Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle

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Submitted 5 March 2007; accepted in final form 3 June 2007

SEX DIFFERENCES EXIST in a variety of cardiovascular and cardiopulmonary disorders. In most cardiovascular diseases, premenopausal women have a significantly better prognosis than men (23). In addition, females fare better than their male counterparts after cardiac ischemia-reperfusion injury (50, 51). On the other hand, women are more susceptible to alcohol-related heart disease and also have a poorer prognosis than males when they are affected by idiopathic dilated cardiomyopathy (23).

The effects of sex on the pulmonary vasculature have not been clearly established yet. Pulmonary arterial hypertension, a disabling condition characterized by pulmonary artery (PA) vasoconstriction and remodeling as well as in situ thrombosis and eventually right heart failure, occurs twice as frequently in females compared with males (11, 29, 39). However, in the setting of chronic hypoxia, females have been noted to exhibit less severe pulmonary hypertension than their male counterparts (35, 44). Interestingly, chronically hypoxic ovariectomized rats develop more severe pulmonary arterial remodeling and right ventricular hypertrophy than chronically hypoxic rats with intact ovaries (36).

Few studies have investigated the effects of sex hormones on PA vasoreactivity (6, 35). Both estrogen and testosterone have been demonstrated to acutely affect vasoreactivity in various vascular beds (28, 43). In an isolated PA model, the acute administration of estrogen or testosterone caused vasorelaxation under normoxic conditions (7). Interestingly, pulmonary vasodilation was more pronounced with testosterone compared with estrogen (7).

The vasomotor effects of sex hormones are mediated through genomic as well as nongenomic mechanisms. The nongenomic effects of estrogen include decreased expression of endothelin-1 and increased production of nitric oxide (NO) and prostaglandin as well as effects on MAPK and ERK signaling pathways (6, 12–14, 21, 54). A calcium-antagonistic effect has been described as well (10). Additionally, estrogen possesses anti-inflammatory properties (18, 50). Testosterone, while shown to have proinflammatory and proapoptotic effects in the myocardium (4, 52), also has vasodilator properties in the coronary and pulmonary vasculature (8, 20). These vascular effects are mediated through a calcium-antagonistic mechanism (8, 20).

The effects of endogenous sex hormones on hypoxic pulmonary vasoconstriction (HPV) have not clearly been elucidated yet. Early investigations were performed by Wetzel et al. (56, 57) and Gordon et al. (14) in isolated sheep lungs. However, it has not yet been determined whether the menstrual cycle affects PA vasoreactivity and acute hypoxic vasoconstriction. This is of interest since the menstrual cycle has
already been shown to affect various conditions associated with increased vascular reactivity in other vascular beds, such as migraine headaches (27), Raynaud’s phenomenon (15), and vasospastic angina (37). A better understanding of the effects of sex hormones on the pulmonary vasculature may allow for therapeutic interventions in the future.

We hypothesized that sex and menstrual cycle affect pulmonary vasoreactivity under normoxic and under hypoxic conditions. To test this hypothesis, isometric force displacement was measured in isolated PA rings from proestrus, estrus, and diestrus female, as well as male, Sprague-Dawley rats.

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 age-matched male and female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 230–310 g were allowed ad libitum access to food and water up to the time of experimentation. The menstrual cycle of the female rats was determined using vaginal smears as described previously by Hubscher et al. (17). The estrous cycle of the female rat consists of four phases and lasts 4–5 days. The proestrus phase is characterized by high estrogen levels, whereas the estrogen level is minimal in the estrus, metestrus, and diestrus phases (17). Female rats were divided into proestrus and estrus or diestrus animals. No metestrus animals were identified.

Isolated PA ring preparation. Rats were anesthetized with intraperitoneal injections 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. The right and left main branches 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 KH 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, the PA rings were stretched to a predetermined optimal passive tension of 750 mg. The rings were allowed to equilibrate for 60 min, during which time the KH solution was changed every 15 min. Viability of PA rings was determined by measuring maximum contractile response to 80 mmol/l KCl. The dosage of KCl was determined to produce maximal contractile response in previous experiments. PA rings were precontracted using phenylephrine (1 μmol/l) after washing out of acetylcholine. Due to its very brief and transient nature, phase I vasoconstriction was measured as the difference between the tension measured when hypoxia was induced and the lowest force preceding phase II vasoconstriction. Maximum vasodilation was measured as the difference between phenylephrine precontraction and the lowest force displacements during hypoxia and expressed as a percentage of maximum phenylephrine precontraction.

