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Ovarian hormones modulate endothelin A and B receptor expression

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Abstract

Aims: The study aims to determine the modulatory roles of ovarian hormones, estrogen (E₂) and progesterone(P), on the expression of endothelin A (ET_A) and B (ET_B) receptors in lung, liver and kidney tissues.

Main methods: Female Sprague–Dawley rats were subjected to bilateral ovariectomy and divided into four groups ovariectomized (OVX), OVX + E₂, OVX + P, and OVX + E₂ + P. A separate group of rats underwent sham surgery and served as a control. Three weeks after OVX or sham surgery, tissues from lungs, liver, renal cortex, and inner medulla were collected, snap-frozen, and kept at –80 °C for assessment of ET_A and ET_B receptor expression using real-time PCR.

Key findings: E₂-treated OVX animals had significantly lower expression of ET_A receptors in the lungs, compared to OVX rats. Pulmonary ET_B receptor mRNA was not measurably affected by any of the interventions. Hepatic ET_A and ET_B were significantly increased in OVX + E₂ + P rats, compared to sham rats. Renal inner medullary ET_A and ET_B receptor expressions were significantly elevated in OVX compared to sham, an effect that was prevented by co-supplementation of OVX with E₂ and P. Additionally, both ET_A and ET_B receptor expression in the renal cortex were significantly attenuated by ovariectomy, and this reduction was not evident in OVX + E₂ rats.

Significance: These data suggest that ovarian hormones regulate ET receptor expression and may contribute to sex differences in cardiovascular and renal health.

Keywords

Endothelin; ET_A receptors; ET_B receptors; Estrogen; Progesterone

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Conflict of interest

The authors declare that there are no conflicts of interest.

1. Introduction

Premenopausal women are largely protected from hypertension and kidney-related diseases compared with men of similar age [15,25,28,31]. This phenomenon is gradually lost after menopause, pointing to a pivotal role of ovarian hormones in the maintenance of cardiovascular and renal health in women [11,34,37]. Endothelin-1 (ET-1), a potent vasoactive peptide found in cardiovascular and renal tissues, has been implicated in sex-differences in blood pressure control and kidney function [17–19,38]. The actions of ET-1 are mediated through activation of the G-protein-coupled receptors, ET_A and ET_B, which are found in a variety of cells in the cardiovascular and renal systems [38]. The activation of ET_A receptors by ET-1 results in strong and sustained vasoconstriction and promotes oxidative stress [3,6], inflammation, and proteinuria [30]. On the other hand, the activation of ET_B receptors by ET-1 causes vasorelaxation [12] and inhibits sodium reabsorption in the renal collecting ducts [20]. Additionally, ET_B receptors in kidney and lung may serve to clear ET from circulation by receptor-mediated uptake and subsequent lysosomal degradation [7]. Therefore, ET-1 appears to have paradoxical effects on blood pressure regulation and renal function by acting either on ET_A or ET_B receptors.

Sex differences in ET-1 signaling system have been observed in renal [14,17,19], vascular [33], pulmonary [8], as well as hepatic tissues [13]. Despite the important role for the ET-1 system in mediating sex-differences in blood pressure and renal diseases, the roles of female sex steroids, estrogen (E₂) and progesterone (P), in regulating this critically important system are unclear. Similar to E₂, ET receptor antagonists have vasculoprotective effects in ovariectomized rats [16]. E₂ suppresses renal ET-1 overproduction and the consequent renal damage in acute kidney injury [35]. In the study reported by Lamping and Nuno, P alleviates the vasoconstrictor effect of ET-1, but is less effective than E₂ [23]. Additionally, Zhang et al. showed that P treatment in OVX mice enhances renal ET_A receptor mRNA expression [40]. In the present study, we hypothesized that ovarian hormones provide cardiovascular and renal benefit that may be related to increased ET_B and decreased ET_A activity. To test this hypothesis, we studied the effect of ovarian hormonal depletion (by ovariectomy) and replacement (by E₂ and/or P supplementation in OVX rats) on ET_A and ET_B receptor mRNA expression in lung, liver, renal cortex and inner medulla.

