Published in final edited form as:

J Surg Res. 2008 May 1; 146(1): 121–126. doi:10.1016/j.jss.2007.05.023.

The Effects of Endogenous Sex Hormones and Acute Hypoxia on Vasoconstriction in Isolated Rat Pulmonary Artery Rings

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Abstract

Background—Studies have noted gender differences in various models but have not investigated whether hormone depletion will abolish these differences. Therefore, we measured isometric force displacement in normal males, castrated male, normal females, and ovariectomized females.

Materials and Methods—Adult males, adult females, castrated males, and ovariectomized females (250–350g) Sprague-Dawley rat pulmonary arteries (n=7–8/group) were isolated and suspended in physiologic organ baths. Force displacement was continuously recorded for 60 minutes of hypoxia. Data (mean±SEM) was analyzed with two-way analysis of variance with post-hoc Bonferroni test or students t-test.

Results—Maximum vasodilation of normal males was $-79.47 \pm 3.34\%$ while normal adult females (n=7) exhibited maximum vasodilation of $-88.70 \pm 6.21\%$ (P=.8149). In addition, delayed, phase II vasoconstriction of male PA rings was $89.79 \pm 7.25\%$ while adult females had maximum phase II vasoconstriction of $95.90 \pm 14.23\%$ (P=.9342). Hormone depletion of males (n=7) exhibited a maximum vasodilation of $-70.45 \pm 5.08\%$ for castrated males as compared to $-79.47 \pm 3.34\%$ for normal adult males (P=.3805). Castrated males exhibited a maximum phase II vasoconstriction of $86.20 \pm 15.76\%$ compared to $89.79 \pm 7.25\%$ exhibited by normal adult males (P=.9516).

Conclusions—Hormone depletion in males and females did not alter pulmonary vasoreactivity in acute hypoxia.

Keywords

Hypoxic pulmonary vasoconstriction; gender differences; sex hormones; estrogen; testosterone

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DISCLOSURES

None.

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INTRODUCTION

Gender differences exist in a variety of cardiovascular and cardiopulmonary disorders. In most cardiovascular diseases, premenopausal women have a much better prognosis than men [1]. It has also been demonstrated that females fare better than their male counterparts after cardiac ischemia-perfusion injury [2,3]. On the other hand, women with idiopathic dilated cardiomyopathy have a poorer prognosis than males, and women are also more susceptible to alcohol-related heart disease [1]. Pulmonary arterial hypertension occurs twice as frequently in females as compared to males [4–6]. However, in the setting of chronic hypoxia, females have been noted to exhibit less severe pulmonary hypertension than their male counterparts [7,8]. Chronically hypoxic ovariectomized rats develop more severe right ventricular hypertrophy and pulmonary arterial remodeling than chronically hypoxic rats with intact ovaries [9].

Several studies have investigated the role of sex hormones with regards to vasoreactivity of the pulmonary vasculature [7,10]. Both testosterone and estrogen have been shown to acutely affect vasoreactivity in various vascular beds [11,12]. Acute administration of estrogen or testosterone to pulmonary arteries causes vasorelaxation under normoxic conditions [13]. Interestingly, pulmonary vasodilation was greater when testosterone was administered compared to estrogen [13].

The vasomotor effects of estrogen are mediated through genomic and nongenomic mechanisms. These include increased production of nitric oxide and decreased expression of endothelin-1 as well as MAPK and ERK effects [10,14–17]. Additionally, estrogen has been shown to have anti-inflammatory properties [2,18]. Testosterone, on the other hand, has pro-inflammatory and pro-apoptotic effects in the myocardium [19,20]. However, vasodilator effects of testosterone in the coronary and pulmonary vasculature have been demonstrated as well [21,22]. These effects are mediated through a calcium-antagonistic mechanism [21,22].

The effects of endogenous sex hormones on HPV were studied by Wetzel et al [23,24] and Gordon et al [25] in isolated sheep lungs. However, these studies did not examine the effects of estrogen depletion in adult animals. It is not known whether depletion of endogenous hormones neutralizes the previously observed gender difference in the response to acute hypoxia. To study this, isometric force displacement was measured in isolated pulmonary artery rings from normal male and female rats as well as from castrated males and ovariectomized females.

METHODS

Animals

All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* [National Institutes of Health (NIH) publication no. 85-23, revised 1985]. All of the animal protocols were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine. Adult male and female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g were allowed access to food and water up to the time of experimentation. Castration and ovariectomy were performed 4–6 weeks prior to experimentation. This time frame has been shown to adequately deplete endogenous sex hormones [19,26].