Hypoxia. To measure the effect of hypoxia on PA, we gassed phenylephrine-precontracted PA rings with 95% N2-5% CO2 for 60 min. This produced a PO2 of 30–35 mmHg in the organ bath, which was measured with a blood-gas analyzer (Synthesis 20; Instrumentation Laboratory, Lexington, MA). As described in previous experiments, hypoxia caused a biphasic PA vasoconstriction: an early contraction (occurring 2–3 min after exposure to hypoxia) followed by a transient vasodilation and a late phase II (occurring 15–20 min after hypoxia exposure) contraction (Fig. 1). Due to its very brief and transient nature, phase I vasoconstriction was not measured. Maximum phase II vasoconstriction was measured as the difference between the highest and lowest force displacements during hypoxia and expressed as a percentage of maximum phenylephrine precontraction. Maximum vasodilation was measured as the difference between phenylephrine precontraction and the lowest force displacements during hypoxia and expressed as a percentage of maximum phenylephrine precontraction.

RESULTS

Effects of sex and menstrual cycle on KCl-induced vasoconstriction. PA rings from proestrus females demonstrated a significantly attenuated vasoconstrictor response to KCl compared with rings from estrus and diestrus females (75.58 ± 3.2% vs. 92.43 ± 4.24%, P < 0.01) (Fig. 2). PA rings from male animals also exhibited attenuated KCl-induced vasoconstriction compared with rings from estrus and diestrus females (79.34 ± 3.2% vs. 92.43 ± 4.24%, P < 0.05). There was no difference in the vasoconstrictor response between PA rings from male and proestrus animals.

Effects of sex and menstrual cycle on phenylephrine-induced vasoconstriction. Similarly to the response to KCl, PA rings from proestrus females exhibited an attenuated vasoconstrictor response to phenylephrine compared with rings from estrus and diestrus females (3.2% vs. 92.43 ± 4.24%, P < 0.01) (Fig. 2).

Fig. 1. Pressure tracing of hypoxic vasoconstriction in isolated pulmonary arteries (PAs). PAs precontracted using phenylephrine (1 μmol/l) were exposed to hypoxia (PO2 = 30–35 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 min of hypoxia.
response to phenylephrine compared with estrus and diestrus rings (59.61 ± 2.98% vs. 70.03 ± 4.61%, \( P < 0.05 \)) (Fig. 3). Male PA rings did not demonstrate a difference in vasoconstriction (65.16 ± 2.71%) compared with either proestrus or estrus and diestrus rings.

**Effects of sex and menstrual cycle on HPV.** Maximum vasodilation did not significantly differ between male, proestrus, and estrus or diestrus rings. Male PA rings exhibited a maximum vasodilation of 80.8 ± 4.11%, whereas proestrus rings exhibited a maximum vasodilation of 81.12 ± 5.48%. Estrus and diestrus rings demonstrated a maximum vasodilation of 82.8 ± 4.76%.

In contrast, phase II vasoconstriction was significantly attenuated in PA rings from proestrus females compared with their male counterparts (64.10 ± 7.10% vs. 83.91 ± 5.97%, \( P < 0.05 \)) (Fig. 4). Phase II vasoconstriction did not differ between proestrus and estrus or diestrus rings (64.10 ± 7.10% vs. 69.20 ± 6.80%). In addition, there was no significant difference between male and estrus or diestrus rings (83.91 ± 5.97% vs. 69.20 ± 6.80%).

**DISCUSSION**

The results of this study demonstrate that even physiological increases in circulating estrogen levels attenuate PA vasoconstriction under both normoxic and hypoxic conditions. PA rings from proestrus females, characterized by physiologically increased estrogen levels, compared with estrus, diestrus, and male animals, exhibited an attenuated vasoconstrictor response when stimulated with vasoactive agents (KCl and phenylephrine) or hypoxia. Even though we did not directly measure circulating estrogen levels, the Hubscher method (17) of determining the estrous cycle based on vaginal smears is a validated and well-accepted strategy (17).

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**Fig. 2.** Effects of sex and menstrual cycle on vasoconstriction after stimulation with KCl (80 mmol/l). Values are normalized within each group to their respective resting tension. PA rings from proestrus females demonstrated a significantly attenuated vasoconstrictor response compared with rings from estrus or diestrus animals. \#\( P < 0.05 \) vs. estrus/diestrus. *\( P < 0.01 \) vs. estrus/diestrus.