2. Materials and methods

2.1. Animals

Studies were performed on female (13–15 weeks old) Sprague Dawley rats, purchased from Harlan Laboratories (Indianapolis, IN). Rats were maintained on standard rodent diet (NIH-31 Open Formula, 7017 (0.3% Na), Teklad Diets, Madison, WI) with free access to water. During the entire experimental period, animals were housed in a temperature and humidity-controlled room with a 12:12-h light–dark cycle. All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care animals and were approved by the Institutional Animal Care and Use Committee at University of Alabama at Birmingham.

2.2. Ovariectomy and hormonal replacement

Under isoflurane (2%) anesthesia, bilateral ovariectomy was performed through dorsal bilateral incisions. Ovaries were exposed, tied off and removed. The muscle layer was sutured and the incisions were closed with wound clips. An additional group of rats underwent sham operation (dorsal bilateral incisions and ovarian exposure without removal of ovaries) and served as a control group. At the time performing ovariectomy, OVX rats were subcutaneously implanted with controlled-release pellets (21-day release, Innovative Research America, Sarasota, FL, USA) containing: (1) 17 β -estradiol (0.35 mg/pellet, OVX + E₂), (2) progesterone (25 mg/pellet, OVX + P), or (3) both (OVX + E₂ + P). A separate group of OVX rats were implanted with cebo pellets.

2.3. Tissue collection and real time PCR

On day 21 after OVX or sham surgery, rats (16–18 weeks old) were anesthetized (isoflurane) and tissues from lung, liver and different parts of the kidney were harvested, snap frozen in liquid nitrogen and stored at –80 °C until molecular assays were performed.

RNA from different tissues was isolated using Purelink Mini RNA traction kit (Ambion) according to manufacturer's instructions. Then, the isolated RNA was reverse transcribed using QuantiTect Reverse Transcription kit (Qiagen). Finally, the resulting cDNA was used to quantify mRNA by real time PCR (CFX96 Real-Time System, BIORAD) using TaqMan primer gene expression assays with ET_A receptor (catalog no. Rn00561137_m1), ET_B receptor (catalog no. Rn00569139_m1), and GAPDH (catalog no. Rn01775763_g1) primers. ET receptor gene expression was quantified relative to GAPDH using 2^{–Ct} method [2].

2.4. Estradiol and progesterone assay

The levels of serum estradiol and progesterone were measured the ELISA (CALBIOTEC® Mouse/Rat Estradiol ELISA, Spring Valley, CA) and (ALPCO® Progesterone rat/mouse ELISA, Salem, NH), following the manufacturer instructions.

2.5. Statistics

Values are presented as means $\pm$ SEM. Statistical comparison between several groups was done by one-way ANOVA followed by post hoc analysis with *t*-test and Bonferroni correction. P values <0.05 were considered significant.

3. Results

Figs. 1–3 illustrate the effects of ovariectomy and supplementation of OVX rats with different hormone replacement regimens (E₂ and each one alone or combined together) on the mRNA expression of ET and ET_B receptors in lung, liver and kidney tissues of female Sprague Dawley rats. Compared with sham values, pulmonary ET_A mRNA levels were not affected by ovariectomy or supplementation of OVX rats with P alone or combined with E₂. However, hormone replacement of OVX rats with E₂ significantly attenuated ET_A receptor expression in lung, compared to OVX or sham values (Fig. 1A). On the other hand, expression of ET_B receptor mRNA in lungs were not significantly different among sham, OVX, OVX + E₂, OVX + E₂ + P, OVX + P groups of rats, (Fig. 1B). The expression of ET_A and ET_B

receptor mRNA appeared to be enhanced in liver tissues obtained from OVX rats, however this increase did not reach statistical significance (Fig. 2A & B). Compared to sham rats, OVX rats co-treated with E₂ and P showed significant increases in the expression of hepatic ET_A and ET_B receptor mRNA. The increase in ET_B receptor mRNA levels was also evident in rats treated with each hormone individually, compared to sham values, as demonstrated in Fig. 2A & B.

