The Influence of Sex Hormones on Pulmonary Vascular Reactivity: Possible Vasodilator Therapies for the Treatment of Pulmonary Hypertension

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Abstract: Pulmonary hypertension is a rare disease of the pulmonary vasculature defined as a mean pulmonary artery pressure >25 mmHg at rest or 30 mmHg with exercise. Recent therapies such as epoprostenol, bosentan and sildenafil are directed at the arterial vascular bed, causing vasodilatation and reducing pulmonary vascular resistance. However idiopathic pulmonary artery hypertension (IPAH) occurs predominantly in women, with three times the incidence compared to men and this suggests that sex hormones may be involved in the pathogenesis. 17\textbeta-estradiol is a pulmonary vasodilator, proposed to act via an endothelium-dependent pathway, involving nitric oxide (NO) and has also been shown to alter responses to hypoxia. Progesterone is also a pulmonary vasodilator but differs from 17\textbeta-estradiol in having endothelial-dependent and independent processes implicated. Interestingly testosterone has been shown to be a vasodilator in both the coronary and pulmonary circulation with a mechanism of action involving calcium channel blockade of the vascular smooth muscle and without endothelial involvement. Clinical trials testosterone confers symptomatic benefits in patients with coronary heart disease and heart failure, acting as a vasodilator. These observations lend support to the notion that testosterone could be a potential treatment for patients with PAH as vasodilator therapy remains the mainstay of treatment. Other potential beneficial effects of testosterone in the pulmonary circulation include immuno-modulation, altering expression of cytokines and an anti-thrombotic action. In this review the influence of sex hormones on the pulmonary vasculature will be discussed, with specific focus on pulmonary hypertension and the potential treatment of this condition.

Keywords: Pulmonary hypertension, oestrogen, progesterone, testosterone.

INTRODUCTION

Pulmonary hypertension is a rare disease of the pulmonary vasculature defined as a mean pulmonary artery pressure >25 mmHg at rest or 30 mmHg with exercise [1]. It has recently been reclassified by the WHO into five classes [2]:

1) pulmonary arterial hypertension (PAH), of which idiopathic PAH (IPAH) is a subgroup (previously called primary pulmonary hypertension);

2) pulmonary hypertension with left sided heart disease;

3) pulmonary hypertension with lung disease and/or hypoxaemia;

4) pulmonary hypertension due to thrombotic and/or embolic disease (CTPH); and

5) miscellaneous.

The pathogenesis in the development of PAH is proposed to be multi-factorial, involving genetic factors such as the transforming growth factor beta (TGF-\beta) superfamily of genes, vasoactive mediators such as prostacyclin, nitric oxide (NO), serotonin, angiotensin II and endothelin 1 (ET-1), as well as cytokines and other growth factors such as vascular endothelial growth factor (VEGF) (reviewed in [3]). These conspire to induce vascular remodeling and vasoconstriction and the characteristic pathological changes. In patients with PAH these include medial hypertrophy of the muscular pulmonary arteries, concentric laminar intimal fibrosis, plexiform lesions, fibrinoid degeneration and thrombotic lesions [4-6]. The plexiform lesions are thought to represent irregular canalization of obliterated arterioles.

IPAH occurs predominantly in women, with three times the incidence compared to men [7] and this suggests that sex hormones may be involved in the pathogenesis. Interestingly in the 1960’s and 70’s there were a number of case reports of patients presenting with IPAH soon after starting the oral contraceptive pill (OCP), and also severe deterioration in patients presenting with IPAH soon after starting the oral contraceptive pill (OCP) [8, 9]. However, in a registry of patients with IPAH, the frequency of OCP use was not different between IPAH patients and the general population [10]. In fact the deterioration seen in these case reports may be linked to the increase risk of venous thromboembolism with the OCP [11], and current advice is to prescribe a progesterone only pill to patients with IPAH. In reality, very little evidence exists in the literature regarding the influence of sex hormones upon the pulmonary vasculature, and has yet to be summarized collectively. The aim of the
The present review is to summarize what data is known and speculate about the possible novel therapeutic role of testosterone for the treatment of pulmonary hypertension.

**OESTROGENS AND THE PULMONARY CIRCULATION**

Females are less likely to develop cardiovascular disease, with the lower incidence in pre-menopausal women rising after the menopause. This gender difference is not confined to the systemic circulation, but also involves the pulmonary circulation. Examples of this include the higher incidence of pulmonary hypertension in females, as discussed above. However, a lower incidence of chronic mountain sickness and high altitude pulmonary oedema is seen in women [12]. This has been proposed to occur by a protective effect of oestrogens.