Isolated PA ring preparation

Rats were anesthetized with intraperitoneal injection of pentobarbital (150 mg/kg). Median sternotomy was performed and the heart and lungs were removed en bloc and placed in modified Krebs-Henseleit (KH) solution at 4°C. Under a dissecting microscope, extralobar PA

branches were dissected out and cleared of surrounding tissue. Right and left main branch PA were cut into 2- to 3-mm wide rings and suspended on steel hooks connected to force transducers (ADInstruments, Colorado Springs, CO) for isometric force measurement. Care was taken during the entire process to avoid injury to the endothelium. PA rings were immersed in individual water-jacketed organ chambers containing modified Krebs-Henseleit solution bubbled with 95% O₂/5% CO₂ at 37°C. Force displacement was recorded using a PowerLab (ADInstruments) eight-channel data recorder on an Apple iMac PowerPC G4 Computer (Apple Computer, Cupertino, CA).

Experimental protocol and groups

Before starting experimental protocols, rat pulmonary arteries (PA) were stretched to an optimal tension of 750mg and allowed to equilibrate for 60 min. Viability of PA rings was determined by measuring maximum contractile response to 80mmol of KCl. The dosage of KCl was determined to produce maximal contractile response in previous experiments. After KCl washout, the integrity of each PA endothelium was evaluated by dilation with acetylcholine (1 μmol/l) after phenylephrine (1 μmol/l) precontraction. Rings demonstrating <200 mg contraction to phenylephrine were discarded. In endothelium-intact PA, rings demonstrating <50% vasorelaxation to acetylcholine were discarded. After washout of acetylcholine, PA rings were allowed to equilibrate. Following equilibration, PA rings were precontracted with phenylephrine. Following precontraction, hypoxia was induced by changing the bubbled gas to 95% N₂/5% CO₂ producing a PO₂ of 30–35 mm Hg. Each experiment was terminated after 60 min of hypoxia.

Presentation of data and statistical analysis

Force displacement during hypoxia is expressed as percent change from the amount of phenylephrine precontraction. All reported values are means ± SE ($n = 7-10/\text{group}$). Experimental groups were compared by two-way analysis of variance (ANOVA) with post hoc Bonferroni test or student's *t* test. (Prism 4; Graphpad Software, San Diego, CA). Differences at an alpha level of 0.05 ($P < 0.05$) were considered statistically significant.

RESULTS

Hypoxic Pulmonary Vasoconstriction

To measure the effect of hypoxia on PA, we gassed phenylephrine-precontracted PA with 95% N₂/5% CO₂ for 60 min. This produced a PO₂ of 30–35 mm Hg in the organ bath, which was measured with a blood-gas analyzer (Synthesis 20; Instrumentation Laboratory, Lexington, MA). Hypoxia caused a biphasic PA vasoconstriction: an early contraction (occurring 2–3 min after exposure to hypoxia) followed by a transient vasodilation, and then a late phase II (occurring 15–20 min after hypoxia exposure) contraction was observed (Fig. 1). Maximum phase II vasoconstriction was measured as the difference between the highest and lowest force displacements during hypoxia and presented as a percentile of maximum precontraction. Maximum vasodilation was measured as a percentile of equilibrated PA ring tension.

Gender effects on HPV

Male PA rings did not significantly exhibit differences in maximum vasodilation and phase II vasoconstriction as compared to females. Normal adult male PA rings ($n=8$) exhibited maximum vasodilation of $-79.47 \pm 3.34\%$ while normal adult females ($n=7$) exhibited maximum vasodilation of $-88.70 \pm 6.21\%$ ($P=.8149$) (Fig. 2). In addition, adult male PA rings exhibited maximum phase II vasoconstriction of $89.79 \pm 7.25\%$ while adult females had maximum phase II vasoconstriction of $95.90 \pm 14.23\%$ ($P=.9342$) (Fig. 3).

Effects of hormone depletion on HPV

In order to determine the isolated effects of hormone depletion in the male gender, adult castrated male PA rings were also subjected to acute hypoxia and compared to normal adult males. Castrated males (n=7) exhibited a maximum vasodilation of $-70.45 \pm 5.08\%$ for castrated males as compared to $-79.47 \pm 3.34\%$ for normal adult males ($P=.3805$) (Fig. 4). Castrated males exhibited a maximum phase II vasoconstriction of $86.20 \pm 15.76\%$ compared to $89.79 \pm 7.25\%$ exhibited by normal adult males ($P=.9516$) (Fig. 5).

Female hormone depletion was evaluated and compared to normal adult females. Ovariectomized females exhibited a maximum vasodilation of $-88.11 \pm 2.29\%$ compared to $-88.70 \pm 6.21\%$ for normal females ($P=.9259$) (Fig. 6). In addition, ovariectomized females exhibited a maximum phase II vasoconstriction of $97.31 \pm 7.66\%$ compared to $95.90 \pm 14.23\%$ in normal adult females ($P=.8928$) (Fig. 7).