Ovariectomy significantly decreased the expression of ET_A as well as ET_B receptor mRNA within the renal cortex (Fig. 3A & B). This reduced expression of cortical ET_A and ET_B receptors caused by ovariectomy was not observed when OVX rats were supplemented with E₂ alone. In contrast, supplementation of OVX rats with P alone or combined with E₂ did not restore the OVX-induced changes in cortical ET-1 receptor expression (Fig. 3A & B). Within renal inner medullary tissue, the expression of ET_A receptor mRNA was significantly enhanced in OVX compared to sham rats (Fig. 3C). Similar increases in the expression of inner medullary ET_B mRNA were observed also in OVX rats, compared to sham rats (Fig. 3D). These elevations in the expression of both receptors were slightly decreased in OVX rats supplemented with E₂ or P but differences were not statistically significant except that ET_B receptor mRNA expression was significantly decreased in OVX + E₂ rats (Fig. 3D). Interestingly, the elevated expression of cortical ET_A and ET_B receptor mRNA induced by ovariectomy was significantly reduced to sham levels by co-supplementation of OVX rats with E₂ and P (Fig. 3C & D).

Similar serum E₂ levels were observed in OVX and OVX + P rats (2.2 ± 0.2 and 2.3 ± 0.2 pg/ml) that were significantly lower than those of sham rats (13.7 ± 5.3 pg/ml). Circulating E₂ levels in OVX + E₂ and OVX + E₂ + P rats were increased to levels significantly higher than sham levels (58.5 ± 6.2 and 42.5 ± 4.9 pg/ml, respectively). Assessment of serum levels of progesterone showed that ovariectomy significantly reduced the level of serum progesterone compared to sham rats (42.9 ± 3.9 vs. 92.2 ± 5.5 ng/ml, respectively), and this was restored to sham levels in OVX + E₂ + P rats (82.5 ± 8.2 ng/ml). Surprisingly, serum progesterone concentrations in OVX + P rats (42.4 ± 8.4 ng/ml) were similar to those of OVX rats and lower than those in OVX + E₂ + P rats (91.9 ± 8.1 ng/ml).

4. Discussion

The main finding of the present study is that ET-1 receptor expression is modulated by the ovarian hormonal status, particularly in the kidney, and this finding may contribute to the cardiovascular and renal protection demonstrated in premenopausal females that is lost after menopause. Our data further suggest a regulatory role for sex-hormonal replacement on ET-1 receptors profile.

As a potent endothelium-derived vasoactive peptide, ET-1 plays a pivotal role in regulating vascular tone and appears to play an important role in the development of pulmonary hypertension [4], liver cirrhosis and portal hypertension [29]. In addition, administration of selective ET_A or mixed ET_{A/B} antagonists reduces pulmonary [4] and portal pressure [32]. In the present study, E₂ supplementation to OVX rats had an attenuating effect on pulmonary ET_A receptor mRNA expression. This observation is in line with previous data suggesting

that raloxifene, having estrogen agonistic actions in the cardiovascular system [1,24], effectively prevents the development pulmonary hypertension in OVX rats, at least in part, by suppressing ET-1 overproduction [27]. These two findings demonstrate an important interaction between E_2 and the ET-1 system in the lung, suggesting a protective effect of E_2 on ET-1-induced control of pulmonary vascular tone. On the other hand, the expression of pulmonary ET_B receptors that function mainly as clearance receptors for circulating ET-1 was not significantly affected by ovariectomy or hormonal replacement to OVX rats. Additionally, our results show that neither hepatic ET_A nor ET_B receptor mRNA is significantly changed in response to ovarian hormone depletion. However, replacement of OVX rats with the dual hormonal replacement regimen showed significant increases in the hepatic ET_A and ET_B receptor mRNA expression levels compared to sham levels. Individual supplementation of E_2 or P also caused significant increases only in hepatic ET_B receptor mRNA levels, compared to sham rats. Thus, recent observations by Ho et al. [13] demonstrating that E_2 attenuates intrahepatic vasoconstriction to ET-1 may be linked to E_2 -induced enhancement of ET_B receptor expression in the liver. However, we used whole liver extracts and did not specifically examine intrahepatic vascular tissue.

