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Estrogen modulation of epithelial permeability in cervical-vaginal cells of premenopausal and postmenopausal women

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Abstract

Objective—To understand how menopause affects estrogen regulation of epithelial permeability.

Design—Experimental study using human normal epithelial vaginal-ectocervical cells obtained from premenopausal and postmenopausal women. Endpoints were paracellular permeability (determined in terms of the resistance of the lateral intercellular space [R_{LIS}] and tight junctions [R_{TJ}]); cellular G-actin; nonmuscle myosin type II-B (NMMII-B) filamentation and magnesium-adenosine triphosphatase activity; and occludin expression (in terms of expression of the functional 65-kd and truncated 50-kd forms).

Results—Estrogen induced an early transient decrease in R_{LIS} that correlated in time with increases in cellular G-actin and NMMII-B magnesium-adenosine triphosphatase activity and with decreases in NMMII-B filamentation and a slower decrease in R_{TJ} that correlated with up-regulation of the 50-kd form of occludin. Estrogen modulation of G-actin NMMII-B and occludin could be described in terms of interaction with the estrogen receptor mechanism. The potency of estrogen effects was similar in cells of premenopausal and postmenopausal women, but the effects occurred earlier in cells of premenopausal women. R_{LIS} returned to baseline faster in cells of postmenopausal women, and the effect was associated with faster reversal of estrogen changes in NMMII-B despite the continued presence of estrogen in the culture medium, suggesting that desensitization of the actin-myosin effects to estrogen actions occur distal to the estrogen receptor.

Conclusions—The results suggest that the transient estrogen decrease in R_{LIS} is mediated by modulation of actomyosin, and it is affected by the aging process. In contrast, the late persistent decrease in R_{TJ} is mediated by occludin degradation and is unrelated to aging.

Keywords

Resistance; Tight junction; Intercellular space; Cervix; Vagina

An important role of estrogen is regulation of epithelial permeability and the control of fluid transudation into the cervical and vaginal lumens. Our present understanding of transepithelial transport in relatively leaky types of epithelia, such as the cervical and vaginal epithelia, is based on the pioneering works of Ussing and Zerahn¹ and Spring and Hope.² The Ussing-Zerahn model predicts that luminal fluids are derived from the blood compartment; that plasma moves through the epithelial paracellular (intercellular) space (Fig. 1B), and that paracellular transport of the fluid is controlled by the resistance of the epithelial tight junctions (R_{TJ}) and

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of the epithelial lateral intercellular space (R_{LIS}). The R_{TJ} and the R_{LIS} contribute in series to the total resistance (R_{TE}) so that $R_{TE} \approx R_{TJ} + R_{LIS}$. The R_{TJ} is a relatively high resistance and is determined by occlusion of the intercellular space through the interactions of extracellular loops of transcellular tight junctional proteins such as the claudins³ and occludin⁴ (Fig. 1D-E). The R_{LIS} is considered a low transepithelial resistance and is determined by the proximity of neighboring epithelial cells (Fig. 1A). The Spring-Hope model² predicts that the driving force moving the fluid from the blood into the lumen is the capillary blood pressure, which creates a subluminal to luminal hydrostatic gradient (Fig. 1A and B). Subluminal to luminal hydrostatic gradients dilate the lateral intercellular space, thereby decreasing the R_{LIS} (Fig. 1B). Dilation is restricted to the basolateral intercellular space because of the apical location of adherens intercellular junctions, which prevent lateral separation near the apical regions of the cells (Fig. 1B).

Estrogen increases fluid transudation by a number of known mechanisms. Estrogen decrease in capillary resistance increases blood flow, which would tend to increase the subluminal to luminal hydrostatic gradient (Fig. 1C).⁵ However, this effect contributes relatively little to net fluid transudation,^{6,7} and the main estrogenic control of transepithelial transport is by modulation of epithelial permeability through regulation of the R_{TJ} (Fig. 1C-E) and the R_{LIS} (Fig. 1C, F, G). Estrogen decrease in R_{TJ} is thought to be mediated by matrix metalloproteinase-7-dependent modulation of occludin,⁸⁻¹⁰ and the decrease in R_{TJ} is explained by loss of gating of the intercellular space due to degradation of occludin extracellular loops.¹⁰ Estrogen decrease in the R_{LIS} involves at least three known molecular mechanisms: actin depolymerization and the formation of monomeric-dominated G-actin cytoskeleton¹¹; decreased dimerization of nonmuscle myosin type II-B (NMMII-B), the predominant cytoskeletal epithelial myosin^{12,13}; and increased magnesium-adenosine triphosphatase (MgATPase) activity of the NMMII-B.¹⁴ Collectively the actin and NMMII-B effects of estrogen would tend to fragment the cortical actomyosin ring and induce formation of a dynamic cytoskeleton (Fig. 1E-G). This would facilitate dilation of the basolateral intercellular space (ie, decrease the R_{LIS}) in response to the capillary subluminal to luminal hydrostatic gradients (Fig. 1C).