17β-oestradiol is known to be a pulmonary vasodilator. A recent study [13] utilized coronary and pulmonary arteries obtained from male and female rats to compare the vasodilatory effects of 17β-oestradiol, testosterone, progesterone and cortisol. These steroid hormones all caused an acute dose-dependent vasodilatory response, but significant gender and vascular specific differences were observed [13]. 17β-oestradiol-mediated vasodilatation was significantly greater in the coronary arteries compared to pulmonary arteries, and the coronary arteries from female animals were also more sensitive to 17β-oestradiol than those obtained from males [13]. Testosterone induced greater vasodilatation in the coronary arteries compared to pulmonary arteries, and the pulmonary arteries from male rats were more sensitive to the effects of testosterone than vessels from females [13]. Progesterone and cortisol caused acute vasodilatation with the response greatest in the coronary arteries, although no significant gender difference was observed [13]. In the pulmonary arteries the order of activity was progesterone > testosterone > cortisol > 17β-oestradiol [13].

Gender differences in the effects of oestrogens in the pulmonary circulation have been reported in differing responses to hypoxia. For example, female rats have been shown to develop less pulmonary hypertension when exposed to chronic hypoxia than males [14]. Similarly, hypoxic pulmonary vasoconstriction (HPV) of isolated perfused sheep lungs is reported to be greater in males compared to females [15]. Subsequent studies investigated whether this gender difference existed before puberty [16]. HPV of perfused and ventilated lungs obtained from pre-pubertal sheep was similar in male and female animals, and the response was comparable in pre- and post-pubertal males [16]. However, HPV was diminished in post-pubertal compared to pre-pubertal females [16]. To study whether this difference was due to the presence of endogenous female sex hormones, four further groups of animals were studied: pre-pubertal female sheep and castrated post-pubertal male sheep, subsequently treated with a long acting oestrogen, 17β-oestradiol or a placebo. 17β-oestradiol treatment reduced HPV in both male and female animals compared to their respective placebo-treated controls, and both treated groups did not differ in their response compared to the post-pubertal female sheep [16]. This study therefore concluded that endogenous oestrogens attenuate HPV. Similarly HPV is reduced in pregnant dogs and in post-gestational dogs treated with oestrogen [17].

The vasodilatory mechanism of action of oestrogens also depends on the vascular bed studied and also on the animal species used [18]. Moreover, as with testosterone, the acute pulmonary vasodilatory effect is unlikely to be mediated by endogenous genomic oestrogen receptors due to the rapid onset of action [19]. In contrast to testosterone, research into the pulmonary vasodilatory mechanism of action of 17β-oestradiol has suggested that this occurs via triggering NO release by endothelial nitric oxide synthase (eNOS) [20]. Extensive research has been done looking at the effects of oestrogen on endothelial cells from different animal and vascular beds [21]. Endothelium-dependant vasodilatation is principally due to NO production and oestrogen has been shown to potentially up-regulate this by seven mechanisms [21]:

1. transcriotional stimulation of NOS gene expression
2. inhibition of cytokine-induced down regulation of NOS gene expression
3. post-translational modification of NOS protein
4. increased co-factor availability
5. non-genomic activation of second messengers
6. modulation of NO degradation
7. translocation from the membrane to intracellular sites.

However, the effect of oestrogen in increasing NOS expression is thought not to be as important as its effect on NOS activity and NO release by nontranscriptional mechanisms [22]. Oestrogen-induced activation of eNOS is proposed to involve the oestrogen receptor and phosphatidylinositol-3-OH kinase [23] and requires mitogen-activated protein kinase [24]. The vasodilatory action of oestrogens on endothelial cells is also proposed to involve prostaglandins and cyclo-oxygenase (COX), an enzyme involved in their biosynthesis. It has been shown that 17β-oestradiol increases production of prostacyclin (PGI2), a vasodilator prostaglandin in ovine uterine arteries, ovine fetal pulmonary artery endothelial cells, human umbilical vein endothelial cells and rat mesenteric and cerebral blood vessels [21]. These increased levels of PGI2 are also associated with increased levels of the COX enzyme and this enzyme exists as two forms COX-1 and COX-2, with both playing a role in oestrogen-induced vasodilatation [25, 26].

