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Review

Estrogen regulation of epithelial ion transport: Implications in health and disease

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ABSTRACT

Estrogen, 17 β -estradiol (E2), has been shown to modulate the activity of ion channels in a diverse range of epithelial tissues. The channel activation or inhibition responses to E2 are often rapid, occurring in seconds to minutes, independent of protein synthesis and gene transcription ('non-genomic' response). These rapid effects of E2 require activation of specific protein kinases or changes in intracellular calcium and pH which in turn modulate the conductance, open probability or number of channels in the plasmamembrane. Estrogen has also been shown to affect the expression of ion transporters over days ('genotropic' response) causing long-term sustained changes in transepithelial ion transport. It is now accepted that so called non-genomic responses are not stand-alone events and are necessary to prime the latent genomic response and even be critical for the full latent response to occur. In a number of epithelia the non-genomic and genotropic responses to estrogen are sex-specific and variable in potency and sensitivity to E2 depending on the stage of the estrous cycle. Of increasing interest is the effect these rapid and latent actions of E2 on ion transporters have on the physiological functions of epithelia. For example, estrogen regulation of a class of voltage-gated K⁺ channels (KCNQ1) can determine the rate of Cl⁻ secretion in the intestine. In whole-body terms, the combined effects of estrogen on a variety of ion channels which control fluid and electrolyte transport in the kidney, intestine and lung may be necessary for endometrial expansion and implantation of the blastocyste.

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1. Introduction

The physiology and pathophysiology of estrogen effects on ion channels and transporters in epithelial tissues is of topical interest.

Abbreviations: E2, 17 β estradiol; ENaC, Epithelial Sodium Channel; CCD, cortical collecting duct.

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Epithelial ion channels play a critical role in the maintenance of whole body fluid balance and in the progression of common diseases such as secretory diarrhea and cancer cell proliferation. The extra-gonadal effects of estrogen on ion channels and transporters are female-sex specific and both rapid 'non-genomic' and genomic in their cell signaling patterns. In healthy tissues, estrogen modulation of ion channel activity and transporters appear to have beneficial effects. For example, estrogen produces a rapid onset and sustained anti-secretory response in intestinal epithelia by inhibiting chloride ion secretion via inactivation of KCNQ1:KCNE3 channels (Fig. 1). The positive effects on organ physiology may be

