Biochemical and Biophysical Research Communications **276,** 40–44 (2000)
doi:10.1006/bbrc.2000.3426, available online at http://www.idealibrary.com on IDEAL®

Suppression of CFTR-Mediated Cl^- Secretion by Enhanced Expression of Epithelial Na^+ Channels in Mouse Endometrial Epithelium

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Received July 31, 2000

The present study investigated the effect of enhanced expression of epithelial Na^+ channels (ENaC) on the cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl^- secretion in the mouse endometrium using the short-circuit current technique. The amiloride sensitivity of the basal current of the cultured endometrial epithelia was found to vary with the magnitude of the basal current, the higher the basal current the greater its sensitivity to amiloride, indicating possible elevation of ENaC expression. However, the magnitude of the forskolin-induced Isc, previously demonstrated to be mediated by CFTR, decreased as the amiloride sensitivity of the basal current increased, suggesting a possible inhibitory effect of elevated expression of ENaC on CFTR-mediated Cl^- secretion. The Matrigel concentration for culturing the endometrial epithelia was found to affect the amiloride sensitivity of the basal current as well as the forskolin-induced Isc in opposite directions. However, competitive RT-PCR demonstrated that the expression of both ENaC and CFTR was enhanced in Matrigel-treated culture, suggesting that the reduced forskolin-induced Isc with enhanced amiloride sensitivity was not due to a reduction in CFTR expression, but rather suppression of CFTR function by enhanced ENaC expression. In addition to the previously demonstrated inhibition of ENaC by activation of CFTR, the present results reveal possible regulation of CFTR by ENaC. The interaction between the two may be one of the underlying mechanisms for balancing Na^+ absorption and Cl^- secretion across epithelia. © 2000 Academic Press

Key Words: **endometrium; mouse; CFTR; ENaC; Cl^- secretion; Na^+ absorption; forskolin; Matrigel.**

Many epithelia are capable of both active Na^+ absorption and Cl^- secretion, thereby regulating fluid balance in our bodies. The switching between Na^+ absorption and Cl^- secretion is fundamental for epithelial functions; however, the mechanism governing these opposing processes is poorly understood. Recently demonstrated inhibition of epithelial Na^+ channels (ENaC) by activation of CFTR in a number of epithelia (1–7) including the mouse endometrium (8) has begun to explain how salt and water transport may be regulated by switching from basal Na^+ absorption to Cl^- secretion upon stimulation. Defective regulation of such process gives rise to pathophysiological conditions as seen in cystic fibrosis airways where defective CFTR results in impaired Cl^- secretion and hyperabsorption of Na^+. On the other hand, under normal physiological conditions mechanisms are likely to exist by which epithelia could maximize Na^+ absorption with minimal Cl^- secretion. The present study investigated this possibility and demonstrated suppression of CFTR-mediated Cl^- secretion by enhanced expression of ENaC in the mouse endometrial epithelium.

MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's 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 Laboratory (Grand Island, NY), while *N*-methyl-D-glucamine (NMDG), D-gluconic acid, penicillin–streptomycin, aldosterone were from Sigma (St. Louis, MO). Forskolin and amiloride hydrochloride were purchased from Research Biochemical International (Natick, MA). Matrigel was purchased from Collaborative Biomedical Products (Bedford, MA). Diphenylamine-2,2′-dicarboxylic acid (DPC) was bought from Riedel de Haen Chemicals (Hanover, Germany).

Cell culture. Endometrial epithelial cells were enzymatically isolated from the mouse uterus according to the method described by McCormack and Glasser (9) with slight modifications (10). The isolated endometrial cells were plated on nitrocellulose Millipore filters (0.45 cm^2) without or with Matrigel coating. 8× dilution of Matrigel

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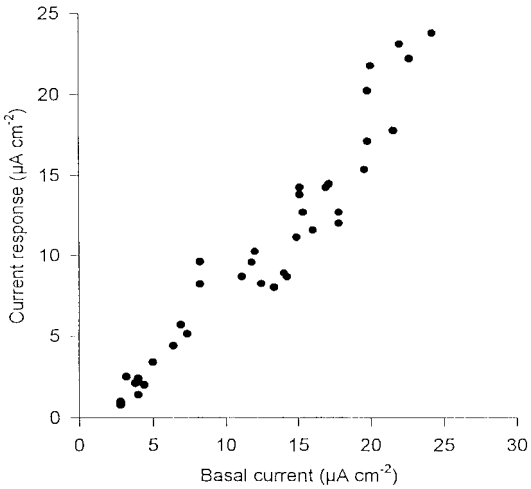

FIG. 1. Correlation of amiloride sensitivity with basal Isc. Basal current magnitude is plotted against he current response to amiloride (1 μM).

was used to coat the filters (single-coated) and a second layer of Matrigel was applied to some filters after the first layer was air-dried (double-coated). Cultures were incubated at 37°C in 95% O_2/5% CO_2 and reached confluence in 3 days.