Oestrogens have also been shown to have endothelial-independent mechanisms of action involving potassium and calcium channels. White et al. [27] studied porcine coronary arteries using patch clamp techniques and found that 17β-oestradiol had a stimulatory action on large-conductance, Ca2+ and voltage-activated K+ (BKCa) channels. Further work showed that this effect was blocked by inhibiting cGMP-dependant protein kinase activity and was mimicked by exogenous cGMP. The authors therefore proposed that 17β-oestradiol relaxed coronary arteries by opening BKCa channels via cGMP-dependant phosphorylation [27]. Kitazawa et al. studied the effect of oestrogens on calcium channels [28] and showed that 17β-oestradiol inhibited voltage dependant L-type Ca2+ channels, in vascular smooth muscle.
Noradrenaline (NA), therefore suggesting a mechanism of with KCl and inhibited pulmonary arterial contraction to vasodilatation of rabbit pulmonary arteries preconstricted with KCl and in inhibited pulmonary arterial contraction to noradrenaline (NA), therefore suggesting a mechanism of action involving blockade of voltage gated calcium channels (VGCCs) and receptor operated calcium channels (ROCCs), as proposed for testosterone [36]. As with 17β-oestradiol, incubation with L-NNA and methylene blue significantly reduced the relaxation induced by progesterone, as well as endothelial denudation [35], indicating the response is mediated in part by NO-induced production of cGMP. The vasodilatory action of progesterone has been studied in other vascular beds and with different species. In rat aorta and rabbit coronary artery [37,38] this mechanism was also proposed to be due to blockade of calcium channels. Work done by Glusa et al. [37] studied the acute effect of progesterone on the vascular reactivity of male thoracic aorta. They found an endothelium-independent relaxation, which was not affected by indomethacin, a COX inhibitor or glibenclamide, an inhibitor of ATP-sensitive K+ channels. Subsequent work showed that verapamil, a calcium channel blocker, and progesterone reduced the maximal contractile response and also shifted concentration-response curves for calcium-induced contractions to the right. The authors hypothesized that progesterone reduces vascular tone by potentially blocking VGCC or ROCCs [37]. Work by Baragallo et al. studied the effect of progesterone on calcium channels [39] using rat VSMCs with patch clamp experiments and showing that the hormone reversibly blunted the L-type calcium channel inward current. The potential endothelium-dependent mechanism of action of progesterone has also been studied [40] using rat aortic strips and showing that progesterone increased NOS activity, as well as COX and PGI2 production in a dose-dependant manner. This evidence was supported by work done by Zhang et al. using rat aortic rings [41], showing progesterone induced dose-dependant relaxation which was partially blocked by NOS antagonist, L-NMMA.

The role of progesterone in pulmonary hypertension in humans has only been considered in one paper. Pribylova et al. [42] reported that the extent of pulmonary hypertension was negatively correlated to serum progesterone in patients with chronic lung diseases. Subsequent administration of progesterone reduced pulmonary artery pressure and also improved myocardial contractility [42]. So far there has been no further work undertaken on progesterone in patients with pulmonary hypertension.

**TESTOSTERONE AND THE PULMONARY CIRCULATION**

Historically testosterone was thought to have detrimental effects upon the cardiovascular system, since males are more than twice as likely as females to develop coronary artery disease (CAD) [43]. However current evidence suggests that low levels of testosterone may increase the risk of atherosclerosis [44]. Similarly testosterone replacement therapy improves the ischaemic threshold in hypogonadal men with angina [45], and causes acute haemodynamic effects at physiological concentrations, increasing cardiac output by reducing the systemic vascular resistance [46]. A long-term study of physiological testosterone therapy in men with chronic heart failure also reports positive effects on exercise capacity as assessed by shuttle-walk testing [47]. Testosterone has been shown to have a vasodilatory action in the coronary circulation, an action that is proposed to occur via a calcium antagonistic action [47-49]. This vasodilatory action...
is proposed to contribute to the clinical benefits associated with testosterone therapy in men with CAD and congestive cardiac failure (CHF). Testosterone has also been demonstrated to induce vasodilation in the pulmonary circulation [13, 36] and there is therefore the potential for testosterone to improve the haemodynamics, exercise capacity, symptoms, quality of life and survival in patients with PAH. As in other vascular beds, the vasodilatory mechanism of action of testosterone in the pulmonary circulation does not involve the nuclear androgen receptor since testosterone-induced vasodilation occurs within minutes of application [36], unlike protein synthesis initiated by the hormone-receptor complex binding to nuclear DNA. Furthermore, the response is insensitive to the androgen receptor antagonist flutamide [36]. Similarly, the involvement of endogenous prostaglandins and NO is excluded by the observation that indomethacin and L-NAME (blockers of cyclo-oxygenase and nitric oxide synthase respectively) do not affect testosterone-induced vasodilation [36].