Within renal tissues, our study revealed a high potential for ovarian hormones to regulate ET_A and ET_B receptor mRNA expression in both the cortex and inner medulla. Overall expression of both ET_A and ET_B receptor levels in the cortex typically represent vascular structures, while ET_B receptor expression in renal medulla is heavily weighted towards tubular structures due to high levels of expression in the inner medullary-collecting duct (IMCD). Taking into consideration that the kidney is very sensitive to the vasoconstrictor properties of ET-1 [5] and plays a critical role in water and Na^+ homeostasis, changes in renal ET-1 receptor expression may have considerable effects on sex differences in renovascular resistance as well as water and electrolyte balance. Our findings that OVX reduced cortical ET_A and ET_B expression that was not evident with E_2 replacement suggests that E_2 normally functions to maintain renal cortical expression of both receptor sub-types, most likely in vascular structures. Further studies will be needed to determine to what extent the effects of E_2 on ET_B receptor expression are endothelial versus epithelial.

It is well established that the inner medullary ET_B receptors are major contributors to the maintenance of water and Na^+ balance [20], however the role of inner medullary ET_A receptors is not clear. It is generally believed that inner medullary ET_A receptors oppose ET_B effects and are anti-natriuretic by virtue of their vasoconstrictor properties [14,26]. In contrast, some studies suggest that the ET_A receptor may contribute to the natriuretic response to ET-1 [9] and that blockade of ET_A receptors leads to fluid retention through effects on tubular reabsorption [21]. Sex-differences in renal medullary ET_A and ET_B receptor function have been previously reported [14,17–19,26,36]. Interestingly, our lab has demonstrated increased ET_A receptor binding and intracellular Ca^{2+} signaling in IMCD obtained from male, compared to female rats [14]. In line with the former study, our current data show that inner medullary ET_A receptor expression was enhanced by ovariectomy and restored back to sham-levels by supplementation of E_2 and P to OVX rats. Our data, together with the previously published sex-difference in ET_A receptor expression in IMCD [14], highlight a central role for ovarian hormones in attenuating inner medullary ET_A receptor

expression in females, that may be linked to decreased incidence of salt-sensitive hypertension premenopausal females vs. males and postmenopausal females.

Our results also showed that inner medullary ET_B receptor mRNA expression was increased in response to depletion of endogenous ovarian hormones and normalized by E₂ and P treatment to OVX rats. Thus it is apparent that the E₂ and P work co-operatively to modulate inner medullary ET_A and ET_B receptor expression, because individual supplementation of each hormone was less effective in restoring receptor expression. This is consistent with previous studies reporting synergism between of E₂ and P on renovascular tone control [10]. Overall, this work provides evidence that the ET-1 system is highly influenced by the sex-hormonal status in females. These effects are not general to all tissues since we observed that renal cortex and lung tissues are restored by E₂ replacement alone, whereas, inner medullary tissues are more sensitive to the combined effect of E₂ and P.

Collectively, our data pinpoint that ovariectomy affected renal and hepatic ET receptor mRNA expression, however, changes in the hepatic ET receptors with OVX did not reach statistical significance levels. On the other hand, ovariectomy did not affect pulmonary ET receptor mRNA expression. This might be related to many factors including the use of whole lung extracts that did not specifically examine pulmonary vascular tissue. In addition, although our results did not show effects on receptor expression in response to ovariectomy, changes might be detected at the receptor protein level.