Short-circuit current measurement. The measurement of I_{SC} has been described previously (11, 12). The Krebs–Henseleit solution used 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.

Competitive RT-PCR. 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 (13). The specific oligo nucleotide primers for Na^+ channel (γ subunit) were 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 (14). The conditions were: denaturation at 94°C for 45 s; annealing at 58°C for 90 s; extension at 72°C for 90 s; 40 cycles. The intensities of the bands of CFTR and ENaC were normalized to that of GAPDH (15) which was amplified simultaneously.

RESULTS AND DISCUSSION

Amiloride Sensitivity of the Basal Isc and the Forskolin-Induced Isc

Previous studies have shown that cultured endometrial epithelia exhibit a basal Isc which is predominantly amiloride-sensitive, indicative of Na^+ absorption which could be switched to predominant CFTR-mediated anion secretion upon stimulation (10, 16, 17). In the present study, variations in basal Isc were observed for different batches of cultured endometrial epithelia. Interestingly, when the distribution of the level of basal Isc was plotted against the Isc response to amiloride (Fig. 1), a correlation between elevated basal Isc and increased amiloride sensitivity was observed, indicating enhanced Na^+ absorption probably due to enhanced ENaC expression. We further studied the changes in the forskolin-induced anion secretion in the presence of amiloride by analyzing data from three groups of cells exhibiting an averaged basal Isc of 7.9 $\pm$ 0.5, 15.1 $\pm$ 0.7 and 22.8 $\pm$ 0.3 μA/cm^2. As shown in Fig. 2, the increase in amiloride-sensitive Isc corresponded to the increase in basal Isc, but the forskolin-induced Isc in the presence of amiloride, which was mediated predominantly by Cl^- secretion, decreased with elevated basal Isc. It should be noted that inhibition of high-rate Na^+ influx across the apical membrane may hyperpolarize the apical membrane, and therefore, increase the driving force for Cl^- secretion, the stronger the inhibition by amiloride the stronger the driving force for Cl^- secretion. However, a decrease in the forskolin-induced Cl^- secretion was observed as the amiloride sensitivity of the basal current increased, which ruled out the possibility that the changes in the forskolin-induced Cl^- secretion was due the changes in the driving force induced by amiloride blockage of Na^+ influx. Instead, the inverse relationship between the forskolin-induced Cl^- secretion and the amiloride sensitivity of the basal Isc suggests that the cAMP-activated anion secretion may be downregulated by enhanced ENaC expression.

Effect of Matrigel on ENaC and CFTR Expression

The variation in the level of amiloride sensitivity of the basal Isc observed in different cultures of epithelial cells suggested possible variation in ENaC expression in different cultures. This was probably due to variations in culture cell density since isolated cells formed clumps preventing accurate cell counting and thus affecting the final formation of the epithelia. Observations have been reported on airway epithelial cells that different culture conditions, such as different culture substrates (plastic vs filter) and hormone treatments, give rise to differential expression of ENaC (18). We tried to define the endometrial cultures using Matrigel which resembles the extracellular matrix facilitating rapid epithelial reconstitution and differentiation (19, 20). The amiloride sensitivity of endometrial cells cultured on different permeable supports, i.e., non-Matrigel-coated, single- and double-Matrigel-coated, was examined and compared. As shown in Fig. 3A, different Matrigel coating conditions gave rise to different levels of basal Isc and amiloride sensitivity, with non-Matrigel-treated cultures exhibiting lowest amiloride sensitivity and double-Matrigel-coated the highest. On the contrary, the magnitude of the forskolin-induced Isc obtained from the double-Matrigel-coated cultures was lower compared to that obtained from signal-Matrigel-coated cultures (Fig. 3B). These results again indicate a reduced cAMP-activated