However, the pulmonary vasodilatory mechanism of action of testosterone does differ from that observed in the systemic and coronary circulations, which is proposed to occur via inhibition of both VGCCs and store-operated calcium channels (SOCCs) [48-50]. In vitro testosterone is an efficacious vasodilator in isolated human pulmonary and mesenteric arteries and veins, but testosterone mediated vasodilatation is approximately 50% lower in isolated human pulmonary arteries and veins compared to systemic human arteries [51]. In vivo testosterone causes vasodilatation of systemic resistance arteries in men with heart failure, but had no effect on pulmonary haemodynamics [46]. This discrepancy would appear to be due to a lack of inhibitory effect upon SOCCs in the pulmonary vasculature. Evidence of an antagonistic action of testosterone upon VGCCs arises from the observation that the vasodilatory efficacy of testosterone in rat pulmonary arteries is dependent upon the mechanism of action of the agonist used to pre-constrict the vessels. Prostaglandin F₂α (PGF₂α) triggers vasoconstriction via activation of ROCCs and VGCCs, while potassium chloride (KCl) disrupts the pulmonary artery vascular smooth muscle cell (PAVSMC) membrane potential causing depolarization with subsequent opening of VGCCs. BAY K8644 directly activates VGCCs. In contrast, thapsigargin inhibits the calcium pumps of the sarcoplasmic and endoplasmic reticulum (SERCA), leading to intracellular calcium store depletion, which in turn triggers extracellular calcium entry via the SOCCs and hence contraction. Testosterone caused marked vasodilatation in vessels preconstricted with PGF₂α, KCl and BAY K8644, yet was ineffective in dilating vessels preconstricted with thapsigargin [36]. This suggests that testosterone causes pulmonary vasodilatation via blockade of ROCCs and VGCCs, but does not inhibit SOCCs in the pulmonary circulation.

The long-term effects of testosterone on vascular reactivity have also been studied (reviewed in [52]). This is studied by using Doppler ultrasound to monitor changes in brachial artery diameter in response to an increase in blood flow, either after release of a blood-pressure cuff inflated over the forearm, or in response to nitrate administration. A study looking at the effect of a 12-week course of oral physiological testosterone replacement found increased flow and nitroglycerin mediated brachial artery vasodilatation as compared to placebo [53]. As testosterone improves both endothelial dependent flow-mediated and endothelial independent nitrate-mediated vasodilatation, it is proposed [53] that this action may be intrinsic to the VSMCs and not a result of signaling pathways in the endothelial cells. This finding also correlates with another study [54] showing improvements in myocardial ischaemia in men with CAD following 3-month physiological testosterone replacement.

These observations lend support to the notion that testosterone could be a potential treatment of PAH. Previous studies have investigated the acute and chronic effects of calcium channel blockers (e.g. nifedipine, diltiazem and amiodipine) in patients with PAH. These drugs inhibit VGCCs, and as mentioned previously approximately 25% of patients respond. At right heart catheterization, amiodipine was shown to reduce pulmonary artery pressure (47.7 ± 4.2 mmHg to 41.7 ± 4.4 mmHg) and pulmonary vascular resistance (8.6 ± 2.1 Wood units to 7.1 ± 1.8 Wood units) [55]. The reduction in pulmonary vascular resistance was also demonstrated with nifedipine [56] and this effect was observed on long-term follow up. This beneficial action was translated into improved long-term survival in a study using diltiazem and nifedipine [57]. As testosterone appears to cause vasodilatation by the same or similar mechanism as calcium channel blockers, studies are now being undertaken using testosterone in patients with PAH to see if a similar beneficial effect is observed.