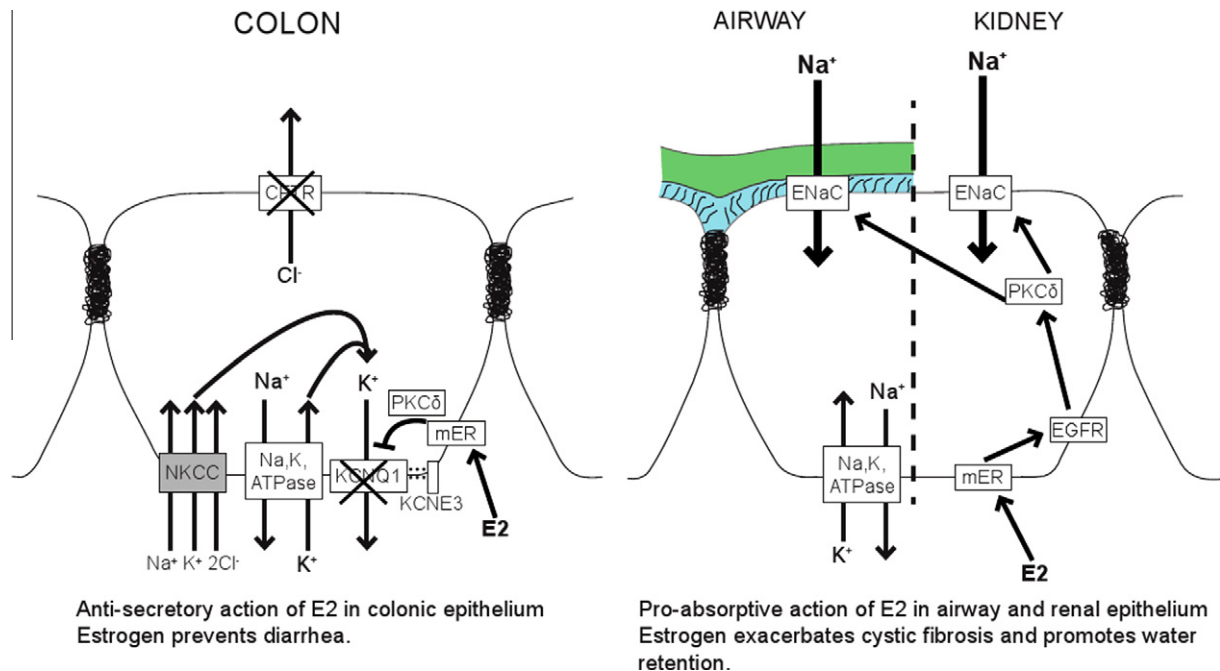


Fig. 1. Actions of 17 β -estradiol (E2) on ion channels in distal colon, airway and renal epithelia. In the colon, E2 activates PKC δ leading to a decrease in K⁺ efflux through the basolateral KCNQ1:KCNE3 channel. As K⁺ recycling provides the driving force for Cl⁻ secretion, E2 inhibits this process, preventing diarrhea in the cases of infection by *V. cholerae* or *E. coli*. In the airways, E2 dehydrates the airway surface liquid through the activation of PKC δ and an increase in Na⁺ absorption via ENaC, impairing mucociliary clearance and therefore exacerbating the lung pathophysiology in patients with cystic fibrosis. As in the airways, E2 targets PKC δ and ENaC to increase Na⁺ absorption in the kidney, showing that E2 is an important player in the regulation of the electrolyte and fluid transport in this organ.

to prevent secretory diarrhea in high estrogen states when extracellular fluid (ECF) volume needs to be conserved (for example during blastocyst implantation into the swollen endometrium). Reproductive females experience the inconvenience of fluid retention during high estrogen states in mid-estrus cycle and ‘bloating’ or excessive fluid retention is a common feature of estrogen treatment in assisted fertilization. It has been postulated that the anti-secretory effects of estrogen in the intestine contribute to ECF volume expansion during the implantation window [1]. Such an effect may be short-circuited by compensatory Na⁺ and water excretory mechanisms in the kidney or insensible transpiration in the lung. However, estrogen exerts pro-absorptive effects in bronchial airway epithelium and in kidney cortical collecting duct cells by rapidly activating ENaC and transepithelial Na⁺ absorption (Fig. 1). Thus the major ECF regulatory organs contribute to the overall physiological response to estrogen, causing ECF expansion to permit implantation (Fig. 1). The deleterious consequences of estrogen action on epithelial ion channels appears to be confined to diseased tissues where ion absorption/secretion is already compromised such as the airways in cystic fibrosis or in breast cancer where ion channel activation may amplify proliferative signals. Thus for example, the influence of estrogen on ion channel activity in epithelia may change the course of a disease, such as in the gender gap of cystic fibrosis where the pro-absorptive and anti-secretory effects of estrogen can reduce airway fluid dynamics thus exacerbating mucociliary clearance and lung function (Fig. 1). We are currently at the pioneering stage of understanding estrogen actions on ion channels and transporters in general and the consequences in health and disease. Much is yet to be uncovered in the underlying physiology and molecular mechanisms of rapid estrogen responses in epithelia, in particular, with respect to the sexual dimorphism of extra-gonadal effects of estrogen on ion channels. In this review we focus on estrogen and ion channels in kidney, lung and intestinal epithelia, in health and disease, giving an insight into the current state-of-the-art as well as promising areas for future research.