The objectives of the present study were to better understand estrogen modulation of the R_{TJ} and R_{LIS} and how menopause affects estrogen actions. The results revealed that estrogen increases epithelial permeability by inducing an early transient decrease in R_{LIS} followed by a slower persistent decrease in R_{TJ} . The potency of estrogen actions was similar in cells of premenopausal and postmenopausal women, but the effects on NMMII-B filamentation and MgATPase activity were shorter in cells of postmenopausal women, resulting in faster reversal of the decrease in R_{LIS} .

METHODS

Cell cultures

The experiments used cultured normal human epithelial vaginal-ectocervical cells (hVEECs) on filters. Primary cultures of normal hVEECs were generated from discarded minces of the vagina/ectocervix. Tissue samples were collected from eight premenopausal women ages 37 to 46 and from nine postmenopausal women ages 55 to 63. Menopause was defined as amenorrhea for more than 12 months and plasma follicle-stimulating hormone greater than 25 mIU/mL. Women underwent hysterectomy for medical indications unrelated to the present study. None of the women were treated with steroid hormones for at least 3 months before surgery. Tissue samples were collected by the Cooperative Human Tissue Network at University Hospitals of Cleveland and Case Western Reserve University according to institutional review board protocol 03-90-TG. hVEECs (also referred to in the past as hECECs) were previously characterized as phenotypically resembling the stratified squamous hVEECs.

^{15,16} Cells chosen for experiments were those obtained from histologically normal tissues reported as human papillomavirus negative, and cultures were routinely tested for mycoplasma. Methods of cell culturing, subpassages, and plating on filters were described previously.¹⁶ For experiments with estrogen-deficient conditions, cells on filters were shifted to steroid-free medium for 3 days as described.¹⁷

Determinations of transepithelial electrical resistance (R_{TE})

Filters containing cells were washed three times and preincubated for 15 minutes at 37°C in a modified Ringer's buffer. Changes in paracellular permeability were determined as changes in the R_{TE} across filters mounted vertically in a modified Ussing chamber. Successive measurements were made of the transepithelial potential difference (ΔPD , lumen negative) and the transepithelial electrical current (ΔI , obtained by measuring the current necessary to clamp the offset potential to zero and normalized to the surface area of the filter), and R_{TE} was determined as $R_{TE} = \Delta PD / \Delta I$. In some experiments determinations of R_{TE} were done using the EVOM epithelial voltohmmeter.^{10,18} The contributions of R_{TJ} and R_{LIS} to the R_{TE} were quantified from the equation of $R_{TE} \approx R_{TJ} + R_{LIS}$, whereupon changes in R_{TE} were determined as described above. The contribution of R_{TJ} was determined from measurements of R_{TE} after lowering extracellular calcium from 1.2 to 0.4 mM. In hEVECs, R_{TJ} depends on Ca^{2+} , and lowering extracellular calcium abrogates the R_{TJ} without significantly affecting the R_{LIS} .¹⁹ The estimated R_{TE} at extracellular calcium <0.1 mM yields $R_{TE} \approx R_{LIS}$.^{18,19} Changes in R_{TJ} were confirmed by measurements of the dilution potential and determinations of the relative mobilities of Cl^- and Na^+ in the intercellular space (U_{Cl}/U_{Na}).¹⁸

Actin assays

Effects of estrogen treatment on cellular G-actin were determined by the DNase-I inhibition assay.¹¹ Briefly, cells on filters were lysed in situ, and DNase-I activity in the lysate was assayed by measuring DNase-I-dependent degradation of DNA. Total actin was measured by the guanidine-HCl method after depolymerization of F-actin to monomeric G-actin. Estrogen modulation of actin is described in terms of the percentage of G-actin (G-actin content per total cellular actin).

