluid volume and its Na^+ concentration during preimplantation are relatively low as compared to other stages, suggesting that Na^+ and water are reabsorbed, leading to the "closure" of the lumen to promote successful embryo implantation (Hoversland & Weitlauf, 1981; Van Winkle, Campione & Webster, 1983). Recent electrophysiological studies have shown an amiloride-blockable short-circuit current in primary cultured mouse and human uterine epithelia that is indicative of ENaC function (Matthews et al., 1993; 1998; Chan et al., 1997b). However, the expression of α-, β- and γ-ENaC subunits has not been reported in any female reproductive tract tissue.

To elucidate the origins and mechanisms underlying fluid formation and to provide a physiological basis for the observed changes in fluid volume and composition in the female reproductive tract, the present study investigated the distribution and cyclic variations in the expression of ENaC subunits and CFTR along the female mouse reproductive tract by in situ hybridization and RT-PCR analysis. The functional role of these ion channels in uterine electrolyte transport was also demonstrated using the short-circuit current technique.

Materials and Methods

Materials

Paraformaldehyde (PFA) was obtained from Electronic Microscopy Sciences (Fort Washington, PA). Optimal Cutting Temperature (OCT) compound was from Sakura Fine Tec Co., (Torrance, CA). 20 × SSC was from USB (Cleveland, OH), proteinase K and tRNA were from Boehringer Mannheim (Indianapolis, IN). DEPC, EDTA, Tris, triethanolamine (TEA), acetic anhydride and dithiothreitol were from Sigma (St. Louis, MO). The restriction enzymes (RE) kit and in vitro transcription kit were from Promega (Madison, WI). Hematoxylin and eosin stains were from Richard-Allen Scientific. ^{35}S-UTP was from NEN Life Science Products (Boston, MA). Developer, fixer and nuclear tract emulsion (NTB-2) were from Kodak (Rochester, NY).

Dulbecco's modified Eagle medium with nutrient mixture F-12 (D-MEM/F-12), phosphate-buffered saline (PBS), fetal bovine serum, non-essential amino acids, pancreatin, RT-PCR kit, and CFTR and Na^+ channel primers were purchased from Gibco, penicillin-streptomycin was from Sigma. Forskolin and amiloride hydrochloride were purchased from Research Biochemical International. Matrigel was purchased from BD Labware (Lincoln Park, NJ).

In Situ Hybridization

Female reproductive tract tissues were isolated from B6/C57 adult mice, embedded in OCT compound, and frozen on dry ice. Frozen tissue blocks were sectioned (8 μm thick) and mounted on glass slides. Sections were fixed in 4% PFA for 1 hour, dehydrated, air-dried and stored at −20°C until use. An in situ hybridization protocol was carried out as previously described (Rochelle et al., 2000). Briefly, tissue sections were digested with proteinase K to promote riboprobe entry and acetylated to reduce the background signal Antisense and sense CFTR probes were prepared from mouse CFTR cDNA (1346–1706 bp), subcloned into pBluescript KS plasmids, and linearized with *Bam* H or *Kpn* I. Antisense and sense ENaC probes were prepared from mouse ENaC cDNA (α: 1263–1927 bp; β: 63–511 bp; γ: 583–1270 bp), subcloned into pCR II vector (Invitrogen), and linearized with *EcoR* V or *Hind* III. The sections were hybridized with ^{35}S-UTP probe (α-, β-, γ-ENaC) or ^{35}S-UTP and ^{35}S-CTP-labeled mCFTR RNA probe. Slides were washed and coated with NTB-2 autoradiography emulsion and then exposed for 4–6 days at 4°C. After the exposure period, slides were developed and counterstained with hematoxylin and eosin. The epithelia of each tissue were examined for the presence (+) or absence (−) of silver grains (Table) using bright- and dark-field microscopy. Each experiment was repeated at least three times.

Semi-Quantitative RT-PCR

Experiments were performed and repeated at least three times on mRNAs obtained from mouse uteri at different estrus stages. The specific oligo nucleotide primers for GAPDH were: GAC CAC AGT CCA TGA CAT CAC TGC (sense) and GCT GTT GAA

Table. Summary of the regional distribution of α, β, γENaC and CFTR in the female murine reproductive tract

Tissue	Stage	ENaC			CFTR
		α	β	γ	
Cervix and vagina	Proestrus	+	+	+	+
	Estrus	+	+	+	–
	Metestrus	+	+	+	–
	Diestrus	+	+	+	–
Uterus	Proestrus	–	–	–	–
	Estrus	–	–	–	+
	Metestrus	–	–	–	–
	Diestrus	+	–	–	–
Ovary and oviduct	Proestrus	–	–	–	+
	Estrus	–	–	–	+
	Metestrus	–	–	–	+
	Diestrus	–	–	–	+

GTC GCA GGA GAC AAC (antisense), corresponding to nucleotides 565–904 with expected cDNA of 340 bp. The specific oligo nucleotide primers for CFTR were: CAT CTT TGG TGT TTC CTA TGA TG (sense) and GTA AGG TCT CAG TTA GAA TTG AA (antisense), corresponding to nucleotides 1655–2135 with expected cDNA of 481 bp; αENaC: TCA CTT CAG CAC ATC TTC CAC AGC TGC (sense) and GTA TCT GCC TAG CTG GTC CAA GTG GGA (antisense), corresponding to nucleotides 2171–2960 with expected cDNA of 790 bp; βENaC: CCC CAC CCA GCA ACT AGT GAA CTC AAA (sense) and AAA GCA CGT GTT CCC CTT TCA AGA CTT (antisense), corresponding to nucleotides 1961–2380 with expected cDNA of 420 bp; γENaC: GAC TCT CTT CCT GAC ACA AAT GGT CCT (sense) and ACA CAC ATT CTC ACA CAT ACA CAT ACT (antisense), corresponding to nucleotides 2070–2793 with expected cDNA of 724 bp. The conditions were: denaturation at 94°C for 45 sec; annealing at 53°C, 58°C, 62°C, 53°C, 59°C for 60 sec; extension at 72°C for 60sec; 25, 30, 33 cycles for GAPDH, CFTR and γENaC respectively. Optimal amplification cycles were determined based on the linear relationship between the amount of PCR product detected and the number of amplification cycles. The intensities of the bands of CFTR and ENaC subunits were normalized to that of GAPDH, which was amplified simultaneously. Experiments in the absence of reverse transcriptase were conducted as negative control. PCR products were sequenced to make sure they were the target mRNA.

