The amiloride-sensitive epithelial Na\(^+\) channel (ENaC), which is made of three different but homologous subunits, controls the rate of transepithelial Na\(^+\) absorption in a variety of epithelia. The present study investigated the functional role of its subunits in regulating ENaC activity, measured as amiloride sensitive short-circuit current \((I_{SC})\), in the mouse endometrial epithelium under different culture conditions. The treatment of the cultured epithelia with aldosterone (1 \(\mu\)M) or culturing cells on filters coated with concentrated Matrigel resulted in an increase in the amiloride-sensitive \(I_{SC}\). Semiquantitative RT-PCR demonstrated that the expression of \(\alpha\) and \(\beta\) subunits was not significantly altered by these treatments, but an increase in the \(\gamma\) subunit expression was observed. An 11-fold increase, induced by aldosterone, in the expression of the \(\gamma\) subunit, but not in the \(\alpha\) and \(\beta\) subunits, was confirmed by capillary electrophoresis with laser-induced fluorescence (CE-LIF). The treatment of endometrial cells with antisense against the \(\gamma\)ENaC subunit abolished the aldosterone-enhanced amiloride-sensitive \(I_{SC}\). The results indicated an important role of \(\gamma\)ENaC subunit in determining ENaC activity, and a possible role of the \(\gamma\)ENaC subunit in interacting with CFTR was also discussed.

**Key words:** aldosterone, \(\gamma\)ENaC, Matrigel.

The amiloride-sensitive epithelial Na\(^+\) channel (ENaC) controls the rate of transepithelial Na\(^+\) absorption in a variety of epithelia including the kidney, colon, airways, and secretory ducts of several glands [1, 2]. ENaC is made of three different but homologous subunits (\(\alpha\), \(\beta\), and \(\gamma\)), each of which is believed to play a role in regulating ENaC activity [3–6]. The importance of these individual subunits can be highlighted by human diseases such as Liddle’s syndrome or pseudohypoaldosteronism, caused by mutations in individual ENaC subunits resulting in gain or loss of the ENaC function [7, 8]. However, the precise role of each ENaC subunit in regulating ENaC activity is far from understood; in particular, no evidence has been provided linking ENaC activity directly to specific ENaC subunits in tissues where they are normally expressed. In the present study, ENaC activity in the mouse endometrial epithelium, measured as amiloride-sensitive short-circuit current \((I_{SC})\), was upregulated by changing culture conditions. The enhanced ENaC activity was correlated with an increase in the mRNA expression of \(\gamma\) subunit and abolished by antisense against \(\gamma\)ENaC.

**Materials and Methods**

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\(^2\) for the short-circuit current preparation and 7.10 cm\(^2\) for the RT-PCR experiments. The filters were...
either with or without Matrigel coating. An 8× dilution of Matrigel was used to coat the filters (single-coated). In one set of experiments, a second layer of Matrigel was applied after the first layer was air-dried (double-coated). The cultures were subjected to the short-circuit current \( I_{SC} \) measurement as described previously [11, 12].

**Increased ENaC activity by culture conditions.** Previous studies have shown that cultured endometrial epithelia exhibit a basal \( I_{SC} \) that can be effectively blocked by amiloride, indicative of predominant \( Na^+ \) absorption [10, 13]. We recently found that the ENaC activity of endometrial cultures could be affected by coating the permeable support with Matrigel [14], which resembles the extracellular matrix facilitating rapid epithelial reconstitution and differentiation [15, 16]. As shown in Fig. 1A, the amiloride-sensitive \( I_{SC} \) obtained from endometrial cells cultured on double–Matrigel-coated filters (\( n = 15 \)) was significantly increased \((p < 0.001)\) by more than eightfold in comparison with that obtained from non–Matrigel-treated cultures (\( n = 27 \)). When the cultures were treated with Matrigel (single-coated, \( n = 3 \)), a further increase \((p < 0.05)\) in the amiloride-sensitive \( I_{SC} \) was observed when aldosterone (1 \( \mu M, n = 10 \)) was added to the culture medium (Fig. 1B). These results suggest that ENaC activity could be regulated by different mechanisms, either by factors such as Matrigel affecting cellular differentiation or hormones such as aldosterone.

**Differential expression of ENaC subunits.** The expression of ENaC subunits in non–Matrigel-treated and double–Matrigel-coated cultures or aldosterone-treated cultures was examined by semiquantitative RT-PCR using primers designed from mouse sequences for ENaC subunits \((\alpha: 2,171 \text{ to } 2,960 \text{ bp}; \beta: 1,961 \text{ to } 2,380 \text{ bp}; \gamma: 2,070 \text{ to } 2,793 \text{ bp})\) [17]. The intensities of the bands of ENaC subunits were normalized to that of GAPDH [18], which was amplified simultaneously. RT-PCR products were further measured by the CE-LIF technique, described previously [19]. All experiments were repeated three times, and the same results were obtained. As shown in Fig. 2A, bands at 790, 420, and 724 bp as expected for ENaC \( \alpha \), \( \beta \), and \( \gamma \) subunits, respectively, were obtained. The expression of both \( \alpha \) and \( \beta \) subunits was not significantly altered with the treatment of either Matrigel or aldosterone. However, prominent change in the \( \gamma \)ENaC expression was observed. In cultures without treatment of Matrigel, the expression of \( \gamma \)ENaC was hardly detectable in agarose gel (Fig. 2A), corresponding to a minimal amiloride-sensitive \( I_{SC} \) (Fig. 1A). A much-enhanced expression of \( \gamma \)ENaC was observed with double–Matrigel-treated cultures (Fig. 2A), corresponding to an eightfold increase in ENaC activity (Fig. 1A). Single–Matrigel-treated cultures gave rise to a detectable level of \( \gamma \)ENaC, which was further enhanced by treatment with aldosterone (Fig. 2B). The quantitative measurement of mRNAs of ENaC subunits was further carried out by using CE-LIF, which has been shown to be a sensitive method of detection and quantitation of PCR products [20]. As demonstrated in Fig. 3, CE-LIF revealed that the PCR product levels of \( \alpha \)ENaC and \( \beta \)ENaC were not altered by aldosterone, but an 11-fold increase was observed with \( \gamma \)ENaC. The correlation between the expression level of \( \gamma \)ENaC and the measured ENaC activity indicates that the effect of Matrigel and aldosterone on ENaC activity may be mediated by a transcriptional regulation of the \( \gamma \)ENaC subunit.