Myosin assays

Effects of estrogen treatment on NMMII-B filamentation were determined in terms of NMMII-B critical concentration.^{12,13} Briefly, purified NMMII-B filaments at concentrations of 20 nM to 10 μM were used for assembly assays in the presence of $MgSO_4$, NaCl, and 0.1 mM $CaCl_2$.¹² The critical concentration of NMMII-B necessary to induce spontaneous dimerization was calculated from the curve of percent nondimerized NMMII-B versus added protein as the concentration of NMMII-B that induced maximal oligomerization. NMMII-B steady-state MgATPase activity was determined in terms of adenosine triphosphate hydrolysis in the presence of F-actin.¹⁴ Briefly, purified NMMII-B filaments were suspended in a solution composed of KCl and $MgCl_2$ plus F-actin (0-200 μM). Assays were started by the addition of adenosine triphosphate (0-7.5 mM) and incubated at 37°C for 30 minutes. Rates were obtained from the average of three time points at the steep increase of inorganic phosphorous (P_i) release, and P_i was determined by spectrometry ($OD = 4.1/\mu mol P_i$ read at 720 nm). Data are presented as the V_{max} of the reaction, where V_{max} values were determined from fitting the data of P_i release (per second) to the Michaelis-Menten equation.¹⁴

Occludin assays

Expression of the tight junction protein occludin was assayed by Western immunoblots using rabbit anti-occludin antibody (Cat. 71-1500, Zymed Laboratory Inc, San Jose, CA). In lysates of hEVECs, the antibody immunoreacts with the functional full-length 65-kd occludin form

and with the truncated estrogen-modulated 50-kd form.^{8,9} Expression of the occludin 65-7 and 50-kd bands in Western blots was assessed by densitometry using an AGFA Arcus II scanner (AGFA, New York, NY), and images were evaluated using version 5.1 of Un-Scan-It gel automated digital software (Silk Scientific, Orem, OR). Estrogen modulation of occludin was determined in terms of the densitometry ratio of the 50-kd/65-kd bands.

Statistical analysis

Data are presented as means of three to five experiments. Data were analyzed using GraphPad InStat (GraphPad Software Inc, San Diego, CA). Significance of trends among groups was estimated by one-way analysis of variance with Tukey-Kramer multiple comparisons posttest analysis. Significance of differences between groups was estimated by two-way analysis of variance.

Chemicals and supplies

All chemicals and supplies, unless specified otherwise, were obtained from Sigma Chemical (St. Louis, MO).

RESULTS

Estrogen regulation of permeability (R_{TE})

The main objective of the study was to better understand the effects of menopause status on estrogen modulation of epithelial permeability, and the experimental model was hEVEC cultures on filters. Estrogen-depleted cells were treated with 17β -estradiol at the physiological submaximal concentration of 10 nM for durations of 16 hours to 4 days (cells of premenopausal women) or for 2 to 5 days (cells of postmenopausal women). The rationale was the different lengths of treatment required to reach maximal effects in the two cell types (see below). Changes in R_{TE} were determined using electrophysiological measurements, and the contributions of the R_{TJ} and R_{LIS} to the total resistance were determined by probing levels of Ca^{2+} during the electrophysiological measurements (described in "Methods").

Baseline levels of R_{TE} were $40 \pm 5 \Omega \cdot \text{cm}^2$ across cells of premenopausal women and were determined by R_{TJ} of $28 \pm 3 \Omega \cdot \text{cm}^2$ and R_{LIS} of $12 \pm 3 \Omega \cdot \text{cm}^2$ (Fig. 2A and B). Baseline levels of R_{TE} across cells of postmenopausal women were $66 \pm 9 \Omega \cdot \text{cm}^2$ and were determined by R_{TJ} of $48 \pm 7 \Omega \cdot \text{cm}^2$ and R_{LIS} of $18 \pm 3 \Omega \cdot \text{cm}^2$ (Fig. 3A and B).