**Fig. 3.** Effects of sex and menstrual cycle on vasoconstriction after stimulation with phenylephrine (1 \( \mu \)mol/l). Values are normalized within each group to their respective resting tension. PA rings from proestrus females demonstrated a significantly attenuated vasoconstrictor response compared with rings from estrus or diestrus animals. *\( P < 0.05 \) vs. estrus/diestrus.

**Fig. 4.** Effects of sex and menstrual cycle on phase II hypoxic vasoconstriction. Maximum vasoconstriction was normalized for each group against phenylephrine-precontraction. A: maximum phase II vasoconstriction was significantly attenuated in PA rings from proestrus females compared with rings from male animals. B–D: Comparison of tracings of phase II hypoxic vasoconstriction for males vs. proestrus females (B), males vs. estrus/diestrus females (C), and estrus/diestrus vs. proestrus females (D), *\( P < 0.05 \) vs. males.
Our data are in concordance with the studies by Wetzel et al. (56, 57), who demonstrated sex differences in HPV in isolated sheep lungs. The hypoxic pulmonary vasomotor response was attenuated in the lungs of female sheep, and it was speculated that this effect was due to the effect of female sex hormones. However, in these experiments, the menstrual cycle was not taken into account. English et al. (7) demonstrated the vasodilatory effects of exogenous estrogen on the pulmonary vasculature.

Packer et al. (33) demonstrated sex differences between male and female rats with pulmonary hypertension and their ability to vasodilate when exposed to various vasodilator stimuli. These authors found similar vasodilatory responses to acetylcholine and calcitonin gene-related peptide but demonstrated that females exhibited greater vasodilation when exposed to adrenomedullin. Thus, these data suggest a sex difference in PA vasodilation (33). It is interesting that we did not find any sex differences with regards to vasodilation. However, it is conceivable that sex hormones affect vasoconstrictor mechanisms more than vasodilator mechanisms. Alternatively, it is possible that because of the relatively short duration of the vasodilatory phase during HPV, potential subtle differences may not have been detected in our model.

We were able to demonstrate that PA vasoreactivity is not only affected by sex and exogenous sex hormones, but also by the menstrual cycle, which is characterized by fluctuations in endogenous estrogen levels. PA rings from the animals with the highest endogenous estrogen levels consistently showed a decreased vasoconstrictor response compared with the groups characterized by lower estrogen levels. To our knowledge, this is the first study to demonstrate that the menstrual cycle affects PA vasoreactivity. Our data may explain why in an earlier study (unpublished observations), in which the menstrual cycle was not taken into account, we were not able to demonstrate sex differences in HPV. These data also emphasize the importance of considering the menstrual cycle when examining sex differences, as pointed out by Leinwand (23) and Chaudry et al. (19, 62). Zellweger et al. (62) demonstrated that proestrus females with sepsis maintained their splenic immune functions compared with males. In addition, females tolerated the sepsis better than their male counterparts. Along these lines, Jarrar et al. (19) showed that the female reproductive cycle is an important variable in the response to trauma-hemorrhage.

Several vasospastic disorders such as migraine headaches (27), Raynaud’s phenomenon (15), and vasospastic angina (37) have been shown to be affected by the menstrual cycle. To our knowledge, an influence of the menstrual cycle on PA vasoreactivity has not yet been described.

Decreasing estrogen levels may induce or worsen vasospasm in various vascular beds, leading to a phenomenon of “estrogen withdrawal” (27, 37). Our data are in support of this theory, since estrus and diestrus females, as well as males (both characterized by low endogenous estrogen levels), demonstrated a more pronounced PA vasoconstrictor response after stimulation with vasoactive agents than proestrus females (characterized by high endogenous estrogen levels). Similarly, an estrogen withdrawal phenomenon in the pulmonary vasculature may lead to vasoconstriction and a subsequent increase in PA pressure, therefore contributing to the development of pulmonary arterial hypertension (PAH). Interestingly, idiopathic PAH is much more common in females (11, 29, 39). It is conceivable that this mechanism may contribute to the pathogenesis of this disease.

On the other hand, females have been noted to exhibit less severe pulmonary hypertension than their male counterparts in the setting of chronic hypoxia (35, 44). It is tempting to
speculate that the genomic and nongenomic effects of estrogen may contribute to this sex difference in disease severity.

In addition, our findings of attenuated vasoconstriction after stimulation with vasoactive agents may be of importance for the treatment of shock, in which there is concern that vasoconstriction may induce or worsen pulmonary hypertension. While many factors contribute to the morbidity and mortality associated with trauma, shock, and sepsis (1, 3, 25, 26, 34, 41, 48, 49, 55, 58), decreased vasoconstrictor-induced pulmonary hypertension may account for the improved survival observed in females in these conditions (2, 5, 40, 45, 59).