Interestingly, the effect of the steroid hormone dehydroepiandrosterone (DHEA), a precursor of testosterone in its biosynthesis from cholesterol, has studied upon HPV in isolated perfused and ventilated ferret lungs [58]. Exposure to hypoxia triggered a triphasic alteration in the pulmonary artery pressure, with an initial contraction followed by a relaxation phase and lastly a sustained contraction [58]. When DHEA was added to the perfusate, the initial hypoxic contraction was reversed and the sustained contraction was abolished [58]. The authors hypothesized that this was due to DHEA increasing Ca²⁺-activated K⁺ (KCa) channel activity, leading to vasorelaxation. Evidence suggests that testosterone may also increase KCa channel activity [59], which suggests testosterone may too be efficacious in inhibiting HPV. VGCCs are also postulated to be involved in HPV [60] and since testosterone is recognized to inhibit these channels in the pulmonary circulation, then this too adds weight to this hypothesis. HPV is involved in the pathogenesis of a number of pulmonary diseases characterized by global alveolar hypoxia, and sustained HPV is proposed as a trigger for hypertensive remodeling within the pulmonary circulation. An inhibitory effect of testosterone upon HPV may therefore also be beneficial against the development of PAH. However, such an effect has yet to be demonstrated.

Further studies have looked at the role DHEA plays in modulating the chronic effect of hypoxia upon the pulmonary circulation [61]. Rats were maintained under hypoxic conditions for between 1-3 weeks it was found that there was a raised pulmonary artery pressure (PAP) and an increase in the right ventricular thickness. Treatment with DHEA reduced the PAP in a dose dependant manner, whilst treatment with DHEA during the 1-3 week hypoxic treatment pre-
vented the increase in pulmonary artery pressure and right ventricular hypertrophy [61]. The mechanism of action was again proposed to be via modulation of \( K_C \) channel activity. Again similar effects of testosterone upon changes induced following chronic exposure to hypoxia have yet to be reported.

**TESTOSTERONE AND PAH – OTHER BENEFICIAL EFFECTS**

As previously discussed, the pathogenesis of PAH is complex and has been proposed to involve inflammatory mechanisms. Inflammatory lung parenchymal changes have been shown in monocrotaline-induced pulmonary hypertension in rats [62-64] and inflammatory mechanisms appear pivotal in the development of PAH associated with connective tissue diseases and HIV [65]. Raised levels of the pro-inflammatory cytokines, IL-1\( \beta \) and IL-6 are observed in the serum of patients with severe IPAH [66]. Testosterone has been demonstrated to suppress the pro-inflammatory cytokines TNF-\( \alpha \), IL-1\( \beta \) and IL-6 in vitro, while increasing expression of the anti-inflammatory cytokine IL-10 [67-69]. This has also been shown in human studies in patients with symptomatic androgen deficiency; physiological testosterone replacement therapy reduced levels of TNF-\( \alpha \) and IL-1\( \beta \), while increasing IL-10 levels [67]. There is therefore potential for testosterone therapy in PAH patients to have an immunomodulatory role, altering expression of cytokines.

The pathological findings in PAH include thrombotic lesions [4-6]. The thrombotic process is complicated with various pro- and anti-thrombosis mediators determining the coagulation status. The major pro-thrombotic factors are plasminogen activator inhibitor-1 (PAI-1), fibrinogen, alpha-2-antiplasmin and factor VIIc, while tissue plasminogen activator (tPA), protein C and anti-thrombin III are important anti-thrombotic factors. In PAH it is widely accepted that shear stress or lung vessel injury generates a thrombogenic surface and this combined with elevated levels of PAI-1 and factor V\( \text{IIc} \) that are found in PAH [70] leads to thrombotic lesions. Cross-sectional studies have highlighted a positive association between testosterone levels and tPA and a negative association with PAI-1, V\( \text{IIc} \) and fibrinogen [71-73]. Replacement of testosterone in hypogonadal men and treatment of normal men with dehydroepiandrosterone (DHEA) reduces PAI-1 serum levels [74], while testosterone administration in healthy men also reduces plasma levels of the acute phase protein fibrinogen [75]. This anti-thrombotic action of testosterone may also play an important role in the treatment of PAH.

**CONCLUSIONS**

In summary, there is evidence that the sex hormones testosterone, 17\( \beta \) oestradiol and progesterone exhibit a vasodilatory action in the pulmonary circulation. Testosterone is thought primarily to act via inhibition of membranous VGCCs. The mechanism of action of 17\( \beta \) oestradiol and progesterone is hypothesized in part to also increase NO in addition to an effect upon membranous ion channels. Future research on the acute and chronic effects of the sex hormones in human studies may be able to further explain the gender differences seen in the incidence cardiorespiratory disorders, for example pulmonary hypertension and potentially develop novel treatments for these conditions.

**REFERENCES**


