Estrogen Paradox in Pulmonary Hypertension Current Controversies and Future Perspectives

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Although the incidence of pulmonary hypertension is higher in female patients, numerous experimental studies have demonstrated better outcome in female animals, exacerbation of the disease after ovariectomy, and a strong protective effect of estrogen: a phenomenon known as the “estrogen paradox” of pulmonary hypertension. On the other hand, some clinical studies have indirectly linked estrogen to increased risk of portopulmonary hypertension, whereas others implicate increased estrogen metabolism and high levels of certain estrogen metabolites in promoting pulmonary vascular remodeling in familial pulmonary arterial hypertension. In this review we investigate the estrogen paradox through highlighting the differential receptor-mediated effects of estrogen. Although estrogen and estrogen receptor-based therapies have shown promise in rescuing preexisting pulmonary hypertension in animals, their role is yet to be defined in humans.

Keywords: estrogen paradox; pulmonary hypertension; estrogen receptors; estrogen metabolites

The role of sex and estrogen (E₂) in pulmonary hypertension (PH) is not fully known. The majority of animal studies thus far have suggested a protective role of female sex and a preventive as well as therapeutic role of E₂ in PH (1–6). E₂ exerts most of its biological effects via two receptors that are abundantly expressed in the cardiopulmonary system: estrogen receptor α (ER α) and estrogen receptor β (ER β). These two receptor subtypes have been shown to have opposing effects in different tissues. For example, ER β has cardiopulmonary protective properties and confers the antifibrotic, antihypertrophic, and vasodilatory properties of E₂ in the heart and lungs (5, 7, 8). By contrast, ER α has proliferative properties in certain tissues and cancers whereas ER β exerts antiproliferative effects (9). Because of these contrasting properties, ER-specific agonists may help to clarify E₂-related effects on different organs and in different disease states. ER β -specific agonists have shown promise in the treatment of experimental PH (5). Naturally occurring ER β agonists such as phytoestrogens have also been successfully used to treat experimental PH (10, 11). Further studies involving genetically modified ER α and

ER β mice are needed to clarify their exact role in the pathogenesis and treatment of experimental PH. Furthermore, human data are not conclusive at this time on the precise role of sex, E₂, E₂ metabolites, and ERs in pulmonary arterial hypertension (PAH). The estrogen paradox still exists (12) and more preclinical and clinical data are needed to resolve this paradox and to determine whether there is a potential benefit to ER-based therapies for human PAH.

ESTROGEN METABOLISM AND ESTROGEN RECEPTORS

17 β -Estradiol or estrogen (E₂), a steroid hormone, is the primary active sex hormone in the female. Steroid hormones are synthesized in the gonads and adrenal glands and are derived from cholesterol. Cholesterol is first converted to pregnenolone that is either metabolized through 17-hydroxypregnenolone to dehydroepiandrosterone and then androstenedione. Pregnenolone can also be metabolized to progesterone and further downstream to testosterone and, via aromatase enzyme, to estradiol. Estradiol can be converted to estrone, or be metabolized through many different pathways. CYP1B1 and CYP1A1 convert estradiol to the catechol estradiols, such as 2-hydroxyestradiol (2-HE), which can then be converted via catechol-O-methyltransferase (COMT) to the methoxyestradiols, such as 2-methoxyestradiol (2-ME). Estrone is metabolized to 16 α -hydroxyestrogen (13–15) (Figure 1).

Estradiol exerts most of its biological effects through its receptors ER α or ER β . Both these receptors are expressed in the lung and heart tissues (16, 17). ER β has been shown to protect the lungs in the trauma-hemorrhage model (16). ER β -knockout mice demonstrated abnormal vascular function and hypertension as well as increased mortality (18, 19). The role of these estrogen receptors has also been highlighted in cancer. More recently it has been shown that ER α has proliferative properties in certain types of cancers whereas ER β exerts antiproliferative effects (9). It has been shown that the antihypertrophic properties of estrogen in the heart are mediated mainly via ER β (5, 7). ER β is also the key receptor involved in the antifibrotic properties of E₂ against cardiac fibrosis (8).