Sex hormones have well-documented effects on the pulmonary vasculature. The vasodilator effects of both estrogen and testosterone are mediated through genomic and nongenomic mechanisms. Estrogen has been shown to increase prostaglandin release and to enhance the production of NO, the latter by upregulation of endothelial NO synthase (12–14, 21). Additionally, estrogen downregulates endothelin-1, a potent vasoactive and mitogenic mediator (6). Estrogen also regulates phosphorylation of proteins in the MAPK and ERK signaling pathways (54). However, the exact mechanisms of this are not entirely clear.

There is also evidence that anti-inflammatory mechanisms contribute to the vasodilatory properties of estrogen. PA vaso-reactivity is influenced by the integrity of the endothelial cell layer and the inflammatory state of the vasculature (9, 24, 46). Acute HPV is associated with smooth muscle cell contraction, inflammation, and cytokine release (30–32, 42, 46, 53). Estrogen has been shown to decrease inflammation and to stabilize cellular integrity (18, 50).

Despite the fact that testosterone has proinflammatory and proapoptotic effects in the myocardium (4, 52), a vasodilator effect in the coronary and pulmonary vasculature is well-documented (8, 20). This effect is mediated through a calcium-antagonistic mechanism at the level of membranous voltage-gated calcium channels (8, 20). While the exact mechanism has not been fully elucidated yet, it seems to be independent of prostaglandins or NO (20), therefore differing from estrogen-mediated mechanisms. It has also been demonstrated that conversion to 17β-estradiol is not responsible for testosterone-mediated vasodilation (8, 61). The vasodilatory properties of testosterone may explain why the PA rings from male animals exhibited less vasoconstriction after phenylephrine and KCl stimulation than the rings from the estrus and diestrus animals, since the latter are characterized by physiologically low levels of any vasoactive sex hormones.

Acute hypoxic vasoconstriction is mediated by multiple mechanisms. These include inhibition of voltage-gated potassium channels, a change in the concentration of reactive oxygen species (whether this is an increase or a decrease is a matter of substantial controversy in the literature), activation of voltage-gated calcium channels, and release of calcium from the sarcoplasmic reticulum. In addition, several intracellular signaling pathways including RhoA/Rho-kinase, protein kinase C, and p38MAPK are involved (30, 47). As mentioned above, both estrogen and testosterone have nongenomic effects on these pathways.

While the hypoxia during the vasoconstrictor experiments in our study may have had a subtle effect on the concentration of reactive oxygen species as well, the integrity of the PAs and their endothelium was well-maintained, as shown in previous experiments (45, 46).

Although long-term estrogen therapy may be associated with significant side effects in females (38), an acute, one-time dose may exert beneficial effects if the PA tone needs to be acutely lowered in critically ill patients. This scenario is frequently encountered after corrective surgery for congenital heart disease in the pediatric population (16) or after lung transplantation (22). In both circumstances, as well as in patients with the acute respiratory distress syndrome (ARDS), HPV can lead to severe pulmonary hypertension with right ventricular decompensation (31). Alternatively, the development of new drugs mimicking some or all of the nongenomic vasomotor effects of estrogen may be of benefit for the treatment of the vasoconstriction and vascular remodeling associated with PAH. In this context, it is of interest that the administration of 17β-estradiol or the estrogen receptor-β agonist diarylpropionitrile has been shown to attenuate lung injury after trauma-hemorrhage in male rodents (60). Figure 5 illustrates the basic mechanisms of normoxic and HPV and demonstrates how estrogen interferes with the underlying mediators and pathways to attenuate PA vasoconstriction.

In conclusion, this is the first study to demonstrate that the estrous cycle affects PA vasoreactivity. Understanding how sex hormones modulate the pulmonary vasomotor response may allow for future therapeutic interventions in PAH.

ACKNOWLEDGMENTS

We thank Dr. Irina Petrace for valuable criticism and insights.

GRANTS

This work was supported in part by National Institutes of Health (NIH) Grant R01-GM-070628 (D. R. Meldrum), NIH NRSA Grant F32-HL-085982 (T. A. Markel), and AHA Postdoctoral Fellowship 0526008Z (M. Wang). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06 RR-015481-01 from the National Center for Research Resources, National Institutes of Health.

REFERENCES

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