Estrus-Stage Identification

Identification of the mouse estrus cycle stages was based on the morphological changes of the vagina described by Yuan and Carlson (1987). The mouse estrus cycle was divided into 4 phases: proestrus, estrus, metestrus and diestrus. After the mice were sacrificed, the vagina was removed and embedded in OCT compound in dry ice. Frozen sections (8 μm) were mounted onto microscope slides. The tissue sections were counterstained with hematoxylin and eosin. In brief, proestrus was identified by the presence of layers of mucus cells, which stain lightly with hematoxylin, in the luminal vaginal epithelia. Estrus was defined by the thick layer of cornified cells, which stain strongly with eosin, in the luminal epithelia. The disappearance of the cornified cell layers and the presence of abundant cornified cells and leukocytes in the lumen were indicative of metestrus. Diestrus was determined by the presence of leukocytes and some epithelial cells in the lumen.

Cell Culture

Endometrial epithelial cells were enzymatically isolated from 3.5- to 4-week old immature mouse uterus so as to avoid the complication of the estrous cycle according to the method described by McCormack & Glasser (1980) with slight modifications (Chan et al., 1997b). In brief, the uteri were sliced longitudinally and treated in 7.5 mg/ml trypsin and 25 mg/ml pancreatin containing PBS at 0°C for 60 min and room temperature for another 45 min. The tissue was gently shaken for 30 sec. Uterine tissue was removed and the filtrate was centrifuged at 1000 × g for 3 min. The supernatant was discarded and the cell pellet was resuspended in D-MEM/F-12 medium containing 10% fetal bovine serum, 1% non-essential amino acids, 100 IU/ml penicillin and 100 μg/ml streptomycin. The isolated endometrial cells were plated on the Matrigel coated nitrocellulose Millipore filters (0.45 cm^2). Cultures were incubated at 37°C in 95% O_2/5% CO_2 and reached confluence in 3 days. 17β-estradiol was added to the cultures 24 hours prior to experiment.

Short-Circuit Current Measurement

The measurement of I_{SC} has been described previously (Ussing & Zerahn, 1951; Wong, 1988). Monolayers grown on permeable supports were clamped vertically between 2 halves of the Ussing chamber. The monolayers were bathed on both sides with Krebs-Henseleit solution, which was maintained at 37°C by a water jacket enclosing the reservoir. The Krebs-Henseleit solution had the following composition (mM): NaCl, 117; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 24.8; KH_2PO_4, 1.2; glucose, 11.1. The solution was bubbled with 95% O_2/5% CO_2 to maintain the pH of the solution at 7.4. Drugs could be added directly to the apical or basolateral side of the epithelium. The epithelium exhibited a basal transepithelial potential difference for every monolayer examined, which was measured by the Ag/AgCl reference electrodes (World Precision Instruments) connected to a preamplifier, which was connected in turn to a voltage clamp amplifier (DVC 1000; World Precision Instruments). The change of I_{SC} was defined as the maximal rise in I_{SC} following agonist stimulation and it was normalized as current change per unit area of epithelial monolayer (in $\mu A/cm^2$).

Statistical Analysis

Results are expressed as mean ± SEM, and *n* indicates the number of experiments. Comparisons between groups of data were made by Student's unpaired *t*-test. A *P* value of less than 0.05 was considered statistically significant.

Results

Cervix and Vagina

All ENaC subunits (α, β and γ) were detected in the cervical and vaginal epithelia throughout the estrus cycle (Table). Figure 1*A–C* illustrates the α, β and γ ENaC mRNA signals in the superficial epithelia of the cervix at proestrus. All ENaC subunits were similarly expressed in vagina throughout the cycle (Table). The cyclic expression pattern of αENaC in the vaginal epithelium is illustrated in Fig. 2*A–D*. As the thinning of the vaginal epithelial layer progressed towards the later stages of the cycle, the intensity of the mRNA signal decreased.

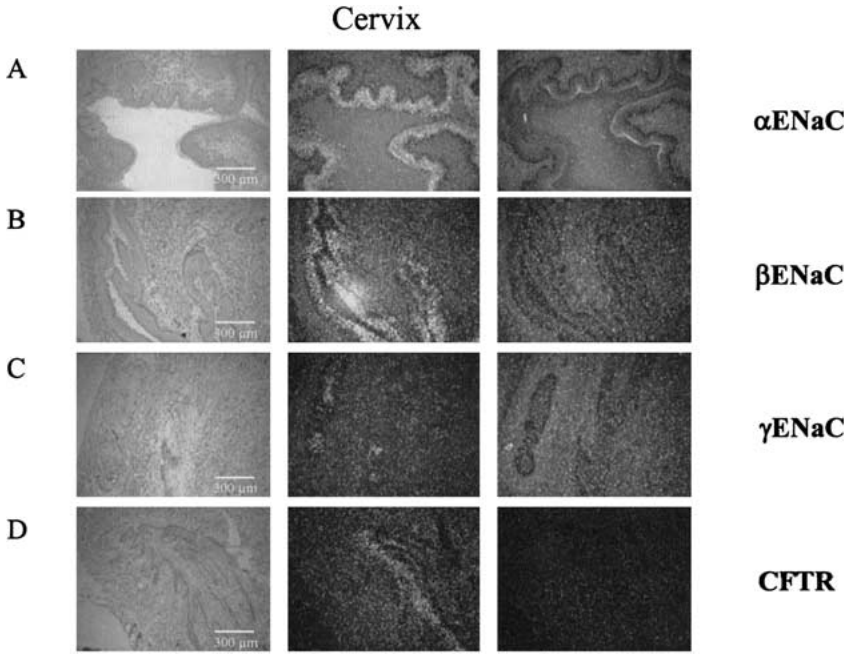

Fig. 1. Demonstration of the expression of mRNA for α epithelial sodium channels (αENaC), βENaC, γENaC and cystic fibrosis transmembrane conductance regulators (CFTR) in mouse cervical epithelia at proestrus by in situ hybridization. Left panels are the bright-field images of sections counter-stained with hematoxylin and eosin (*HE*). Middle panels are dark-field images of sections labeled with ^{35}S-antisense mRNA probes. Right panels are dark-field images of sections labeled with ^{35}S-sense mRNA probes (negative control).