DISCUSSION

The results of this study demonstrate that the response to acute hypoxia does not differ between adult male and female rat pulmonary arteries. The maximum vasodilatory phase and phase II vasoconstriction of HPV were similar between males and females. In addition, it was shown that hormone depletion in males and females by castration and ovariectomy, respectively, does not alter pulmonary vasoreactivity in acute hypoxia.

These results are in contrast to the studies of English et al. [13] and Wetzel et al. [23,24]. Since the experiments by Wetzel et al. were performed in isolated sheep lungs, it is conceivable that the discrepancy between those studies and our study may be due to the fact that different models and different species were investigated. In addition, Wetzel et al. used a perfusate containing autologous blood that may have contributed to non-genomic action of circulating sex hormones and other vasoactive substances. It was the aim of our study to investigate the role of endogenous sex hormone depletion during acute hypoxia. In our model, Krebs-Henseleit solution was used as perfusate, and this enabled us to avoid confounding effects of other circulating vasoactive substances and mediators. A limitation of our model is that the acute nongenomic effects of circulating sex hormones cannot be examined. We will address this in future experiments using exogenous sex hormones that will be added to the organ bath.

While English et al. also examined pulmonary arteries from rats, the experiments were performed under normoxic conditions. Additionally, the effects of exogenous sex hormones were investigated. Therefore, the gender differences that were observed under *normoxic* conditions and after exposure to *exogenous* sex hormones may not be detectable when the effects of *endogenous* sex hormones are examined under *hypoxic* conditions. This discrepancy is consistent with the fact that a female predominance is present in pulmonary arterial hypertension but not in pulmonary hypertension secondary to chronic hypoxia [27].

Sex hormones clearly have a variety of effects on the pulmonary vasculature. The vasomotor effects of both, estrogen and testosterone, are mediated through genomic and nongenomic mechanisms. Also, sex hormones have been shown to have properties affecting smooth muscle cell remodeling of pulmonary arteries [28–30]. One study has shown that exogenous estrogen exposure protected pulmonary hypertensive rats from pulmonary vascular remodeling and right ventricular hypertrophy [28]. Also, DHEA, a testosterone precursor, has been shown to prevent and reverse pulmonary artery remodeling [31].

Estrogen enhances the production of vasodilators such as nitric oxide (NO) by upregulation of endothelial NO synthase [15–17]. Additionally, estrogen has been shown to downregulate endothelin-1, a potent vasoactive and mitogenic mediator [10]. Estrogen also regulates

phosphorylation of proteins in the MAPK and ERK signaling pathways [14]. However, the exact mechanisms of this are not entirely clear. Pulmonary artery vasoreactivity is influenced by the integrity of the endothelial cell layer and the inflammatory state of the vasculature [32–34]. Acute hypoxic pulmonary vasoconstriction is associated with smooth muscle cell contraction, inflammation, and cytokine release [32,35–39]. Estrogen has been shown to decrease inflammation and to stabilize cellular integrity [2,18].

Despite the fact that testosterone has pro-inflammatory and pro-apoptotic effects in the myocardium [19,20], a vasodilator effect in the coronary and pulmonary vasculature is well documented [21,22]. This is mediated through a calcium-antagonistic mechanism at the level of membranous voltage-gated calcium channels [21,22]. These effects are independent of prostaglandins or nitric oxide [22]. It is also known that conversion to 17 β -estradiol is not responsible for testosterone-mediated vasodilation [21,40].

Acute hypoxic vasoconstriction is mediated by multiple mechanisms like inhibition of voltage-gated potassium channels, activation of voltage gated calcium channels and release of calcium from the sarcoplasmic reticulum. In addition several intracellular signaling pathways like RhoA/Rho-kinase, protein kinase C and p38MAPK are involved [32,36]. As mentioned above, both, estrogen and testosterone, have nongenomic effects on these pathways. In our model, HPV was investigated in isolated pulmonary artery rings after en-bloc removal of heart and lungs. This may have attenuated or even abolished the acute nongenomic vasoactive effects of circulating sex hormones on the pulmonary vasculature or the mobilization of vasoactive substances. Obviously, the genomic effects of sex hormones were still present in our model. Therefore, it is possible that the gender differences that were observed in previous studies, are mainly due to nongenomic sex hormone effects. The genomic effects alone may not suffice to cause any obvious gender differences in HPV. This would also explain why hormone depletion did not show any additional effects.