ROLE OF SEX AND ESTROGEN IN THE PREDISPOSITION AND DEVELOPMENT OF PULMONARY HYPERTENSION

Despite advances, the role of sex and E₂ in the pathogenesis of PAH is incompletely understood. Estrogen has well-known protective effects in the cardiovascular system (20). Sex differences do exist in both experimental and clinical forms of PH. However, in various animal models, females have less evidence of PH compared with males and ovariectomy resulted in exacerbation of the disease. Female rats and swine, compared with males (1–3), when exposed to chronic hypoxia develop less severe pulmonary hypertension, right ventricular hypertrophy, vascular remodeling, and polycythemia. These sex differences

(Received in original form January 11, 2012; accepted in final form April 18, 2012)

Supported by NIH grants HL089876 and HL089876S1 (M.E.).

Author Contributions: Contributions to conception and design: S.U. and M.E.; drafting the article and revising it critically for important intellectual content: S.U., M.R., and M.E. All authors approved this version of the manuscript.

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Am J Respir Crit Care Med Vol 186, Iss. 2, pp 125–131, Jul 15, 2012

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Originally Published in Press as DOI: 10.1164/rccm.201201-0058PP on May 3, 2012

Internet address: www.atsjournals.org

Figure 1. Sex hormone biosynthesis, estrogen metabolism, and estrogen receptors. *Sex hormone biosynthesis:* Sex hormone biosynthesis and the conversion of estrogen (E₂) to its downstream metabolites 2-hydroxyestradiol (2-HE), 2-methoxyestradiol (2-ME), the so-called “good metabolite” shown in green, and 16α-hydroxyestrone (the so-called “bad metabolite” shown in red) via CYP1B1 and COMT (13) are shown. *Genomic and nongenomic pathways for the cellular actions of E₂:* Local E₂ biosynthesis from the conversion of testosterone to E₂ via aromatase (CYP450) enzyme is shown. AKT = protein kinase B; COMT = catechol-O-methyltransferase; CYP1B1 = cytochrome P450 1B1; DHEA = dehydroepiandrosterone; E₂ = estrogen; ERα/β = estrogen receptors α and β; ERE = estrogen response element; GPCR = G protein-coupled receptor; 2-HE = 2-hydroxyestradiol; 17β-HSD = 17β-hydroxysteroid dehydrogenase; MAPK = mitogen-activated protein kinase; 2-ME = 2-methoxyestradiol; P = phosphate; PI3K = phosphoinositide-3-kinase; TF = transcription factor.

are attributed to the protective effects of estrogen in the females, as estrogen and its metabolites have been shown to prevent and rescue experimental PH (4, 5, 21, 22).

We found that a single dose of monocrotaline (MCT, 60 mg/kg) induced PH in female rats to a lesser extent compared with male rats (5) as has been previously reported by others in the same model (22), or by vascular endothelial growth factor (VEGF) receptor inhibition followed by chronic exposure to hypoxia and prolonged recovery in normoxia (23). E₂ treatment of female rats starting on Day 21 after monocrotaline resulted in reversal of vascular pathology in the lungs, similar to our findings in male rats (5). These data showed that the beneficial effects of E₂ in females and males with experimental PH are similar. The sex differences and the effects of ovariectomy in the newly established VEGF receptor inhibition/hypoxia/normoxia model still need to be investigated in detail as experiments using different protocols have shown variable results (23–25).

There is ample evidence to support a sex-related role for serotonin in the pathobiology of PH. Serotonin is synthesized in pulmonary artery endothelial cells, and there is cooperative interaction between the 5-HT_{1B} receptor and the serotonin transporter (SERT) to mediate proliferation of pulmonary artery smooth muscle cells. Activity of SERT may modulate the development of PH, and mice overexpressing SERT (SERT⁺) develop PH and severe hypoxia-induced PH that is abolished by ovariectomy. Male SERT⁺ mice do not develop PH whereas female SERT⁺ mice demonstrate increased right ventricular systolic pressure and pulmonary vascular resistance that is abolished

by ovariectomy. Chronic administration of estrogen reestablishes PH in ovariectomized, normoxic, and hypoxic SERT⁺ mice (26). It is of interest that serotonin mediates its effects through up-regulation of S100A4 (27) and that the S100A4-overexpressing mouse shows a female predisposition to severe pulmonary vascular pathology (28). In that study it was shown that 17β-estradiol increases S100A4 and causes smooth muscle cell proliferation.