**Effect of antisense against \( \gamma \)ENaC.** To further test whether \( \gamma \)ENaC subunit was indeed responsi-
able for enhancing ENaC activity, experiments were conducted by using antisense against γENaC subunit. As shown in Fig. 4, the aldosterone-enhanced amiloride-sensitive $I_{SC}$ was abolished by γENaC antisense ($n=6$, $p<0.05$), but not by the mis-sense (control, $n=6$, $p>0.05$), confirming that the γENaC subunit was indeed responsible for the enhancement in ENaC activity induced by aldosterone.

Aldosterone is the key hormone in the regulation of $\text{Na}^+$ homeostasis. Its $\text{Na}^+$-saving action is mediated

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**Fig. 2.** Semiquantitative RT-PCR analysis demonstrating differential expression of ENaC subunits. **A.** Comparison of subunits expression in non-Matrigel-treated (−) and double-Matrigel-treated (++) cultures. **B.** Comparison of subunits expression in single-Matrigel-treated (+) cultures with (+) and without (−) aldosterone treatment. GAPDH was used as an internal standard. The experiments were repeated three times with similar results.

**Fig. 3.** CE-LIF measurement of RT-PCR products of ENaC subunits. **A.** Comparison of the amount of RT-PCR products in control and aldosterone-treated cells. **A.** GAPDH; **B.** αENaC; **C.** βENaC; **D.** γENaC. Measurements were made three times, and similar results were obtained.

**Fig. 4.** Effect of γENaC antisense on aldosterone-enhanced amiloride-sensitive $I_{SC}$. Mean currents±SEM obtained under different conditions were plotted ($n=6$, *$p<0.05$). Antisense (specific oligo nucleotide: TAC AGA TAC TCT CAG TTA AAA GAC) or mis-sense (specific oligo nucleotide: GTT TTC TTC TGC TTG GTC CA) (5 μg/ml) and aldosterone (1 μM) were added to cultures 24 h before experiments.
by the activity of ENaC in the apical membrane aldosterone-responsive tissues such as the kidney and distal colon. A differential expression of different ENaC subunits in response to aldosterone has been observed in different tissues with a marked increase in αENaC mRNA in mouse and rat kidney [21–23], but enhanced β and γENaC mRNA expressions in rat distal colon [24–26]. These differences could be due to tissue-specific transcription efficiency, and no firm conclusion can be drawn as to which specific subunit is responsible for aldosterone-enhanced ENaC activity since no direct demonstration has been made linking transcriptional changes directly to functional variations in ENaC activity.

Results and Discussion

The present study observed an increase in the amiloride-sensitive $I_{\text{SC}}$ associated with an increase in the expression of γENaC, but not α and β subunits, in response to aldosterone stimulation. The enhancing effect of aldosterone on ENaC activity was reversed by antisense against γENaC, demonstrating directly for the first time that the change of γENaC at a transcriptional level was indeed responsible for the enhancement of ENaC activity induced by aldosterone. Another interesting observation made in the present study was that Matrigel, which resembles the extracellular matrix facilitating epithelial differentiation, also enhanced endometrial ENaC activity with an increase in γENaC expression. Although the effect of aldosterone may be mediated by well-known nuclear receptors, the exact mechanism by which Matrigel influenced the ENaC expression is not clear. It seems quite likely that Matrigel may act through an extracellular mechanism, though it cannot be ruled out that the growth factors contained in the Matrigel may be responsible for the enhancement of the ENaC expression. Nevertheless, the upregulated ENaC activity in both cases, aldosterone and Matrigel-treated, was associated with the enhanced expression of γENaC. Therefore the γENaC subunit appears to be critical for regulating the activity of ENaC under different culture conditions in mouse endometrial epithelium.

The importance of a γENaC subunit may be further revealed by our recent demonstration of suppressed CFTR-mediated $\text{Cl}^-$ secretion by an enhanced expression of ENaC in mouse endometrium [14]. In that study, only the γENaC expression was found to be enhanced. Together with the present results, it suggests that γENaC may be involved in regulating ENaC functions, including the upregulation of ENaC activity and an inhibitory effect on CFTR activity as previously observed [14]. It should be noted that the C-terminal of the γENaC subunit has a motif known to interact with a ubiquitous protein-ligase, mutations of which cause Liddle’s syndrome [27–29]. Therefore, the interaction between ENaC and CFTR may also be mediated by γENaC with a potential site for protein–protein interaction.

In summary, the present study has demonstrated the differential regulation of ENaC subunits in mouse endometrial epithelial cells. Only the γENaC subunit mRNA, but no other subunits, was found to be upregulated upon hormone treatment or an extracellular matrix affecting cell differentiation. Therefore the γENaC subunit appears to be important in regulating ENaC activity, and thus the rate of $\text{Na}^+$ absorption across the mouse endometrium. The γENaC subunit may also participate in the interaction between CFTR and ENaC, thereby determining the delicate balance between $\text{Cl}^-$ secretion and $\text{Na}^+$ absorption in the endometrium.

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