2. Estrogen regulation of ion transport in the kidney

One of the main physiological functions attributed to the kidney is the regulation of water and electrolyte content of the body. This function is performed by its basic structural and functional unit, the nephron. In the distal nephron, the cortical collecting duct (CCD) plays a critical role in the hormonal control of electrolyte and fluid homeostasis and it has been shown that these processes can be tightly regulated by the hormones insulin, vasopressin and aldosterone [2–12]. The rapid non-genomic actions of aldosterone on renal ion transporters has been well-described [13–18]. Aldosterone regulates CCD Na⁺ reabsorption in a rapid non-genomic and genomic manner; this regulation involves changes in Epithelial Sodium Channel (ENaC) expression, trafficking dynamics, stability at the plasma membrane and changes in open channel probability [29–31].

For many years, it has been shown that 17 β -estradiol (E2) has an impact in the control of whole body electrolyte and fluid homeostasis [19–22], however, it is only in the last decade that the targets and molecular mechanisms involved in this regulation have started to be identified [23–25]. The kidney has been proposed as an estrogen-responsive target where E2 could exert a pro-absorptive effect [23]. Moreover, a possible role of E2 in stimulation of renal K⁺ secretion [24], Ca²⁺ signaling [15,25,26], pH homeostasis [27] and the rennin-angiotensin pathway [28] has been proposed.

Evidence suggesting a long-term sex-specific pro-absorptive effect for E2 in the kidney have been published in recent years [32,33]. In rat kidney, it has been shown that the abundance of α -, β - and γ -ENaC subunit mRNA was significantly higher in female compared to male rat kidneys. These sex differences were abolished in ovariectomized rats and treatment of ovariectomized rats with E2 increased renal α -ENaC subunit mRNA levels over long periods up to 24 h [32]. Another study, in a diabetic rat model, has shown that E2 causes diabetes dependent and independent

effects on renal electrolyte handling and associated transporter proteins [33]. In mouse collecting tubular cells, E2 or progesterone alone increased ENaC activity and a low concentration of E2 together with progesterone produced an additive stimulation of ENaC activity [34]. Ovariectomized female rats treated with E2 also showed an increase in plasma Na^+ concentration [35]. Furthermore, this study has shown that in aldosterone-infused female rats, plasma Na^+ was increased further than in male rats infused with aldosterone [35], suggesting a regulatory role for E2 on the renin–angiotensin–aldosterone system (RAAS) [28].

The evidence described above involves only long-term effects for E2 on Na^+ reabsorption and, in contrast, little is known about the E2 short-term effects on renal transport processes. Recently, we have found evidence for a rapid E2 pro-absorptive effect in the distal nephron via stimulation of ENaC activity. The rapid E2 activation (<15 min) of amiloride-sensitive Na^+ current in mouse M1 CCD cells was sensitive to inhibitors of PKC δ and ER α (Yusef & Harvey, unpublished).

As for the case of Na^+ reabsorption, K^+ secretion in the kidney can be tightly regulated by aldosterone. The classical mechanism involves an increase in the expression of the Na^+/K^+ -ATPase at the basolateral membrane of principal cells of the CCD and a subsequent increase in the driving force for K^+ secretion through the Renal Outer Medullary Potassium channel (ROMK) [36]. A second and more novel mechanism is related to situations of high plasma K^+ concentration where an increase of plasma aldosterone leads to repression of signaling pathways involved in the inhibition of apical K^+ channels such as ROMK and BK [25]. However, a possible regulation of K^+ secretion by E2 in the kidney has not been established. Inspired by the observation that polycystic kidney disease has worse prognosis in men, a study performed in MDCK cells has shown that testosterone stimulates fluid secretion by increasing cAMP levels but failed in showing any effect for E2 [37]. On the other hand, a more recent study has shown that in aldosterone-infused female rats, plasma K^+ concentration was reduced further than in male rats infused with aldosterone. Furthermore E2 but not progesterone reduced plasma K^+ concentration in ovariectomized female rats [35]. These observations provide another piece of evidence supporting a possible regulatory effect of E2 on RAAS.