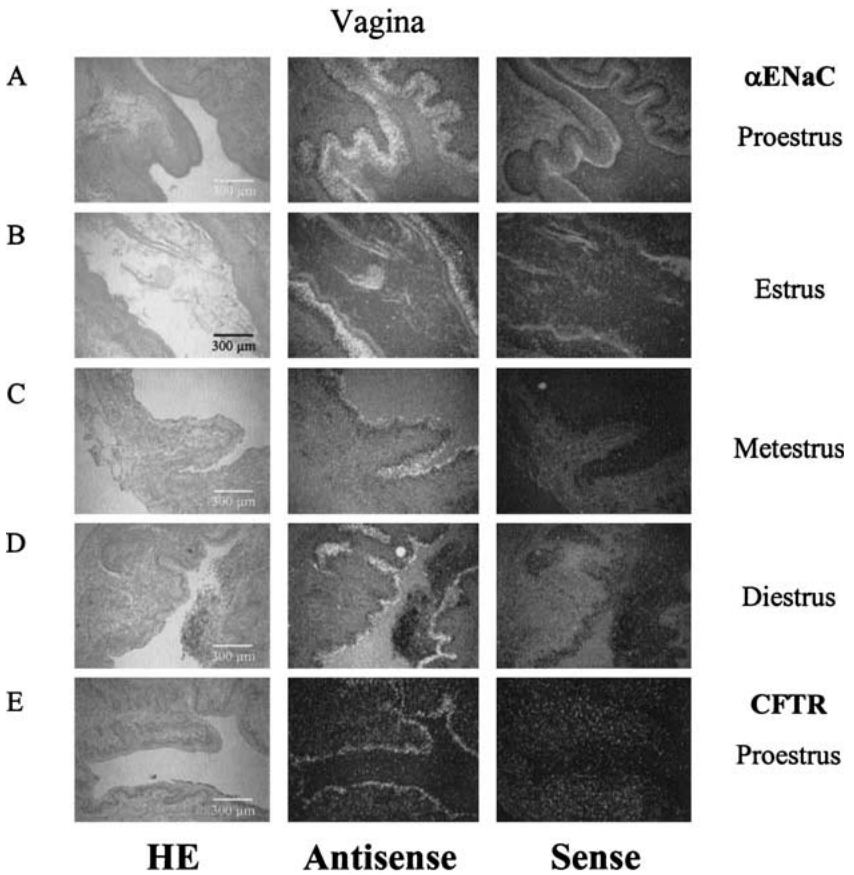

Fig. 2. Demonstration of the expression of mRNA for αENaC and CFTR in vaginal epithelia. Fig. 2*A–D* shows the expression of αENaC of proestrus, estrus, metestrus and diestrus vaginal epithelia. Fig. 2*E* shows the expression of CFTR of proestrus vaginal epithelia.

In contrast to the continuous expression of ENaC throughout the estrus cycle, the expression of CFTR mRNA was detected in the superficial epithelium of the cervix (Fig. 1*D*) and vagina (Fig. 2*E*) at proestrus only.

Uterus

αENaC mRNA was detected in the uterine epithelium during the diestrus (Fig. 3*A*) but not during other estrous stages (*data not shown*). The αENaC signal

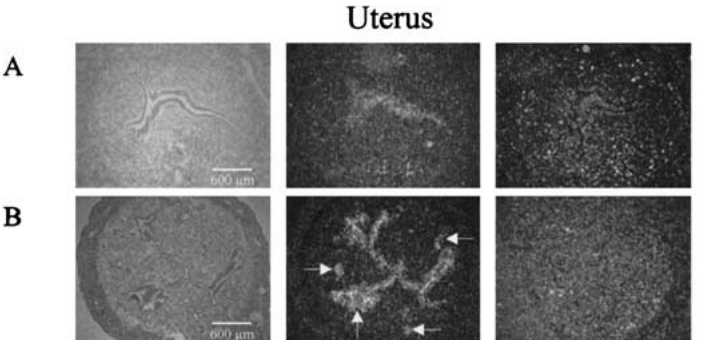

Fig. 3. Demonstration of the expression of mRNA for αENaC and CFTR in the uterine epithelia. Fig. 3*A* shows the expression of αENaC during diestrus. Fig. 3*B* shows the expression of CFTR during early estrus. Arrows indicate uterine glands.

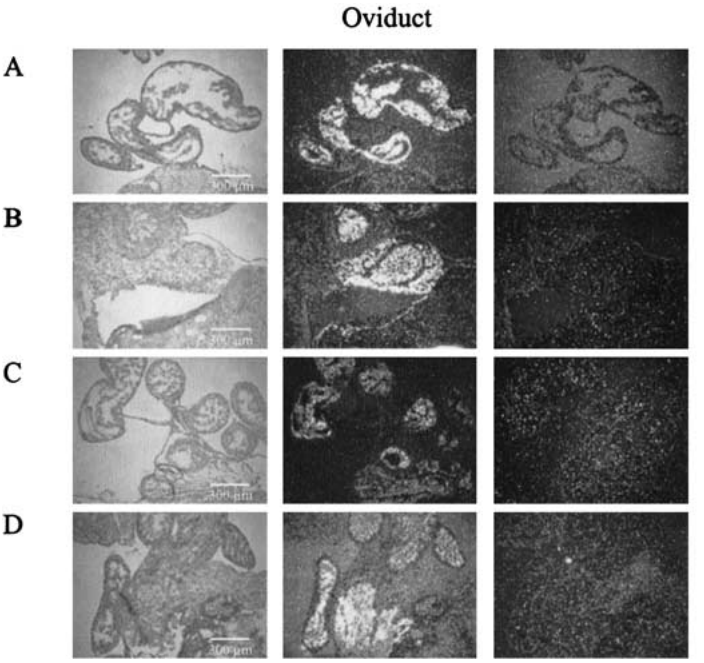

Fig. 4. Demonstration of the expression of mRNA for αCFTR in the oviduct epithelia. Figs. 4*A–D* show the detection of CFTR mRNA signal in oviduct epithelia at proestrus, estrus, metestrus and diestrus respectively.