In cells of premenopausal women, treatment with estrogen for 16 hours decreased R_{TE} from 40 to $24 \Omega \cdot \text{cm}^2$ (ie, an increase in permeability from 25 to approximately $42 \text{ mS} \cdot \text{cm}^2$) (Fig. 2A and B). The effect was monophasic; it began within 6 hours of treatment and reached a peak after 36 to 48 hours (Fig. 2A). Removal of estrogen from the culture medium resulted in reversal of the effect: the increase in R_{TE} began 48 hours after the removal of estrogen, and R_{TE} reached pretreatment levels 4 days after the start of the experiment (Fig. 2A). In cells treated with estrogen for 4 days, a similar initial decrease in R_{TE} was observed, followed by a slight increase in R_{TE} after 3 to 4 days that did not reach pretreatment levels (Fig. 2B). Removal of estrogen after 4 days of treatment did not affect the R_{TE} .

In cells of postmenopausal women, treatment with estrogen induced a biphasic decrease in R_{TE} : an initial decrease within 24 hours from $66 \Omega \cdot \text{cm}^2$ to approximately $50 \Omega \cdot \text{cm}^2$, followed by a secondary decrease to approximately $27 \Omega \cdot \text{cm}^2$ (Fig. 3A and B). Removal of estrogen after 2 days of treatment resulted in reversal of the effect: the increase in R_{TE} began 1 to 2 days after removal of the estrogen, and it reached near baseline levels approximately 7 days from the start of the experiment (Fig. 3A). In the continued presence of estrogen for a total of 5 days, the R_{TE} began to increase slowly after 3 days of treatment, but it did not reach baseline levels

(Fig. 3B). Removal of estrogen after the 5 days of treatment did not seem to affect the changes in R_{TE} , which continued to increase slowly (Fig. 3B).

Estrogen regulation of the R_{TJ} and R_{LIS}

The contributions of the R_{TJ} and R_{LIS} to the R_{TE} were determined by probing extracellular Ca^{2+} during the electrophysiological measurements. In cells of premenopausal women, the initial phase of decrease in R_{TE} was the result of an acute decrease in R_{LIS} (Fig. 2A and B), followed by a slower decrease in R_{TJ} (Fig. 2A and B). Removal of estrogen reversed the decreases in R_{LIS} and R_{TJ} (Fig. 2A and B). In the continued presence of estrogen in the culture medium, the decrease in R_{TJ} persisted (Fig. 2A and B), but the decrease in R_{LIS} reversed (Fig. 2B).

In cells of postmenopausal women, the initial decrease in R_{TE} was also the result of an acute decrease in R_{LIS} (Fig. 3A and B), whereas the secondary decrease in R_{TE} was the result of a decrease in R_{TJ} (Fig. 3A and B). Interestingly, the decrease in R_{LIS} reversed after 24 hours of treatment with estrogen irrespective of the absence (Fig. 3B) or the continued presence of estrogen in the culture medium (Fig. 3A). In the continued presence of estrogen in the culture medium, the decrease in R_{TJ} persisted (Fig. 3B), but after removal of estrogen, the decrease in R_{TJ} reversed, returning to near baseline levels approximately 4 days after estrogen removal (Fig. 3A).

Estrogen regulation of mechanisms that control the R_{LIS} and R_{TJ}

hVEECs cultivated from premenopausal and postmenopausal women were depleted of steroids and then treated with 17β -estradiol at concentrations ranging from 0.1 nM to 100 nM. Treatment with estrogen increased in a dose-related manner cellular G-actin (Fig. 4A and B), NMMII-B MgAT-Pase activity (Fig. 4A and B), and the occludin 50 kd/65 kd ratio (Fig. 4A and B), and decreased NMMII-B filamentation (Fig. 4A and B). For the four endpoints, the effects of estrogen began with 0.1 nM 17β -estradiol and reached saturation at approximately 10 nM. The dose-related trends were similar in cells of premenopausal and postmenopausal women (Fig. 4), and for both groups of cells, the dose-response curves of the means could be fitted into modified Hill equations with a Hill coefficient n of approximately 1, suggesting interaction of estradiol with a single class binding site. The calculated median effective concentration of estradiol was 0.8 nM in cells of premenopausal women and 1.2 nM in cells of postmenopausal women ($P > 0.3$), indicating similar potency of estradiol action for all four endpoints in both groups of cells. These levels also correspond with the reported kilodaltons of the estrogen receptor.²⁰