The possible role of testosterone in establishing the sex difference in PH cannot be overlooked. Although testosterone has vasodilator properties in isolated pulmonary arteries (29–31), male rats with high levels of testosterone do worse than females in various experimental models of PH (1–3, 22, 23). For example, male apoE^{−/−} mice receiving a high-fat diet developed more severe PH associated with insulin resistance and lower plasma adiponectin levels compared with female apoE^{−/−} mice (32). As testosterone inhibits the secretion of adiponectin in adipocytes (33), it was hypothesized that elevation of adiponectin accounted for the less severe vascular phenotype in female apoE^{−/−} mice. When male apoE^{−/−} mice were treated with rosiglitazone, there was an eightfold higher plasma adiponectin level, improved insulin sensitivity, and complete regression of PH (32). Thus, testosterone per se does not seem to offer protection against experimental PH or explain female predominance of PAH (13).

In the clinical setting, there is a higher incidence of PAH in females, and this is particularly evident in idiopathic and familial forms (34). Clinical PAH is considered to be a disease of young

females, although more recently there seems to be a change in the trend and older females are increasingly diagnosed with PAH (34). A sex-related response to treatment has also been described in the clinical setting, as women with PAH derived greater benefit than men from endothelin receptor antagonist therapy (25).

ESTROGEN TREATMENT FOR PULMONARY HYPERTENSION

Estrogen therapy given to animals with PH results in prevention as well as rescue of severe PH and associated right ventricle (RV) dysfunction (4, 5). The beneficial effects of E₂ in PH may result from a multitude of actions of E₂ on both lungs and heart.

E₂ and Inflammation and Pulmonary Hypertension

Inflammation plays a key role in the progression of experimental PH as well as in clinical forms of PAH of various origins (35), and E₂ has shown promising antiinflammatory properties in many tissues such as the lung (36). E₂-induced rescue of PH in animals is associated with significantly reduced pulmonary inflammation (5). Hypoxia-induced pulmonary vascular remodeling requires recruitment of circulating mesenchymal cells that are hematopoietic progenitors (37). Frid and colleagues demonstrated a striking perivascular accumulation of mononuclear cells of a monocyte/macrophage lineage (expressing CD45, CD11b, CD14, CD68, ED1, and ED2) (37). Furthermore, early recruitment and alternative activation of alveolar macrophages in hypoxic lungs appear to be critical for the later development of hypoxic PH (38). In addition, previous studies have shown a relationship between alveolar macrophage number and PH in the monocrotaline (MCT) model (39, 40). E₂ limits pulmonary monocyte/macrophage infiltration associated with PH (5). There are reports suggesting that these antiinflammatory effects of E₂ are mediated mainly via ER β (41–43).

E₂ and Vascular Remodeling and Pulmonary Hypertension

Remodeling of the pulmonary vasculature plays an important role in the progression of PH in animal models as well as in patients with PAH, that is, proliferation of cells with properties of smooth muscle cells that leads to obliteration of the vascular lumen, resulting in increased pulmonary vascular resistance. E₂ inhibits proliferation and migration in human vascular smooth muscle cells (44). Tofovic and colleagues have demonstrated a dose-dependent effect of E₂ on inhibition of human pulmonary artery smooth muscle growth and a biphasic response in endothelial cells *in vitro* (45). In another study, on the other hand, it was shown that 17 β -estradiol increased S100A4 and caused smooth muscle cell proliferation (28). It has been shown that E₂ attenuates hypoxic pulmonary vascular remodeling and decreases proliferation in hypoxic, but not normoxic, cultured rat pulmonary artery endothelial cells in an ER-dependent manner. E₂ effects on PA remodeling and endothelial cell activation are mediated by both ER α and ER β (6).