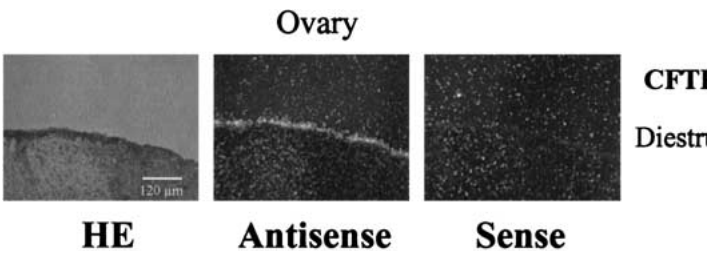

Fig. 5. Expression of CFTR mRNA in the ovarian surface epithelium at diestrus.

was concentrated in the luminal epithelium and hardly detectable in the glandular epithelial cells. Neither β nor γ ENaC was detected in the uterus throughout the estrus cycle (Table). CFTR mRNA was detected in the uterus at early estrus but not other stages (Table). As shown in Fig. 3*B*, CFTR mRNA was expressed both in luminal and glandular epithelia of the endometrium.

Ovary and Oviduct

None of the ENaC subunit mRNAs were detected in ovary and oviduct at any estrus stage (Table), indicating that ENaC does not have a significant role in regulating ovarian and oviduct fluid homeostasis.

In contrast to ENaC, CFTR was detected in both the ovary and oviduct throughout the estrus cycle. Figure 4*A–D* illustrates the abundant CFTR signal in the oviduct throughout the estrus cycle. Note that the CFTR signal in the proestrus and estrus oviduct was considerably stronger than that found at metestrus and diestrus. A representative image of CFTR signal detected in the surface epithelium of the ovary is shown in Fig. 5.

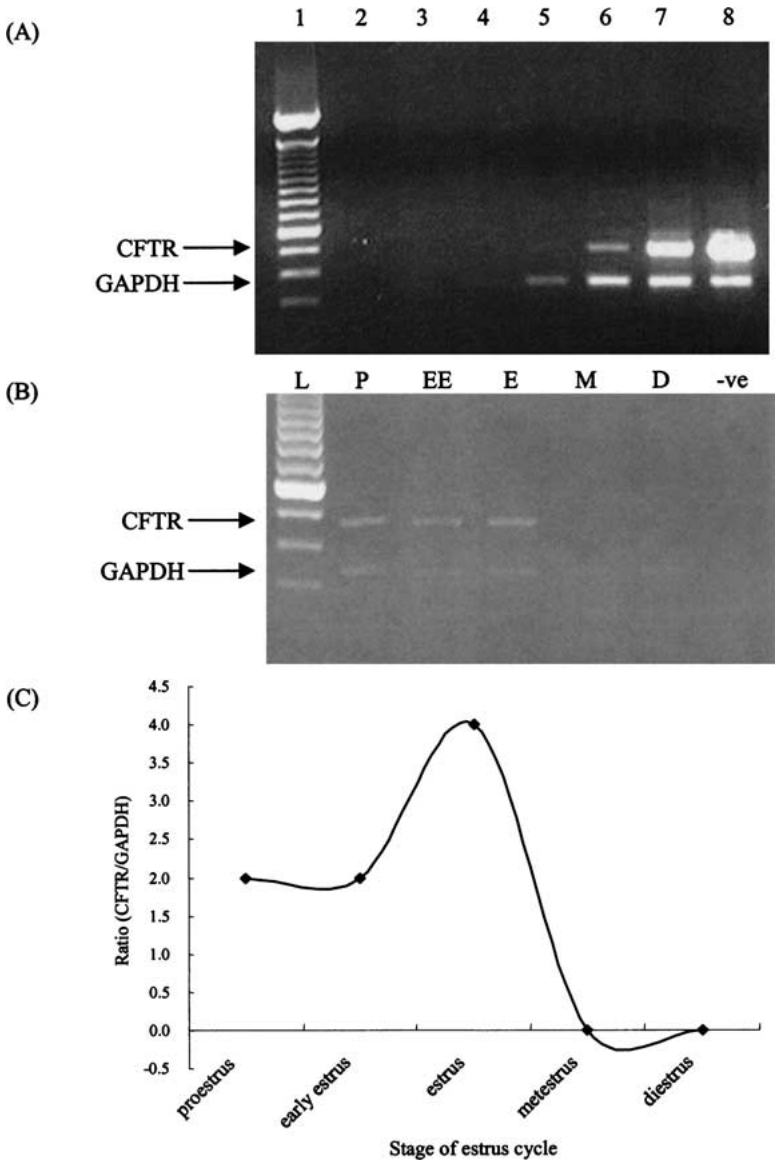

Fig. 6. Semi-quantitative RT-PCR analysis demonstrating the expression of uterine CFTR mRNA throughout the estrous cycle. (*A*) Agarose gel demonstrating the dependence of CFTR and GAPDH mRNA signals on PCR cycles (lane *2*: negative control of the absence of reverse transcriptase; lanes 3–8: 15, 20, 25, 30, 35 and 40 PCR cycles). (*B*) RT-PCR result of CFTR (481 bp) and GAPDH (340 bp) at proestrus (*P*), early estrus (*EE*), estrus (*E*), metestrus (*M*), diestrus (*D*) and in absence of reverse transcriptase (*-ve*), *L* is the DNA ladder. (*C*) Relative expression of uterine CFTR mRNA normalized with the internal marker GAPDH. Experiments were repeated three times, but only one set of results is presented for clear demonstration of the cyclic expression pattern.

Uterine CFTR and ENaC Expression by Semi-Quantitative RT-PCR.