In recent years data supporting a novel role for E2 to rapidly regulate Ca^{2+} homeostasis in the kidney has been shown [25,26,38]. E2 can rapidly modulate intracellular Ca^{2+} levels through a Ca^{2+} entry pathway in kidney M1 cells [15]. Moreover, it has been shown that TRPV5, a Ca^{2+} -permeable cation channel member of the Transient Receptor Potential channel subfamily, is responsible for the rapid and transient increase in whole-cell currents and intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) induced by E2 in rat CCD cells [26]. A more recent study has shown acute actions of E2 on intracellular Ca^{2+} signaling in intercalated cells from isolated distal convoluted tubules, connecting tubules and initial cortical collecting ducts [27]. It has been postulated that the E2 induced $[\text{Ca}^{2+}]_i$ increases and enhanced H^+ -ATPase activity may occur through the activation of the membrane associated estrogen receptor, GPER1 [27].

Although the current literature is scarce on E2 regulation of kidney ion transport recent evidence points to a previously unknown role of E2 as an important player in the regulation of the electrolyte and fluid transport in the kidney, its potential role in the regulation of RAAS and a non-genomic regulation of ENaC.

3. Estrogen regulation of ion transport in airway epithelium

The epithelium lining the lung is covered by a thin layer of fluid that provides a barrier against inhaled pathogens and particles. In the bronchi, the airway surface liquid (ASL) covers the bronchial

epithelial cells and is composed of two layers. The periciliary layer has a low viscosity to allow beating cilia remove inhaled particles and pathogens which are trapped in the second layer, the mucus. The hydration of ASL and its height at $\sim 10 \mu\text{m}$ have to be optimal for an efficient mucociliary clearance [39]. The transepithelial movement of Cl^- , Na^+ and K^+ are mainly responsible for the transport of water and therefore the hydration of the ASL. As in other epithelia, Na^+ is transported from the apical surface to the blood via apical membrane ENaC and the basolateral Na^+/K^+ -ATPase. The recycling of K^+ through potassium-selective ion channels across the basolateral membrane provides the driving force for Cl^- secretion across the apical membrane. Cl^- is secreted from the basolateral to the apical side through the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter and apical Cl^- channels such as the cystic fibrosis transmembrane conductance regulator (CFTR) or Ca^{2+} -dependent Cl^- channels such as TMEM16A.

The pulmonary epithelium is a target for steroid hormones and their effects on lung function, inflammation or immune responses to allergen exposure have been studied in depth. Estrogen has been shown to modulate the innate immune response in the lung. Cystic fibrosis mice when injected with estrogen and then infected with *Pseudomonas aeruginosa*, increased the number of white blood cells and PMN (polymorpho nuclear neutrophils) in the whole lung and in the broncho-alveolar lavage. E2 exposure in the lung of CF mice increased the inflammatory infiltrate, mucin and the mRNA levels of Toll Like Receptor 2, InterLeukin (IL)-23 and IL-17A [40]. In the case of allergen exposure in the lung, E2 may have a beneficial effect. Indeed, male mice, nebulised with ovalbumin for 10 days and then challenged with methacholine, developed airway hyper-responsiveness whereas female mice undergoing the same protocol did not show a hyperactive airway [41]. In relation to asthmatic responses it has also been described that E2 could prevent the cholinergic constriction of tracheal rings [42].

In contrast, little is known about the effect of female hormones on airway epithelial ion transport. The few published studies show that estrogen may modulate ion transport in airway epithelial cells and that this can have beneficial or deleterious effects depending on the tissue compartment and on the physiology/pathophysiology state of the airway. For example, it has been described that after an episode of respiratory distress syndrome (RDS), female patients show a much more efficient airway fluid clearance as well as a higher survival rate [43]. It has also been shown that preterm babies have a much lower plasma concentration of estrogen and progesterone and that the infants given estrogen supplement have less severe RDS and improved survival [44]. These studies suggest a role for female hormones in airway fluid clearance and lung development. This conclusion is strengthened by a study in newborn piglets that prenatal estrogen and progesterone deprivation, by the use of specific estrogen and progesterone receptor antagonists, impaired the formation of the alveolar compartments and decreased the amiloride sensitive airway fluid clearance [45].