The time course of estrogen effects on the four molecular mechanisms involved in regulation of epithelial permeability is shown in Figure 5. In cells of premenopausal and postmenopausal women, treatment with estrogen induced an early transient increase in cellular actin (Fig. 5A), a slower transient decrease in NMMII-B filamentation (evident as a transient increase in the critical concentration of NMMII-B, Fig. 5B), and a slower transient increase in MgATPase activity (Fig. 5C). All three types of effects reversed to near baseline levels despite the continued presence of estrogen in the culture medium. However, for NMMII-B filamentation and MgAT-Pase activity, the return to baseline levels was faster in cells of postmenopausal women than in cells of premenopausal women (Fig. 5B and C).

In both types of cells, treatment with estrogen induced an increase in the occludin 50-kd form (Fig. 5D) that persisted after removal of estrogen from the culture medium and correlated in time with the decrease in R_{TJ} (Figs. 2 and 3). Treatment with estrogen also induced a persistent decrease in the ratio of Cl^- and Na^+ mobilities in the intercellular space (U_{Cl}/U_{Na}) (Fig. 5E)

with a time course similar to that of the effect on the occludin 50-kd form, confirming a decrease in R_{TJ} .

DISCUSSION

The objective of the study was to understand how menopause modulates estrogen regulation of the permeability of female reproductive tract epithelia. The experiments used cultured normal hVEECs that retain phenotypic characteristics of the native epithelia,¹⁶ and the objective was to understand regulation of permeability phenomena and mechanisms involved at the cellular-molecular level. Although there is a strong background of data to suggest similar effects in vivo, extrapolation of the results to the in vivo case needs to be determined.

The results showed that baseline levels of permeability were lower in hVEECs of postmenopausal women than in cells of premenopausal women. The results also showed that treatment with estrogen increased permeability in both types of cells, but the increase in permeability was greater in cells of premenopausal women than in cells of postmenopausal women. Estrogen induced an early and transient decrease in R_{LIS} , which correlated in time with changes in G-actin, NMMII-B filamentation, and MgATPase activity, suggesting that the changes in R_{LIS} are the result of estrogen-induced changes in the cortical actomyosin. Estrogen also induced a slower decrease in R_{TJ} that persisted despite removal of estrogen from the culture medium. The R_{TJ} effect correlated in time with estrogen modulation of occludin, suggesting that the R_{TJ} changes are the result of occluding degradation. The estrogen permeability changes were not the result of toxic effects on the cells because the estrogen R_{TJ} and occludin effects are also reversible after prolonged estrogen withdrawal.²¹

The present data support earlier studies in the field that estrogen modulation of the R_{LIS} and R_{TJ} , and estrogen modulation of actin NMMII-B and occludin, are mediated by estrogen receptor- α (ER α)^{14,22}. Estrogen modulation of G-actin NMMII-B and occludin could be described in terms of activation of the classic estrogen receptor mechanism.²⁰ Interestingly, the potency of estrogen actions was similar in cells of premenopausal and postmenopausal women (Fig. 4), but the time course of estrogen actions was slower in cells of postmenopausal women (Figs. 2 and 3). The likely explanation is the low baseline levels of the ER α in cells of postmenopausal women and the requirement for estrogen up-regulation of the ER α . Accordingly, treatment with estrogen can up-regulate the ER α in cells of postmenopausal women, and, having reached sufficient activity, the ER α can mediate estrogen permeability.

The different time courses of estrogen modulation of the R_{LIS} and R_{TJ} suggest activation of two independent mechanisms. This hypothesis is supported by the different signaling pathways of occludin modulation (mediator of changes in R_{TJ}) and modulation of actin and myosin (mediators of changes in R_{LIS}). ER α -dependent regulation of the R_{LIS} involves nitric oxide/cyclic guanosine monophosphate/cyclic guanosine monophosphate-dependent, protein kinase-dependent G-actin increase¹¹, modulation of NMMII-B filamentation is mediated by epidermal growth factor receptor/extracellular signal-regulated kinase-mitogen-activated protein kinase/casein kinase II, and Rho-associated kinase-dependent modulation of myosin heavy-chain phosphatase¹³; and modulation of NMMII-B MgATPase is mediated by the epidermal growth factor receptor/extracellular signal-regulated kinase-mitogen-activated protein kinase/casein kinase II signaling cascade.¹⁴ At present, little is known about whether activation of the decrease in R_{TJ} is triggered independently of the R_{LIS} or whether activation of the R_{LIS} triggers the decrease in R_{TJ} .