The expression of CFTR- and EnaC-subunit mRNA in the uterus at different estrus stages was also examined by semi-quantitative RT-PCR analysis. Experiments were repeated at least three times, but only one set of representative results is presented for clear demonstration of the cyclic expression pattern. Optimal amplification cycles were determined based on the linear relationship between the amount of PCR product detected and the number of amplification cycles as shown for CFTR (Fig. 6*A*). Experiments in the absence of reverse transcriptase were also conducted to exclude the contamination of plasmids or genomic DNA. As shown in Fig. 6*B*, uterine CFTR mRNA level was high at proestrus and estrus but not detectable at metestrus and diestrus. On the other hand, mRNA for all ENaC subunits was detected in the uterus throughout the estrus cycle (Fig. 7–9). The expression levels of α and βENaC were low at proestrus and estrus, but reached a maximum at metestrus and diestrus (Figs. 7, 8). γENaC mRNA expression was low throughout the cycle except diestrus (Fig. 9). All PCR products were sequenced and an identity of over 98% and 99% with CFTR and ENaC subunits, respectively, was observed.

Effect of 17β-Estradiol on ENaC- and CFTR-Mediated I_{SC}

To demonstrate hormonal regulation of ENaC and CFTR activities in the endometrium, the I_{SC} technique was used in conjunction with cultured endometrial epithelia. The forskolin-induced I_{SC},

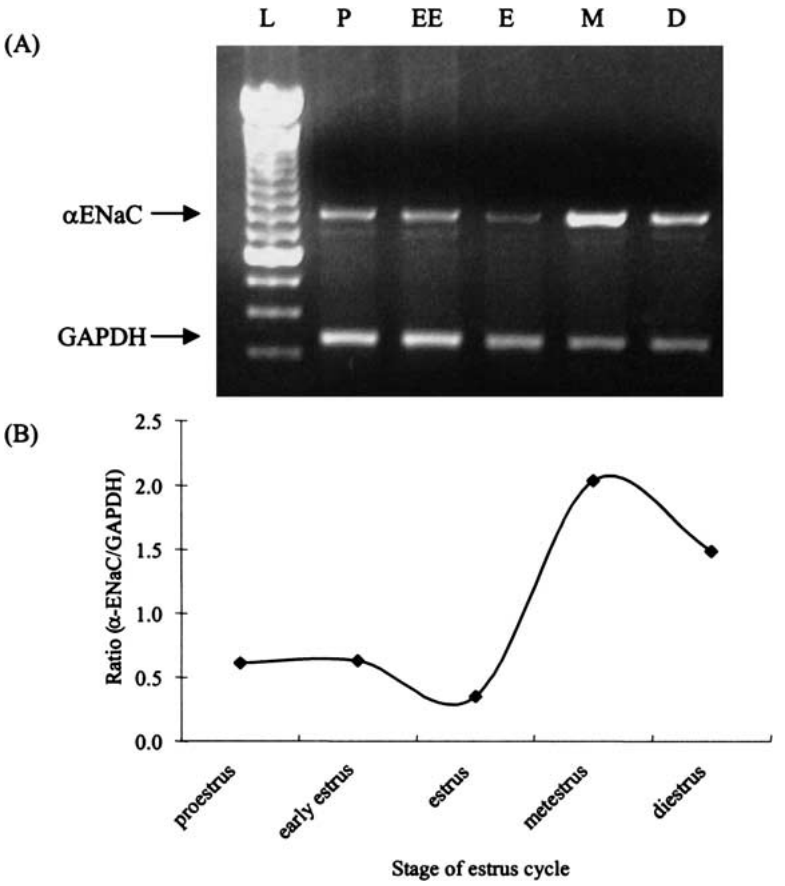

Fig. 7. Semi-quantitative RT-PCR analysis demonstrating the expression of uterine α-ENaC mRNA at different stages of the estrous cycle. (*A*) RT-PCR result of α-ENaC (790 bp) and GAPDH (340 bp). (*B*) Relative expression of uterine α-ENaC mRNA was normalized with internal marker GAPDH. Experiments were repeated three times, but only one set of results is presented for clear demonstration of the cyclic expression pattern.

presumably mediated by CFTR, was significantly increased from 5.1 ± 0.1 μA/cm^2 to 8.3 ± 0.7 μA/cm^2 ($P < 0.05$, $n = 6$, Fig. 10) in cultured epithelia treated with 0.01 μM of 17β-estradiol. However, the amiloride-sensitive I_{SC}, presumably mediated by ENaC, was significantly reduced in 17β-estradiol-treated epithelia (0.1 μM), from 17.87 ± 1.04 μA/cm^2 to 13.92 ± 0.92 μA/cm^2 ($P < 0.05$, $n = 6$, Fig. 11). The results were consistent with the expression patterns of CFTR and ENaC found above.

Discussion

The present study is the first to provide a molecular basis for the absorptive and secretory activities underlying fluid formation throughout the entire murine female reproductive tract. Although nearly 80 years has passed since the first classical study on cyclic changes in fluid accumulation in rat uterus was reported (Long & Evans, 1922), the molecular entities governing the electrolyte transport, which is essential for fluid formation in the female genital tract, remain largely unknown. Early measurements on vaginal and uterine fluid electrolyte composition have shown a low Na^+ concentration in relation to plasma. Studies on in vivo and in vitro transvaginal and transuterine potentials in rats (Levin & Edwards, 1968) and humans (Moghissi, 1979) have also indicated a reabsorptive mechanism of the female genital-tract epithelia. Recent studies on primary cultures of human and mouse endometrial epithelia have further demonstrated a substantial amiloride-sensitive basal short-circuit current under unstimulated conditions, which is indicative of Na^+ absorption (Matthews et al., 1993; 1998; Chan et al., 1997b). These findings have raised a fundamental question: what/where is the source of the Na^+ present in the female genital tract?

To answer this question we have to find the origin(s) of electrolyte and fluid secretion along the female reproductive tract. We undertook the present study with the assumption that active Cl^- secretion would drive both the counter-ion Na^+ and fluid into the lumen and that CFTR would be the key player in active Cl^- secretion across the female reproductive tract as indicated by the recent electrophysiological studies (Deachapunya & O'Grady, 1998; Chan et al., 1999; 2000b).

The present finding of abundant expression of CFTR in the ovary and oviduct throughout the estrus cycle suggests that this part of the female reproductive tract plays a major secretory role and is the constant source of NaCl and fluid in the genital tract.