Thus it would appear that estrogen can affect Na^+ reabsorption and fluid clearance in the lung. The first study to indicate such a role for E2 came from Swezey et al. in 1998 showing an increase in ENaC activity and mRNA copies in isolated alveolar type II epithelial cells [46]. A later study by Laube et al. described that a cocktail of estrogen and progesterone increased basal short-circuit current as well as amiloride- and ouabain-sensitive currents in alveolar epithelial cells. This study also showed that these hormones increased the mRNA expression levels of α - and β -ENaC subunits and the Na^+/K^+ -ATPase $\beta 1$ subunit [44]. These results further indicate a regulatory role for female sex hormones on Na^+ absorption in airway epithelium with a physiological consequence for alveolar airway fluid clearance.

Although estrogen may have beneficial effects on innate immune responses in allergic asthma and on fluid clearance in RDS,

the influence of gender and female sex hormones on lung function in chronic pulmonary diseases is not straight-forward [47]. In some chronic inflammatory lung diseases such as cystic fibrosis (CF), estrogen is reported to exacerbate lung function – the so-called CF gender gap. Moreover, it has been described that lung function changes with the menstrual cycle phase in women with CF [48].

The amiloride-insensitive nasal potential difference (NPD) was found to be increased in CF female patients during the luteal phase (high estrogen + progesterone concentration) of the menstrual cycle when compared to the follicular phase [49], probably due to an altered transepithelial Na^+ absorption. Other groups have studied the effect of estrogen on Cl^- transport in CF female patients and in CF bronchial epithelial cell lines. Fanelli et al. showed an increase of Cl^- efflux in the CF bronchial epithelial cell line CFBE41o after 17β -estradiol treatment. This effect was due to an increase in F508del-CFTR in the apical membrane through the up-regulation of NHERF1 [50]. In 2008, Coakley et al., showed a decrease in UTP induced nasal potential difference variation during high estrogen blood levels in CF and non-CF female patients [51]. From this observation, they studied the effect of estrogen on nucleotide-mediated ASL regulation and found that E2 decreased Ca^{2+} signaling and impaired ASL volume homeostasis through the estrogen receptor $\text{ER}\alpha$. A recent study has shown a compromised IL-8 response in inflammation with estrogen treatment in CF bronchial epithelium [52]. Taken together, these studies in bronchial and alveolar epithelia indicate a physiological role for sex-specific actions of estrogen on airway ion transport and fluid clearance in the normal lung and a pathophysiological role in exacerbation of lung function in cystic fibrosis.

4. Estrogen regulation of ion transport in intestinal epithelium

The large intestine plays a major role in regulation of whole-body water and salt homeostasis. The distal colon is the main site for water conservation in the body. Every day 1–1.5 l of fluid enters the colon with only 0.1–0.2 l being lost in the feces under normal conditions. This fluid movement is supported by two transport processes: Na^+ absorption and Cl^- secretion. Na^+ absorption through the Na^+/H^+ exchanger NHE1 in the small intestine and via ENaC in the colon permits the absorption of water from the feces under physiological conditions. ENaC in the intestine is activated by aldosterone or low plasma Na^+ . This channel constitutes the electrogenic pathway for intestinal Na^+ absorption regulated by steroid hormones. The colonic epithelium is a major site for neurohumoral regulated Cl^- secretion, which serves to ensure the mucosal hydration and the lubrication necessary for the passage of the fecal matter through the colon. Cl^- secretion is a complex mechanism involving different ion channels and transporters. Cl^- is transported into the cell basolaterally through the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transporter NKCC1 and is secreted across the apical membrane through the cystic fibrosis transmembrane conductance regulator (CFTR). The electrochemical driving force for Cl^- secretion is provided by K^+ recycling across the basolateral membrane via two types of channels, KCNN4 and KCNQ1 potassium channels. Cl^- secretion may be activated by two different pathways. The cAMP-mediated Cl^- secretion is activated by secretagogues that increase intracellular cAMP levels which also activate KCNQ1:KCNE3 channels. The calcium mediated Cl^- secretion is activated following a rise in intracellular calcium and a stimulation of calcium-dependent apical Cl^- channels and basolateral KCNN4 channels [53]. Interestingly, estrogen has been described as a salt retaining steroid hormone, high estrogen states produce an increase in whole body fluid and salt retention. Moreover distal colon has been identified as a target tissue for 17β -estradiol; indeed colonic crypts express both estrogen receptor isoforms, $\text{ER}\alpha$ and $\text{ER}\beta$ [54]. Our