E₂ and Cardiopulmonary Fibrosis and Myocardial Hypertrophy

Pulmonary fibrosis can be associated with PH, and myocardial fibrosis can also complicate the disease course in PH. E₂ and its metabolites have been shown to have potent antifibrotic properties in the settings of severe PH (5, 46). Pedram and colleagues have shown that ER β is responsible for the antifibrotic effects of E₂ in the heart. E₂ inhibits the transition of fibroblasts into myofibroblasts via ER β in the heart, hence preventing myocardial

fibrosis (8). E₂ also has ER β -mediated direct antihypertrophic effects in the heart (7, 47–49). Females are less prone to develop myocardial hypertrophy than males and ER β mediates this male–female difference in the development of pressure-overload hypertrophy (50). Female sex and ER β also attenuate cardiac remodeling and apoptosis in the pressure overload model (51).

Effects of E₂ on Cardiopulmonary Angiogenesis

Loss of pulmonary microvessels and impaired regeneration contribute to the progression of PH. In the RV, loss of myocardial microvessels and increased oxygen demand in patients with PH result in RV ischemia (52). MCT-induced experimental PH is also associated with decreased RV capillary density (5). By contrast, hypoxic PH leads to increased RV capillary density, a marker of hypoxia-induced RV remodeling (53). In this setting, E₂ was shown to decrease hypoxia-induced RV capillarization, which was attenuated by nonspecific ER antagonist (6). On the other hand, E₂ has well-known proangiogenic properties in various tissues (54). E₂ regulates proangiogenic VEGF gene transcription via estrogen receptors α and β (55). E₂ also induces endothelial cell migration and proliferation through estrogen receptor-mediated pathways (56). In experimental PH induced by MCT, E₂ stimulates cardiopulmonary neoangiogenesis, resulting in a significantly better prognosis (5). We believe that stimulation of cardiopulmonary angiogenesis by E₂ is a key mechanism involved in E₂-induced rescue of PH. Increased myocardial blood vessels together with increased angiogenesis in the pulmonary circulation by E₂ therapy may underlie the decrease in PH severity leading to improvements in cardiopulmonary structure and function. The fact that in the presence of TNP-470, a potent angiogenesis inhibitor, E₂ failed to rescue MCT-induced PH further supports the role of angiogenesis in E₂-induced rescue of PH (5).

Effects of E₂ on NO-dependent Vasodilation

Accumulating evidence indicates that E₂ stimulates nitric oxide synthase (NOS) activity and therefore improves pulmonary hemodynamics and vascular remodeling (57, 58). Augmented endothelial NOS (eNOS) protein expression in pregnant human myometrium is induced by E₂ via both ER α and ER β (59). Postnatal E₂ up-regulates lung NO synthases and improves lung function in bronchopulmonary dysplasia (60). Exogenous E₂ rapidly attenuates pulmonary artery (PA) vasoactivity and acute hypoxic pulmonary vasoconstriction (61). Lahm and colleagues also concluded that sex and menstrual cycle affect PA vasoconstriction in isolated PA rings. Even physiological increases in circulating E₂ levels attenuated PA vasoconstriction under both normoxic and hypoxic conditions (62). Furthermore, E₂ acutely stimulates NOS activity in fetal pulmonary artery endothelium (57) and improves pulmonary hemodynamics and vascular remodeling in perinatal PH (58). E₂ increases NO-dependent dilation in rat pulmonary arteries (63). Selective ER α and ER β agonists also rapidly decrease pulmonary artery vasoconstriction by an NO-dependent mechanism (64). However, deletion of the eNOS gene also has a greater impact on the pulmonary circulation of male than female mice (65), perhaps because other E₂-dependent mechanisms are induced or because of other sources of NO independent of eNOS, that is, inducible NOS. Both E₂ stimulation of endothelial-dependent vascular relaxation and inhibition of vascular smooth muscle contraction may contribute to the sex-related differences in vascular tone and may represent beneficial vascular effects of hormone replacement therapy (66).