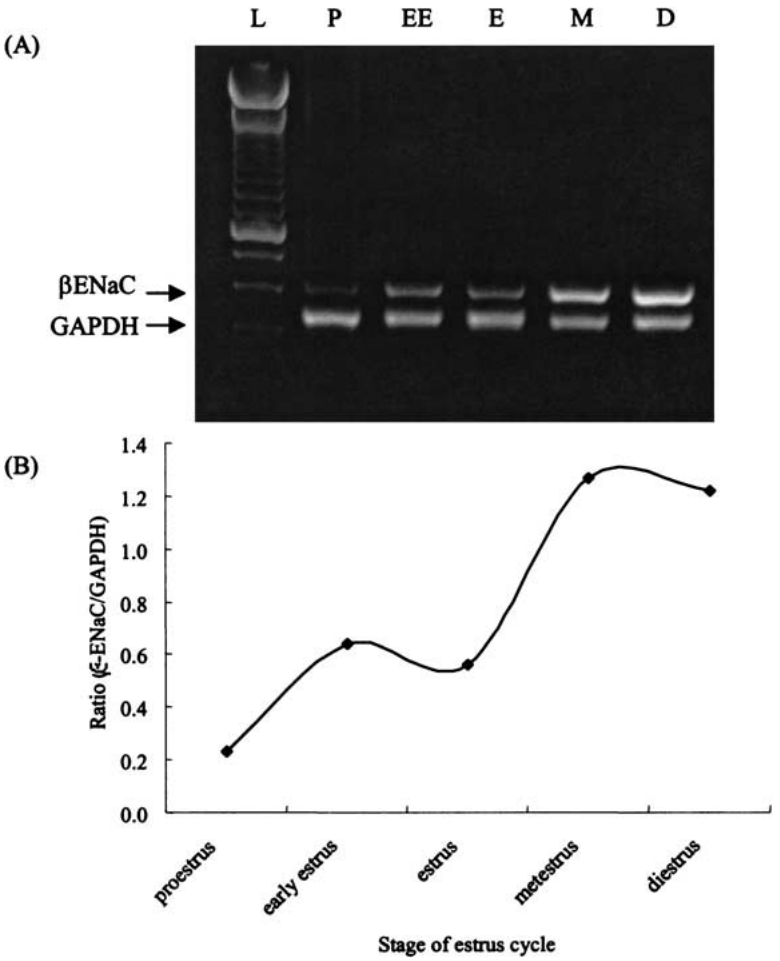

Fig. 8. Semi-quantitative RT-PCR analysis demonstrating the expression of uterine β-ENaC mRNA at different stages of the estrous cycle. (*A*) RT-PCR result of β-ENaC (420 bp) and GAPDH (340 bp). (*B*) Relative expression of uterine β-ENaC mRNA normalized with the internal marker GAPDH. Experiments were repeated three times, but only one set of results is presented for clear demonstration of the cyclic expression pattern.

The lack of ENaC expression in the ovary and oviduct suggests that little Na^+ and fluid reabsorption occurs in this part of the genital tract and, thus, the fluid found in the lower part of the genital tract may be contributed by the fluid of ovarian or ductal origin. On the contrary, the expression of all ENaC subunits in the mouse cervical and vaginal epithelia throughout the estrus cycle indicates a primarily reabsorptive role of the cervix and vagina with ENaC as a main pathway for Na^+, and eventually water reabsorption to prevent salt and water loss from cervical and vaginal discharges. Expression of all α-, β-, and γ-ENaC subunits has also been observed in human cervical epithelium throughout the menstrual cycle (Rochelle et al., Chan et al., unpublished data), indicating an important role of ENaC in both murine and human cervix.

Our molecular evidence for polarized mRNA expression of CFTR and ENaC at the two ends of the genital tract agrees well with findings from previous electrophysiological studies. A transvaginal potential difference with negative luminal polarity consistently observed in women throughout the menstrual cycle (Moghissi, 1979), together with a luminal NaCl concentration lower than that in the plasma (Duncan & Levin, 1976), is consistent with the expression of ENaC in the vagina and cervix. A lack of ENaC expression in the oviduct may account for the relatively small and amiloride-insensitive basal transepithelial potential difference and short-circuit current observed in primary cultures of oviduct epithelia of rabbit (Dickens & Leese, 1994), mouse (Leung et al., 1995) and human (Downing et al., 1997). On the other hand, the presence of the forskolin-sensitive current, presumably mediated by CFTR, observed in normal but not CFTR knock-out mouse oviduct epithelium (Leung et al., 1995) is consistent with the presently observed abundant expression of CFTR in the mouse oviduct. It should be noted that the expression of CFTR in human fallopian tube has also been observed (Rochwerger & Buchwald, 1993; Tizzano et al., 1994).

The present results offer the potential for vaginal, cervical and uterine secretion as evidenced by the expression of CFTR in these regions although its expression in these regions depends on the cycle and is relatively brief as compared to the continuous expression of CFTR in the ovary and oviduct. This finding is consistent with the previous notion that fluid secretion can originate from different regions of