laboratory first described the inhibitory role of estrogen on epithelial Cl^- secretion in the female distal colonic crypts [55]. The anti-secretory response to estrogen in the intestine occurs in a sex specific manner, no effects on secretion were found in male rats, and estradiol inhibited both basal and secretagogue-induced Cl^- secretion in female rats. The potency of the E2 anti-secretory response varied with the stage of estrous cycle and was rapid in onset, non-genomic and involved the activation of specific PKC isoforms [56,57]. This study established a physiological role for estrogen as a regulator of Cl^- secretion in order to contribute to the E2-induced salt and water retention observed during the high estrogen states [58].

4.1. Estrogen regulation of KCNQ1:KCNE3 potassium channels

Studies from our laboratory have determined the molecular mechanisms for E2 inhibition of Cl^- secretion via the modulation of KCNQ1:KCNE3 potassium channels in the basolateral membrane [59]. KCNQ1 is a voltage-gated K^+ channel expressed in a wide range of tissues and can associate with the KCNE family of regulatory subunits, which is made up of five isoform members. The expression of KCNE isoforms is tissue specific. The association between KCNQ1 and a specific KCNE subunit can drastically change the electrophysiological properties of the channel [60]. For example KCNQ1 expressed with KCNE1 produces the delayed rectifier channel expressed in the heart and essential for the cardiac action potential repolarization. In the distal colon, KCNQ1 is expressed with KCNE3 and this association causes KCNQ1 to function as a constitutively activated K^+ channel. This electrophysiological characteristic fits with the function of KCNQ1 in epithelial cell K^+ recycling [61]. The molecular mechanisms leading to KCNQ1 inhibition by estrogen involves PKC δ and PKA activation, and subsequent KCNE3 phosphorylation and dissociation from KCNQ1 causing a rapid decrease in channel conductance [56]. The sexual dimorphism in the E2 response can be explained, at least in part, by the higher expression of PKC δ in female than in the male tissue. Moreover PKC δ and PKA expression were shown to fluctuate throughout the estrous cycle with a correlation between high levels of protein kinase expression and the potency of the anti-secretory effect of E2 [57]. Thus it appears that KCNE3 plays a critical role in the sex-specific actions of estrogen on epithelial Cl^- secretion. In colonocytes KCNE3 increases KCNQ1 channel open probability at the resting membrane potential and KCNE3 is more expressed in male tissue than in female in which expression is also estrous cycle dependent. When plasma E2 levels are high, KCNE3 expression is lower. Importantly, the E2 effects on K^+ channel subunit association appears to be specific to KCNQ1:KCNE3 and other channel subunit isoform complexes are unaffected such as KCNE2 or KCNE1 association with KCNQ1. This specificity should confer a tissue-specific effect of estrogen on K^+ channel activity in the intestine and airway epithelia where KCNQ1:KCNE3 predominates, leaving the heart channel KCNQ1:KCNE1 unaffected. Patch-clamp experiments in CHO cells have demonstrated the molecular mechanism for E2 inhibition of KCNQ1:KCNE3 channel current via PKC δ phosphorylation of KCNE3 at the S82 amino acid site [59] with the subsequent uncoupling between KCNE3 and KCNQ1 leading to KCNQ1 inactivation and loss of the electrochemical driving force for Cl^- secretion.