One of the questions raised by the present data is what mechanism stimulates R_{LIS} return to baseline levels despite the continued presence of estrogen in the culture medium. Of equal interest is what regulates the faster return of R_{LIS} to baseline levels in cells of postmenopausal

women. The estrogen effect on G-actin had a different time course than on NMMII-B filamentation and MgATPase activity (Fig. 5), and the increases in G-actin reversed relatively fast, within 24 hours after reaching their peak levels (Fig. 5). The time course of the G-actin effect was similar in cells of premenopausal and postmenopausal women, suggesting that the decrease in G-actin is not the mechanism that determines the fast reversal of R_{LIS} in cells of postmenopausal women. In contrast, the accelerated return of R_{LIS} to baseline levels in cells of postmenopausal women was associated with faster reversal of the changes in NMMII-B filamentation and MgATPase activity (Figs. 2, 3, and 5). It is therefore possible that the faster return of R_{LIS} to baseline levels in cells of postmenopausal women is the result of desensitization of the $ER\alpha$ -NMMII-B cascades. Because the estrogen-induced decrease in R_{TJ} persisted in the presence of estrogen, the desensitization is likely to have occurred distal to $ER\alpha$.

The present results could be relevant to our understanding of estrogen and aging regulation of epithelial female reproductive tract permeability in vivo because the permeability characteristics of the cultured hVEECs are in the range described for the vaginal-cervical epithelium in vivo, and treatment of cells with estrogen results in changes in permeability that mimic estrogenic effects in vivo.^{6,7} Data showed that baseline permeability was lower in cells of postmenopausal women (Figs. 2 and 3) and that estrogen modulation of the R_{TJ} could explain in part the baseline and age-related changes in R_{TJ} . By extrapolating these findings to the situation in vivo, it is possible to hypothesize that during reproductive years, endogenous estrogens decrease the R_{TJ} , and after menopause and the ensuing hypoestrogenic milieu, R_{TJ} returns to its constitutive relatively high level of resistance. Because R_{TJ} is the main determinant of the transepithelial resistance, the overall epithelial permeability decreases after menopause and leads to decreased plasma transudation and decreased lubrication of the lumen. Interestingly, the R_{TJ} per se is not dependent on the aging process.²³

In contrast to the R_{TJ} , estrogen regulation of the R_{LIS} is mediated by changes in the cytoskeletal actomyosin cortex, and changes in the R_{LIS} are dependent on the aging process.²³ G-actin-mediated estrogen decrease in the R_{LIS} is acute and transient (Gorodeski¹¹ and the present results). By probing signaling steps distal to the $ER\alpha$, it was possible to induce estrogen-independent increases in G-actin that stimulated an acute decrease in R_{LIS} .²³ The estrogen-independent increases in G-actin were smaller in hVEECs of postmenopausal women than in cells of premenopausal women,²³ suggesting aging-dependent modulation of the G-actin system distal to the $ER\alpha$. The present data show that estrogen modulation of NMMII-B filamentation and MgATPase activity were also attenuated in cells of postmenopausal women, suggesting aging dependence as well. A possible explanation for the physiological role of the estrogen decrease in R_{LIS} is stimulation of an acute increase in permeability in epithelia of premenopausal women, such as those observed at the preovulatory phase of the menstrual cycle. Equally important is the speculation, based on the present data, that the aging process modulates actomyosin mechanisms that determine the structure of the cytoskeleton.