Another interesting aspect that may need to be considered is the timing and duration of the hypoxic insult. Exposure to perinatal hypoxia and the development of pulmonary vasoconstriction and hypertension later in adulthood have been witnessed in many studies [41–43]. Hakim et al. found that adult rats exposed to hypoxia in the perinatal period had increased reactivity to hypoxia and elevated pulmonary vascular resistance [41]. In addition to perinatal insults resulting in increased susceptibility to hypoxia in adulthood, studies found that gender differences exist in newborn rats exposed to perinatal hypoxia [30]. Hampl et al. found that late adverse effects of perinatal hypoxia are seen in ovariectomized females, and those same adverse effects are blunted by the presence of ovaries [30]. The results of previous studies support the idea that the timing of the hypoxic insult may affect differences in pulmonary vascular reactivity.

Packer et al. [44] found that gender differences between male and female pulmonary hypertensive rats and the ability to vasodilate only existed in a select treatment group. They found similar vasodilatory responses to acetylcholine and calcitonin gene related peptide, but demonstrated that females exhibited greater vasodilatation when exposed to adrenomedullin. Thus, their evidence suggests a limited gender difference in vasoreactivity of pulmonary arteries [44].

Although the above mentioned investigators have shown gender differences in pulmonary artery reactivity, our data suggest that gender does not affect the response to acute hypoxia. Therefore, the timing and duration of the hypoxic insult as well as the model, in which potential gender differences are investigated, may determine whether gender differences are detectable or not. A possible explanation for our observations may be related to the late and relatively

brief hypoxic insult in our model. Early hypoxia during the perinatal period may result in gender differences that are only appreciated during later hypoxic insults.

A limitation of our study is the fact that the menstrual cycle was not taken into account. The estrous cycle of the female rat consists of four stages (proestrus, estrus, metestrus and diestrus) and lasts 4–5 days [45]. The estrogen level peaks in the proestrus phase and tapers off after that phase. Therefore, it is possible that the exclusion of estrus, metestrus and diestrus females may unmask sex-hormone specific effects. However, neither the studies by Wetzel et al. [23, 24] and English et al. [13] nor previous studies from our lab [2] took the menstrual cycle into account. Yet, these studies demonstrated gender differences. In order to maintain generalization, it was the purpose of this study to look at the female gender as an entity. Whether HPV differs between proestrus female and male rats is currently under investigation.

In conclusion, this study did not reveal any gender differences in the response of isolated pulmonary artery rings to acute hypoxia. Hormone depletion did not significantly affect HPV in males or females. We speculate that the acute, nongenomic effects of sex hormones on HPV may differ between genders. This is currently under investigation.

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Figure 1. Representative pressure tracing of hypoxic vasoconstriction in an isolated pulmonary artery. Pulmonary arteries precontracted using phenylephrine (1 $\mu\text{mol/L}$) were exposed to hypoxia ($\text{P}_{\text{O}_2} = 30\text{--}35$ mmHg) for 60 min. Maximum vasodilation was measured as the difference between the tension measured when hypoxia was induced and the lowest force preceding phase II vasoconstriction. Maximum phase II vasoconstriction was measured as the difference between the lowest force preceding contraction and the highest force during 60 minutes of hypoxia.

Figure 2.

Representative histogram of gender differences in maximum vasodilation. Values are normalized within each group to their respective resting tension. Maximum vasodilation in HPV of normal males did not differ significantly from normal females ($P=.8149$).

Figure 3.

Representative histogram of gender differences in phase II vasoconstriction. Maximum vasoconstriction was normalized for each group against their respective maximum vasoconstriction with phenylephrine. Normal males did not differ significantly from normal females ($P=.9342$).

Figure 4.

Representative histogram of differences in maximum vasodilation amongst normal adult males and hormone depleted castrated males. Maximum vasodilation was normalized within each group with respect to their resting tension. Maximum vasodilation was not significantly different between groups ($P=.3805$).

Figure 5.

Representative histogram of differences in maximum phase II vasoconstriction amongst adult males and hormone depleted castrated males. Values were normalized for each group against their respective maximum vasoconstriction with phenylephrine. Normal males did not significantly differ from hormone depleted males ($P = .9516$).

Figure 6.

Representative histogram of differences in maximum vasodilation amongst adult females and hormone depleted ovariectomized females. Vasodilation was normalized within each group with respect to their resting tension. Maximum vasodilation was not significantly different between groups ($P = .9259$).

Figure 7.

Representative histogram of differences in maximum phase II vasoconstriction amongst normal females and hormone depleted ovariectomized females. Values were normalized for each group against their respective maximum vasoconstriction with phenylephrine. Maximum phase II vasoconstriction was not significantly different between groups ($P=.8928$).