The inhibition of intestinal Cl^- secretion in high estrogen states may be a physiological protective response against secretory diarrhea during the estrous cycle. Secretory diarrhea results in excessive loss of body fluids causing life-threatening dehydration and electrolyte imbalance. The main causes of secretory diarrhea in humans are toxins secreted by *Vibrio cholerae* toxin (CT) and *Escherichia coli* heat-stable enterotoxin (STa). These toxins stimulate cAMP or cGMP production which leads to a pronounced and

sustained Cl^- secretion. A recent study has shown that 17β -estradiol rapidly inhibits and even prevents Cl^- secretion induced by the bacterial toxins CT and STa in female rat colonic crypts. The E2 inhibition of toxin induced intestinal secretion occurs via sex-specific PKC δ dependent modulation of KCNQ1:KCNE3 channels to reduce the driving force for Cl^- secretion [61].

4.2. Estrogen regulation of TRPV6 calcium ion channels

Modulation of intracellular free calcium ion activity is a crucial signal for initiation of many important processes including gene transcription, cell growth and proliferation, neurotransmitter release [62]. Since the whole body Ca^{2+} homeostasis is dependent on gastrointestinal Ca^{2+} uptake, it is essential to understand the regulation of intestinal Ca^{2+} absorption [63]. In rat female colonic crypt, E2 rapidly stimulates a calcium influx through a non-genomic mechanism. It has been shown that this effect was gender-specific and mediated by PKC and PKA dependent signaling pathways [64]. Further studies carried out in another model of colonic epithelium, the T84 cell line, correlated this effect of E2 on Ca^{2+} influx with the E2 modulation of the endogenous Transient Receptor Potential Vanilloid 6 (TRPV6) channel. TRPV6 is an epithelial Ca^{2+} channel strongly expressed in colonic epithelium and has been proposed to be the molecular pathway for capacitative calcium entry and to play a significant role in intestinal Ca^{2+} absorption [65]. Using siRNA directed against TRPV6 it has been demonstrated that TRPV6 is the molecular target for the E2 effect on Ca^{2+} influx in intestinal epithelium [66]. These studies taken together suggest that estrogen is a major hormonal regulator of intestinal Ca^{2+} intake as a result of TRPV6 channel modulation.

5. Conclusions

Estrogen regulation of the epithelial Na^+ channel in kidney cortical collecting duct cells can mimic the effects of aldosterone to enhance Na^+ reabsorption, revealing previously unknown renal responses to estrogen (Fig. 1). In the lung, the combined pro-absorptive action of E2 through the activation of ENaC and the anti-secretory response cause a reduction in the volume of airway surface liquid lining the bronchial epithelium. Such ion transport responses to E2 in airway epithelia are female sex-specific and may underlie the CF gender gap where females with cystic fibrosis have exacerbated lung function after puberty and during the mid-estrous cycle when circulating plasma levels of E2 are highest (Fig. 1). In the intestine, one of E2 main targets is KCNQ1:KCNE3 channel and its inhibition leads to a decrease in Cl^- secretion, thus regulating whole body fluid balance (Fig. 1). Extracellular fluid volume expansion is a feature of high estrogen states such as occur in mid-term of the menstrual cycle, use of oral contraceptives and assisted *in vitro* fertilization. The pro-absorptive, anti-secretory effects of estrogen may underlie this ECF volume expansion. The physiological role of estrogen regulation of ion channels in epithelia of kidney, intestine and lung which regulate whole body fluid and electrolyte balance may be to maintain endometrial expansion necessary for implantation of the blastocyst and to enhance cardiovascular dynamics during pregnancy.

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