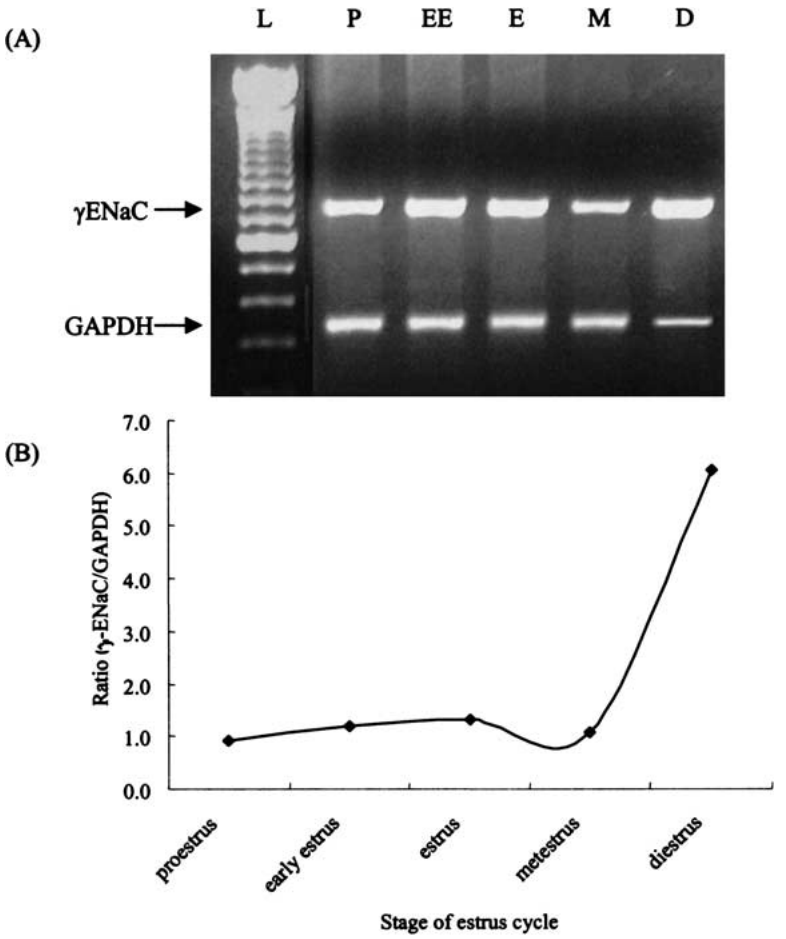

Fig. 9. Semi-quantitative RT-PCR analysis demonstrating the expression of uterine γ-ENaC mRNA throughout the estrous cycle. (*A*) RT-PCR result of γ-ENaC (724 bp) and GAPDH (340 bp). (*B*) Relative expression of uterine γ-ENaC mRNA normalized with the internal maker GAPDH. Experiments were repeated three times but only one set of results is presented for clear demonstration of the cyclic expression pattern.

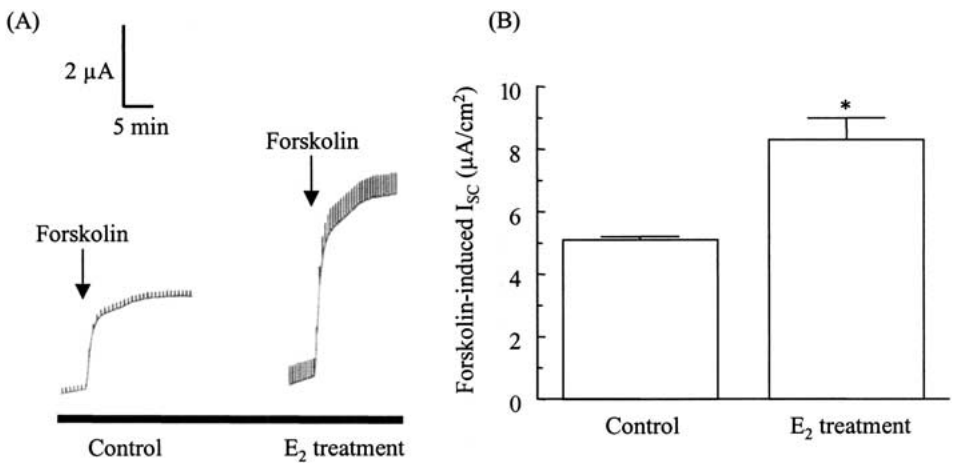

Fig. 10. Effect of 17β-estradiol on the forksolin-induced I_{sc}. (*A*) Representative I_{sc} recording of the forskolin-induced I_{sc} after amiloride pretreatment in control and 17β-estradiol-treated (E_2, treatment, 0.01μM) epithelia. (*B*) Statistical results showing magnitude of the forskolin-induced I_{sc} in control and 17β-estradiol-treated epithelia. Data are means ± SEM. (*$P < 0.05$, $n = 6$).

the female reproductive tract (Armstrong, 1968; Casslen, 1986). The estrus cycle-dependent expression of CFTR, i.e., at proestrus in the vagina and cervix, and at estrus in the uterus, is consistent with observed cyclic changes in ovarian hormones (Tejada et al., 1998). Stimulation of CFTR expression by estrogen both in vivo and in vitro has been demonstrated (Rochwerger & Buchwald, 1993; Rochwerger et al., 1994) and downregulation of CFTR by progesterone has also been reported (Mularoni et al., 1995). The

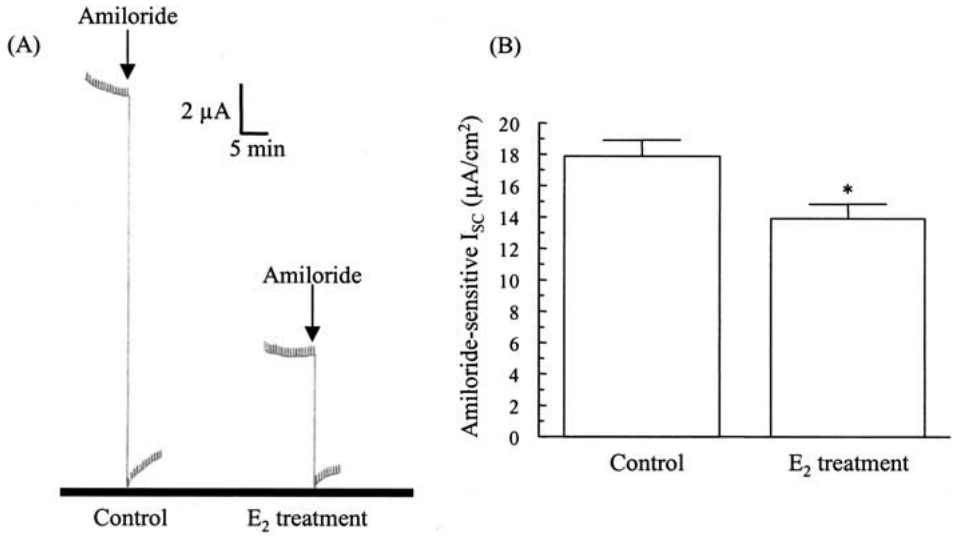

Fig. 11. Effect of 17β-estradiol on the amiioride-sensitive I_{sc}. (*A*) Representative I_{sc} recording of the amiioride-sensitive I_{sc} in control and 17β-estradiol treated (E_2 treatment, 0.1 μM) epithelia. (*B*) Statistical results showing current magnitude of the amiloride-sensitive I_{sc} in control and 17β-estradiol-treated epithelia. Data are means ± SEM. (*$P < 0.05$, $n = 6$).

ovarian hormone-dependent regulation of CFTR expression provides a physiological basis for the cyclic changes in fluid volume, which have long been observed in different regions of the female reproductive tract, most notably with the highest fluid accumulation during proestrus and estrus. CFTR as a Cl^- channel provides apical exit of Cl^- that has accumulated in the cell via a basolaterally located bumetanide-sensitive secondary active transport mechanism (Fong, Liu & Chan, 1998). Therefore, enhanced expression of CFTR at proestrus/estrus is expected to bring about a higher rate of Cl^- secretion and thus greater fluid accumulation.