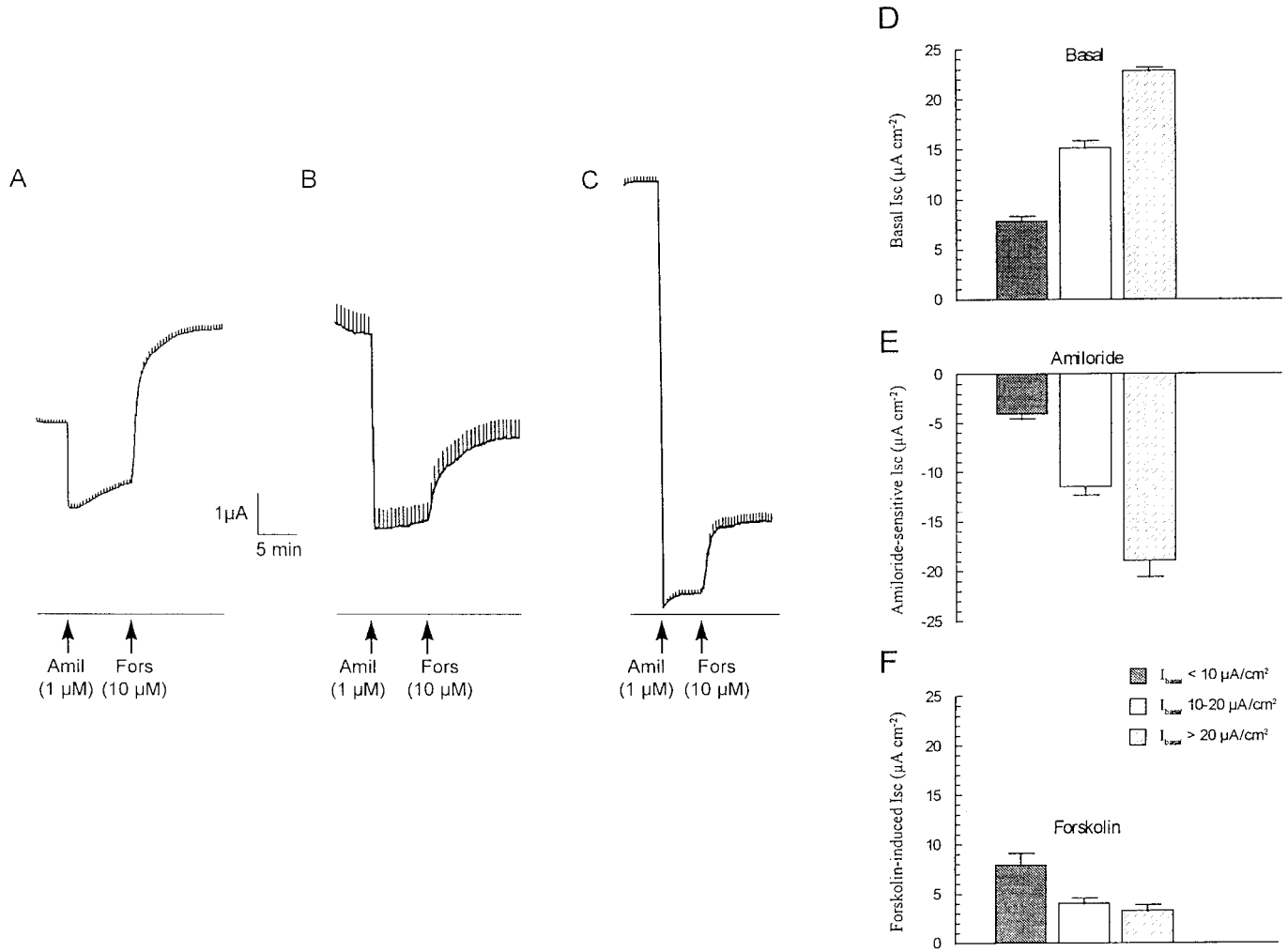

FIG. 2. Reciprocal relationship between amiloride-sensitive Isc and forskolin-induced Isc. Representative Isc recordings of amiloride-sensitive Isc and the subsequent forskolin-induced Isc with basal Isc less than 10 μA cm^{-2} (A), between 10 and 20 μA cm^{-2} (B) and greater than 20 μA cm^{-2} (C). Statistical results obtained from three groups of epithelia showing current magnitude of basal current (D), amiloride-sensitive current (E) and forskolin-induced current (F). Data are means ± SEM ($n > 10$).

anion secretion accompanied with enhanced amiloride sensitivity.

The expression of ENaC and CFTR in non-Matrigel-treated and double-Matrigel-coated cultures was examined by competitive RT-PCR using primers designed from mouse sequences for CFTR and ENaC γ subunit. GAPDH was used as an internal marker for normalization. As shown in Fig. 4, bands at 481 and 724 bp as expected for CFTR and ENaC γ subunit, respectively, were obtained. The expression of both γENaC and CFTR was enhanced in double-Matrigel-coated cultured epithelial cells compared to non-Matrigel-coated cultures. The enhanced expression of ENaC was consistent with the enhanced amiloride sensitivity observed. However, the observed reduction in the forskolin-induced, or CFTR-mediated, Isc could not be explained by the CFTR expression pattern. These data tend to suggest suppression of CFTR function by enhanced ENaC expression, perhaps by mechanisms similar to those suggested for inhibition of ENaC by CFTR (21). These may include interference of exo/endocytosis of CFTR by ENaC and direct protein-protein interaction between ENaC and CFTR or other regulatory proteins at functional level. It is interesting to noted that previous studies in patched and voltage-clamped airway epithelial cells grown on different culture conditions observed the appearance of an amiloride-sensitive Na^+ conductance accompanied by a reduction of the cAMP-activated Cl^- conductance (18). The present finding of enhanced ENaC expression accompanied with a reduction in CFTR-mediated Cl^- secretion in Matrigel-coated cultures tends to suggest