CONCLUSIONS

In summary, the present results suggest that both estrogen and the aging process regulate epithelial permeability. Estrogen increases the permeability by inducing decreases in the R_{LIS} and R_{TJ} . The estrogen transient decrease in R_{LIS} is mediated by modulation of the cellular actomyosin, whereas the late persistent decrease in R_{TJ} is mediated by occludin degradation. The data also suggest that desensitization of the actomyosin effects to estrogen actions occurs distal to the estrogen receptor. In addition, the results suggest that the aging process affects specifically the R_{LIS} through modulation of the cytoskeletal actomyosin. These mechanisms may not be limited to the reproductive tract epithelia and could also operate in other types of leaky epithelium²⁴ and endothelium.²⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Models of estrogen regulation of transepithelial permeability. **A:** The main resistances for free diffusion of fluid from the capillary bed into the lumen through the paracellular (intercellular) space are the tight junctional resistance (R_{TJ}) and the resistance of the lateral intercellular space (R_{LIS}) in series. **B:** The capillary blood pressure generates a subluminal to luminal hydrostatic gradient that dilates the basolateral intercellular space; it decreases the R_{LIS} and induces flow of plasma in the subluminal to luminal direction. **C:** Estrogen increases subluminal to luminal transport (and hence luminal lubrication) by increasing the capillary pressure and by decreasing the R_{LIS} and R_{TJ} . Estrogen decrease in R_{TJ} (**D**) involves modulation of occludin (**E**). Estrogen decreases the R_{LIS} by inducing formation of a dynamic cytoskeleton (**F**), which is more sensitive to the effects of the capillary subluminal to luminal hydrostatic gradient (**C**). Estrogen fragmentation of the cytoskeleton is mediated by three known mechanisms: actin depolymerization, decreased dimerization of nonmuscle myosin II-B (NMMII-B), and increased NMMII-B magnesium-adenosine triphosphatase (MgATPase) activity (**G**).

FIG. 2.

Estrogen modulation of paracellular resistance across human epithelial vaginal-ectocervical cell cultures generated from ectocervical-vaginal tissue of premenopausal women. Estrogen-depleted cells were treated with 10 nM 17 β -estradiol for 16 (**A**) or 96 hours (**B**). At the indicated times, filters containing cells were removed for determinations of transepithelial electrical resistance (R_{TE}), tight junctional resistance (R_{TJ}), and resistance of the lateral intercellular space (R_{LIS}). Shown are means of five experiments. All trends were significant ($P < 0.0001$, analysis of variance). Variability (SD) ranged from 4% to 7%.

FIG. 3.

Estrogen modulation of paracellular resistance across human epithelial vaginal-ectocervical cell cultures generated from ectocervical-vaginal tissue of postmenopausal women. Estrogen-depleted cells were treated with 10 nM 17 β -estradiol for 24 (A) or 120 (B) hours. At the indicated times, filters containing cells were removed for determinations of transepithelial electrical resistance (R_{TE}), tight junctional resistance (R_{TJ}), and resistance of the lateral intercellular space (R_{LIS}). Shown are means of six experiments. All trends were significant ($P < 0.0001$, analysis of variance). Variability (SD) ranged from 2% to 8%.

FIG. 4.

Estrogen dose-dependent modulation of the molecular mechanism that regulates epithelial permeability in human epithelial vaginal-ectocervical cells. Cultures were generated from ectocervical-vaginal tissues of premenopausal (**A**) and postmenopausal (**B**) women. Estrogen-depleted cells (C, control [untreated]) were treated for 24 hours (actin assays) or 48 hours (myosin and occludin assays) with one of the indicated concentrations of 17β -estradiol. Assays are described in "Methods," and data were normalized to baseline levels in estrogen-depleted cells (=10%) versus maximal effects in cells treated with 100 nM 17β -estradiol (=100% effect). Shown are means of three experiments. Variability ranged from 1% to 5%, and the dose-related trends for the four types of endpoints were significant in all cases ($P < 0.0001$, analysis of variance). The differences in estrogen potency among the four endpoints were not significant. NMMII-B, nonmuscle myosin type II-B; MgATPase, magnesium adenosine triphosphatase.

FIG. 5.

Estrogen modulation of G-actin (A), nonmuscle myosin type II-B (NMMII-B) filamentation (B), NMMII-B magnesium adenosine triphosphatase (MgATPase) activity (C), relative expression of occludin 50-kd and 65-kd bands (D), and the relative mobilities of Na^+ and Cl^- in the intercellular space (U_{Cl^-}/U_{Na^+}) (E) in human epithelial vaginal-ectocervical cells generated from ectocervical-vaginal tissue of premenopausal and postmenopausal women. Estrogen-depleted cells (C, control) were treated with 10 nM 17 β -estradiol for 5 days, and at the indicated times, filters containing cells were removed for assays. Shown are means ($\pm$ SD) of three to four experiments. * $P < 0.01$.