It is imperative to comment on the apparently higher levels of circulating E₂ in estrogen-replaced OVX rats compared to sham rats. Representation of the different stages of the estrus cycle, and not only the proestrus stage, in the sham group is very likely to contribute to relatively low E₂ levels in sham rats because the level of E₂ in the diestrus rats is as low as in OVX rats. Additionally, other factors that might be responsible for the comparatively higher E₂ levels in OVX + E₂ and OVX + E₂ + P rats include continuous supplementation of the hormone as sustained release pellets which differs from the natural rhythmic fluctuations in the level of ovarian hormones in sham rats. On the other hand, the unexpected levels of serum progesterone in OVX + P and OVX + E₂ rats might be related to multiple causes. One conceivable candidate is cross-reactivity of the antibodies provided in the commercial assay with a number of related steroid compounds already existing in serum samples [22]. Interestingly, however, the combination of E₂ with P resulted in restored P concentrations. Going forward, it will be important to determine the contribution of free progesterone versus progesterone associated with steroid binding proteins, which was not done in the current study, to get a clearer explanation for serum progesterone measurements.

Finally, our findings suggest that the ability of ovarian hormones to modulate the expression of both ET_A and ET_B receptors within the renal cortex and inner medulla are in the same pattern, thus maintaining the normal balance between the usually contrasting effects of ET_A and ET_B activation within the kidney. We postulate that the differences in cortical ET_A and inner medullary ET_B receptor expression observed in females with different hormonal status might be a compensatory mechanism to changes in cortical ET_B receptor and inner medullary ET_A receptor expression, respectively, rather than a direct effect of ovarian hormones on receptor expression. This idea will require further investigation. Taken

together, these changes in renal ET-1 receptor expression could be related, at least in part, to the normal physiologic responses to keep the balance between the opposing effects of ET_A (vasoconstrictor and anti-natriuretic) and ET_B (vasodilator and natriuretic) receptor function.

5. Limitations of the study

In the current study, we measured the level mRNA expression and not the receptor protein levels. Although, the expression of mRNA has been often interpreted as the concentration of the corresponding protein assuming that the transcript abundances are the main determinant of the protein abundances, however, protein abundances reflect a dynamic balance among a series of linked processes including, but not limited to transcription. Processing of mRNA to translation, localization, modification, and programmed destruction of the receptor protein itself have important effects on the level of receptor protein [39]. So our results are limited to the effect of ovarian hormones on ET_A and ET_B mRNA expression and do not extend to interpret ET_A and ET_B receptor abundances, which could be assessed in future studies using other techniques as binding studies.

6. Conclusion

This preliminary study points to a potential modulatory role of ovarian hormones on ET_A and ET_B receptor expression that may have a considerable impact on cardiovascular and renal health in the female population. Future studies are needed to clarify the effects of the ovarian hormones on vascular versus tubular ET-1 receptors expression, signaling and function.

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Fig. 1. Relative mRNA expression of ET_A (A) and ET_B (B) receptors in lung tissues obtained from sham, ovariectomized (OVX) rats implanted with subcutaneous pellets containing estrogen (OVX + E₂), progesterone (OVX + P), their combination (OVX + E₂ + P) or placebo pellets (OVX). Values represent fold change from sham levels. Data are means ± SE of 4–6 samples in each group. *P < 0.05 vs. sham. †P 0.05 vs. OVX.

Fig. 2.

Relative mRNA expression of ET_A (A) and ET_B (B) receptors in liver tissues obtained from sham, ovariectomized (OVX) rats implanted with subcutaneous pellets containing estrogen (OVX + E₂), progesterone (OVX + P), their combination (OVX + E₂ + P) or placebo pellets (OVX). Values represent fold change from sham levels. Data are means ± SE of 5–7 samples in each group. *P < 0.05 vs. sham.

Fig. 3.

Relative mRNA expression of ET_A and ET_B receptors in renal cortex (A & B) and inner medulla (C & D) obtained from sham, ovariectomized (OVX) rats implanted with subcutaneous pellets containing estrogen (OVX + E₂), progesterone (OVX + P), their combination (OVX + E₂ + P) or placebo pellets (OVX). Values represent fold change from sham levels. Data are means $\pm$ SE of 4–7 samples in each group. *P < 0.05 vs. sham. †P < 0.05 vs. OVX.