We should note that there are discrepancies between the present results and those obtained by others on the CFTR-expression profile along the female genital tract. For example, continuous expression of CFTR has been detected in human cervix using the RT-PCR technique (Hayslip, Hao & Usala, 1997) and rat uteri throughout the estrus cycle using in situ hybridization (Trezise et al., 1993). While the discrepancy could be attributed to species differences, the difference in the sensitivity of the techniques used could result in the apparently different expression profiles. Indeed, the present study also revealed a higher sensitivity of the RT-PCR technique over that of in situ hybridization. It is also possible that a lower efficiency of our probes used as compared to those used by others could limit our detection of CFTR expression by in situ hybridization.

The out-of-phase co-expression of CFTR and ENaC in the uterus provides further evidence supporting an ability of the uterus to both secrete and absorb uterine fluid. Long and Evans (1922) first described the accumulation of fluid that distends the uterine horns of rats at proestrus and estrus and that is absent at diestrus. While there was no doubt that the horns could become distended as a result of estrogen stimulation, there was a debate whether the cervix was the route of uterine fluid loss at diestrus, since the cervical canals could be opened by the action of progesterone. Later experiments with ligation of the cervix while the uterus was under the influence of either estrogen or progesterone, clearly indicated that estrogen was capable of stimulating secretion of fluid while progesterone stimulated absorption of fluid in the rat uterus (Armstrong, 1968; Clemetson, Verma & De Carlo, 1977). The hormone-dependent changes in uterine fluid volume can be explained by differential regulation of CFTR and ENaC expression by ovarian hormones observed in the present study. For example, estrogen stimulates CFTR expression but down-regulates ENaC resulting in enhanced fluid secretion during the early phase of the estrus cycle, as demonstrated by the results obtained from the I_{SC} measurements showing decreased Na^+ absorption and increased Cl^- secretion by estrogen. There appear at least two different mechanisms responsible for the disappearance of uterine fluid at diestrus: 1) downregulation of CFTR to slow down fluid production and 2) upregulation of ENaC to increase the rate of reabsorption. The absence of CFTR at diestrus may further augment ENaC function to facilitate fluid reabsorption, another possible role of CFTR as a negative regulator of ENaC (Szutts et al., 1995).

The out-of-phase co-expression of CFTR and ENaC in the uterus may be of physiological significance. While maximal CFTR expression at estrus may enable a higher rate of uterine fluid production

to facilitate sperm transport and sperm capacitation, downregulation of CFTR and upregulation of ENaC at metestrus and diestrus may reduce the fluid volume in the lumen to enhance close contact between the endometrial surface to facilitate implantation of the embryo.

The expression pattern of ENaC and CFTR in the cervix and vagina suggested that both ENaC and CFTR work closely together to maintain an optimal cervical and vaginal fluid environment for sperm movement and optimal antimicrobial activity. In addition to a primarily reabsorptive role of the vagina and cervix as dictated by the expression of ENaC throughout the cycle, the expression of CFTR at proestrus may serve to lubricate the cervical and vaginal lumen and reduce the viscosity of the mucus for sperm movement towards the oviduct for successful fertilization. In fact, the primary cause for the reduced fertility rate observed in CF women has been suggested to be the formation of thick cervical mucus, which acts as a barrier to sperm penetration (Kopito et al., 1973). The present finding of CFTR expression along the female reproductive tract, particularly in the ovary and oviduct, extends the possible pathophysiological basis for the reduced fertility rate observed in CF women to other regions of the reproductive tract. For example, there may be an important role of CFTR in mediating neurohormonal-regulated secretory responses in the endometrium (Fong & Chan, 1998; Chan et al., 1997a, 1999) and conducting HCO_3^- (Poulsen et al., 1994), an important ion involved in the processes of sperm capacitation (Visconti et al., 1999) and embryo development (Boatman, 1997). While a defect in CFTR appears to result in reduced fertility in CF women, it is not known whether the fertility rate in women with ENaC mutations such as in Liddle's syndrome and PHA1 was affected.

The present study suggests an important role for CFTR in mediating Cl^- secretion in the female reproductive tract; however, the present results do not exclude possible involvement of other Cl^- conductive pathways. In fact, despite the observed CFTR expression in human fallopian tube (Tizzano et al., 1994), no cAMP-stimulated or CFTR-mediated Cl^- current was observed in cultured human fallopian tubule epithelia; instead, an ATP-activated Cl^- current that could be blocked by SITS was found (Downing et al., 1997). It could be reasoned that CFTR expression or function was lost during the culture of human fallopian tubule cells while the alternative Cl^- conductive pathway was preserved. Similarly, the limited expression of CFTR presently observed in the vagina and cervix suggested that CFTR is not the sole secretory mechanism, since the vagina is known to be able to secrete under mechanical and external stimulation at any time. Gorodeski and Goldfarb (1997) have also demonstrated ATP-stimulated secretion in an endocervical epithelial cell line. It would be interesting to identify the molecular entities of other alternative pathways and examine their regulation by hormones.

In summary, the present study has demonstrated for the first time the distribution of ENaC and CFTR mRNA expression in the murine female reproductive tract. The differential expression of CFTR and ENaC at the two ends of the tract indicates a main secretory role of the ovary and oviduct and a predominantly absorptive role of the cervix and vagina. The present results also suggest an ability of the uterus to secrete and absorb at different stages of the estrus cycle. Regional and cyclic variations in the expression of ENaC and CFTR are likely to alter the composition of the genital tract fluid to permit successful reproductive events occurring in the different regions.

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