Figure 2. Summary of the likely protective effects of estrogen against pulmonary hypertension. The possible beneficial effects of estrogen for the treatment of pulmonary hypertension are illustrated, along with the corresponding reference numbers. eNOS = endothelial nitric oxide synthase; ER β = estrogen receptor β ; ERs = estrogen receptors; PA = pulmonary artery; PAECs = pulmonary artery endothelial cells; PASM = pulmonary artery smooth muscle cell; VEGF = vascular endothelial growth factor.

E₂ may also cause myocardial vasodilatation and help the failing RV. E₂ stimulates expression of endothelial and inducible NOS in rat myocardium *in vitro* and *in vivo* (67). Chronic E₂ replacement increases NO-mediated vasodilation of the guinea pig coronary microcirculation (68).

Local Tissue Estrogen Production, Estrogen Metabolism, Aromatase, and PAH

Clinical studies have shown a variable role of estrogen in the development of PAH. Postmenopausal women are at increased risk of developing systemic sclerosis-related PAH (69) and hormone replacement therapy can prevent the development of PAH in these patients (70). Mutations in bone morphogenetic protein receptor type 2 cause familial PAH, but the penetrance is reduced and females are significantly overrepresented. The findings of Austin and colleagues suggest that variations in estrogens and estrogen metabolism may modify familial PAH risk and that the risk may be altered by variations in CYP1B1 (71). In patients with familial PAH, CYP1B1, a highly expressed enzyme in lung tissue that metabolizes estrogen and produces estrogen metabolites, increases the conversion of E₂ into 16 α -hydroxyestrone, a metabolite that promotes pulmonary vascular remodeling (71). In contrast, some estrogen metabolites, such as 2-methoxyestradiol (2-ME), attenuate experimental PH (21, 22). Aromatase is an enzyme that converts endogenous androgens to estrogen. The increased risk of portopulmonary hypertension in female patients with chronic liver disease is associated with high aromatase activity (72).

The lung possesses the required machinery to biosynthesize estrogen from testosterone with the help of aromatase (73). Local regulation of aromatase expression and activity will influence the levels of E₂ and the nature of its metabolites in the lung tissue. However, no studies in the adult lung have reported local aromatase activity or whether local lung E₂ biosynthesis is disrupted in advanced PH. Although high aromatase expression is seen in portopulmonary PAH, it is uncertain whether it originated in the lung tissue and could play a role in replenishing local E₂ biosynthesis.

Circulating Plasma Estrogen Levels in Pulmonary Hypertension

There is a paucity of clinical data correlating circulating plasma estrogen levels in patients with idiopathic PAH with their age,

sex, disease severity, and reproductive state. On the other hand, the results of animal studies showing the efficacy of estrogen replacement therapy (4, 5) suggest that severe PH could well be a low estrogenic state. The effect of menopause and hormone replacement therapy on the disease course and progression is still not clear in patients with PAH.

RV failure is a major cause of morbidity and mortality in patients with PAH. Circulating estrogen levels may also affect cardiac function. Interestingly, higher circulating estradiol levels are associated with better RV systolic function in postmenopausal women using hormone replacement therapy, and higher levels of androgens were associated with greater RV mass and volumes in both sexes (74).

ROLE OF ESTROGEN RECEPTORS IN PULMONARY HYPERTENSION

The combined contribution of specific estrogen receptors and metabolites may clarify the controversies surrounding the role of sex and estrogen in PH.

Our recent data provide evidence that the beneficial actions of E₂ on MCT-induced PH are ER dependent, mainly mediated through ER β because (1) a selective ER β agonist was able to rescue PH as efficiently as E₂; and (2) E₂ failed to reverse PH in the presence of a selective ER β antagonist (5). The ER-mediated action of E₂ has also been reported in hypoxic PH (6). Lahm and colleagues showed that the protective action of E₂ in attenuating hypoxic PH could be reversed by a nonselective E₂ receptor antagonist. However, in addition to ER β , ER α appears necessary for E₂ protection in hypoxic PH. Although a higher dose and longer duration of E₂ were used in the hypoxia study (6), this difference may reflect distinct mechanisms of action of E₂ in hypoxic PH compared with MCT-induced PH, as contributions of each ER subtype may be context specific.