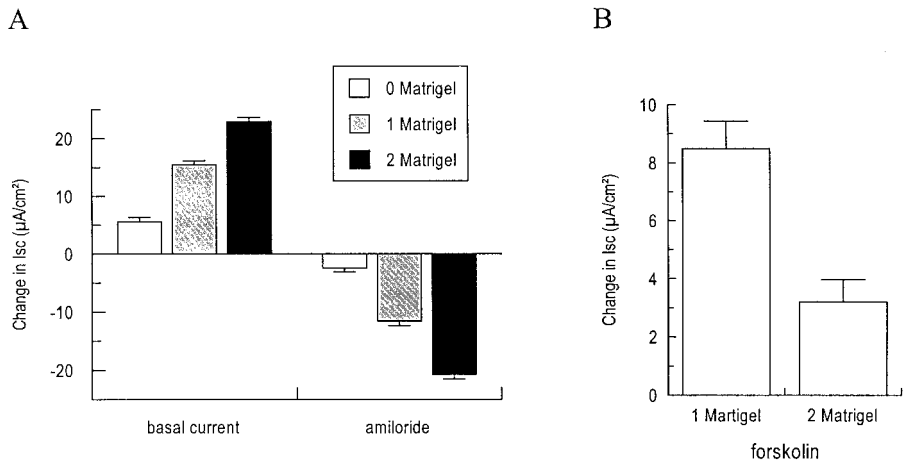

FIG. 3. Effect of Matrigel coating on amiloride sensitivity of basal current and the forskolin-induced Isc. (A) Mean current magnitude ($n > 15$) of basal Isc and amiloride-sensitive Isc obtained from non-Matrigel-coated, single-Matrigel-coated and double-Matrigel-coated cultures. (B) Mean current magnitude of forskolin-induced Isc obtained from single-Matrigel-coated and double-Matrigel-coated cultures (see text for details). Forskolin (10 μM) was added after the addition of amiloride (1 μM).

that the functional expression of ENaC and CFTR may be predetermined by cellular mechanisms governing differentiation such as epithelial polarity.

Therefore, there appears to be an interaction between CFTR and ENaC in the mouse endometrial epithelium. Previously demonstrated inhibition of ENaC upon activation of CFTR may provide a mechanism for switching from predominant Na^+ absorption under basal condition to predominant anion secretion upon stimulation (8). On the other hand, the presently observed suppression of CFTR-mediated Cl^- secretion by enhanced level of ENaC expression may also have physiological significance. While preparing the endometrium for implantation, the expression of ENaC may be elevated with suppressed CFTR-mediated Cl^- secretion, thereby preconditioning the endometrium in favor of overall salt and water absorption as observed during embryo implantation. The interplay between CFTR and ENaC may also determine the delicate balance between Cl^- secretion and Na^+ absorption in other epithelia.

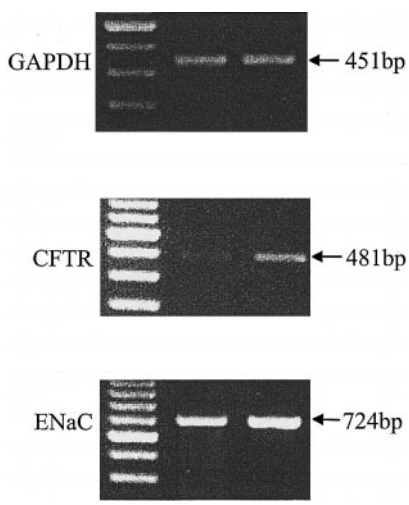

FIG. 4. Competitive RT-PCR analysis demonstrating enhancement of both CFTR and γENaC expression in double-Matrigel-coated cultures compared to that in non-Matrigel-coated cultures. GAPDH was used as an internal marker.

ACKNOWLEDGMENTS

The work was carried out at the Epithelial Cell Biology Research Center and supported by the Direct Grant (2040775) and Strategic Research Program of the Chinese University of Hong Kong and Natural Science Foundation of China.

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