It appears that estrogen-mediated protection against vascular injury, hypertension, cardiac remodeling and apoptosis is ER mediated specifically through ER β (18, 51, 75). However, previous reports have suggested beneficial effects of E₂ metabolites in hereditary PAH and in MCT-induced PH (21, 22, 71), and it has been speculated that the action of E₂ in preventing PH is independent of receptors because E₂ metabolites have weak affinity for ERs (22). This speculation has been challenged because E₂ in the presence of the COMT inhibitor OR-486, which prevents the conversion of E₂ to catechol and methoxyestrogen metabolites, was still able to attenuate the progression of PH (6).

ESTROGEN RECEPTOR-BASED THERAPIES FOR PULMONARY HYPERTENSION

The critical role of ER β in the lung has been previously demonstrated in experiments where ablation of ER β resulted in severe structural abnormalities (73). Because we discovered in our more recent study that E₂ reversal of PH is likely mediated by ER β , it will be important to evaluate the potential clinical use of selective ER β agonists through rigorous preclinical studies. PH is associated with RV myocardial hypertrophy. ER β also seems to play an important role against myocardial hypertrophy because estrogen therapy was able to reduce cardiac hypertrophy in ER α -deficient mice but was ineffective in ER β -deficient mice (49). More studies are warranted in ER α and ER β knockout mice to understand the pathogenesis and the potential of E₂ to rescue PH in these animals. E₂, by binding to ER β , could regulate the transcriptional activity of its target genes (Figure 1). Alternatively, E₂ can activate protective signaling pathways through G protein-coupled receptors (Figure 1). These beneficial actions of estrogen therapy (Figure 2) seem to be maintained or potentiated even after removal of estrogen (5).

Experimental studies show a differential receptor-dependent protective effect of estrogen in experimental models of PH (5, 6). These studies place the estrogen paradox in PAH in an entirely different perspective and highlight the importance of specific estrogen receptor-modulating therapies for PAH. Our findings in rats raise the possibility that E₂ and specific ER β agonists may have significant benefit for patients with preexisting severe PAH. Phytoestrogens are structurally nonestrogenic polyphenolic compounds that specifically bind to ER β with much higher affinity than ER α (76). Experimental studies have shown that phytoestrogens prevent and reverse experimental PH (10, 11). Phytoestrogens have been shown to exert their beneficial effects in the cardiovascular system preferentially through stimulation of ER β (76). Phytoestrogens also restore nitric oxide-mediated relaxation in isolated pulmonary arteries from chronically hypoxic rats (77). Genistein, a natural soybean-derived phytoestrogen, is a vasodilator with cardioprotective and antiinflammatory properties (78, 79) and also promotes apoptosis in pulmonary artery smooth muscle cells (80).

Although females have shown innate protection against experimental PH (1–3, 5, 22), and E₂, ER β -specific agonists, and phytoestrogens have shown promise in treating PH in animal models (4, 5, 10, 11), we must be cautious in translating those findings to humans. The etiological differences in the pathobiology of experimental PH models compared with human idiopathic PAH may bear significance in relation to the sex- and estrogen-related data.

Altogether, ER-based therapies are emerging as promising agents for the treatment of experimental PH. More clinical data will be needed to establish them as a novel and safe treatment modality for patients with PAH.

CONCLUSIONS

Despite being strongly predominant in female patients, PH responds well to E₂ therapy in various experimental settings. The rescue action of E₂ in experimental PH is ER mediated (5, 6). Nonhormonal selective ER ligands may minimize the untoward estrogenic side effects and warrant further preclinical testing as novel treatment options for patients with PAH.

Author disclosures are available with the text of this article at www.atsjournals.org.

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