

## EXOGENOUS ESTROGEN RAPIDLY ATTENUATES PULMONARY ARTERY VASOREACTIVITY AND ACUTE HYPOXIC PULMONARY VASOCONSTRICTION

Tim Lahm,\* Paul R. Crisostomo,<sup>†</sup> Troy A. Markel,<sup>†</sup> Meijing Wang,<sup>†</sup> Yue Wang,<sup>†</sup> Brent Weil,<sup>†</sup> and Daniel R. Meldrum<sup>†‡§</sup>

\*Division of Pulmonary, Allergy, Critical Care and Occupational Medicine,

<sup>†</sup>Departments of Surgery, <sup>‡</sup>Cellular and Integrative Physiology, and <sup>§</sup>Center for Immunobiology, Indiana University School of Medicine, Indianapolis, Indiana

Received 3 Jan 2008; first review completed 16 Jan 2008; accepted in final form 18 Feb 2008

**ABSTRACT**—Chronic estrogen exposure has been shown to affect pulmonary artery (PA) vasoreactivity. However, the immediate effects of exogenously administered 17 $\beta$ -estradiol (E2) on vasopressor-induced PA vasoconstriction and acute hypoxic pulmonary vasoconstriction (HPV) have not yet been investigated. We hypothesized that exogenously administered E2 attenuates PA vasoreactivity and acute HPV through a rapid mechanism. Isometric force displacement was measured in isolated PA rings from proestrus female adult Sprague-Dawley rats, estrus, metestrus, or diestrus female adult Sprague-Dawley rats, and male adult Sprague-Dawley rats. The vasoconstrictor response in the absence of hypoxia (organ bath bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>) was measured after stimulation with 1  $\mu$ M of phenylephrine. Hypoxia was generated by changing the gas to 95% N<sub>2</sub>/5% CO<sub>2</sub>. The E2 was added to the organ bath in 0.1-nM, 0.5-nM, 1- $\mu$ M, 500- $\mu$ M, and 1-mM doses. The 1-mM dose caused an immediate decrease in force in PA rings from estrus, metestrus, or diestrus female adult Sprague-Dawley rats. In addition, 500  $\mu$ M and 1 mM of E2 attenuated phenylephrine- and hypoxia-induced vasoconstriction and potentiated the vasodilatory phase of hypoxia. These effects were immediate and independent of sex or estrous cycle. Lower E2 doses did not mediate any significant effects. We conclude that high doses of exogenous E2 acutely attenuate PA vasoreactivity and acute HPV in a rapid and dose-dependent manner. A better understanding of how E2 modulates the pulmonary vasomotor response may allow for future therapeutic interventions in acute pulmonary hypertensive crises or in pulmonary arterial hypertension.

**KEYWORDS**—Sex hormones, 17 $\beta$ -estradiol, phenylephrine, estrous cycle, sex differences, nongenomic effects

### INTRODUCTION

The effects of sex hormones on the pulmonary vasculature are complex and not fully understood. Idiopathic pulmonary arterial hypertension, a disabling condition characterized by pulmonary artery (PA) vasoconstriction, remodeling, and *in situ* thrombosis, and eventually right ventricular failure, occurs twice as frequently in females as compared with males (1). However, in the setting of chronic hypoxia, females have been noted to exhibit less severe pulmonary hypertension than their male counterparts (2). Interestingly, chronically hypoxic ovariectomized rats develop more severe pulmonary arterial remodeling and right ventricular hypertrophy than chronically hypoxic rats with intact ovaries (3). In addition, ovariectomized rats exposed to monocrotaline (a plant toxin that induces progressive pulmonary vascular injury resulting in pulmonary hypertension and right ventricular failure), exhibit more severe disease than normal female rats (4).

In a previous study (5), we demonstrated that sex and estrous cycle affect PA vasoreactivity and that physiologic increases in circulating estrogen levels attenuate PA vasoconstriction under both normoxic and hypoxic conditions.

Pulmonary artery rings from proestrus female Sprague-Dawley rats, characterized by physiologically increased estrogen levels compared with estrus and diestrus female Sprague-Dawley rats and male animals, exhibited an attenuated vasoconstrictor response when stimulated with vasoactive agents or hypoxia. Few other studies have investigated the effects of sex hormones on PA vasoreactivity (2, 6). In an isolated PA model, the immediate administration of estrogen caused vasorelaxation under normoxic conditions (7).

The vasomotor effects of sex hormones are mediated through genomic and nongenomic mechanisms. The genomic mechanisms of estrogen rely on the production of proteins to mediate its effects, and consequently, such effects are gradual in character. In contrast, the nongenomic effects occur much more rapidly, taking only seconds to minutes, and use existing proteins for effect (8–10). Therefore, any immediate effect that exogenously administered estrogen exerts must be mediated through nongenomic mechanisms.

The immediate effects of exogenous sex hormones on hypoxic pulmonary vasoconstriction (HPV) are incompletely understood. This is of interest because HPV and pulmonary arterial hypertension share common intracellular pathways and signaling mechanisms that may be amenable to therapeutic manipulation (11). Early investigations were performed by Wetzel et al. (12) and Gordon et al. (13) in isolated sheep lungs. However, in these experiments, estrogen was given to living animals 2 to 5 days before experimentation. Therefore, it is not clear which of the described effects were caused by genomic versus rapid nongenomic mechanisms. To our

Address reprint requests to Daniel R. Meldrum, MD, 635 Barnhill Dr, MS 2017, Indianapolis, Indiana 46202. E-mail: dmeldrum@iupui.edu.

This study was supported in part by the National Institutes of Health (grant no. R01GM070628, grant no. R01HL085595, grant no. K99/R00 HL0876077-01, and grant no. F32HL085982), by the American Heart Association Grant-in-Aid, and by the American Heart Association Postdoctoral Fellowship (grant no. 0725663Z).

DOI: 10.1097/SHK.0b013e31816f239f

Copyright © 2008 by the Shock Society

knowledge, no study has described the rapid and immediate effects of exogenous estrogen on acute HPV and PA vasoreactivity. This is not only of interest in light of the data indicating that *endogenous* estrogen attenuates PA vasoreactivity and HPV (2, 3, 5), but also because the administration of *exogenous* estrogen has been shown to be beneficial in the setting of experimental trauma-hemorrhage, shock, sepsis, and acute lung injury (14–16). A better understanding of the rapid effects of exogenous estrogen on the pulmonary vasculature may allow for therapeutic interventions in pulmonary arterial hypertension and pulmonary hypertensive crises in the future.

We hypothesized that exogenous estrogen attenuates PA vasoreactivity and acute HPV through a rapid mechanism. We also sought to find out whether the effects of exogenous estrogen vary between different sexes and between various stages of the estrous cycle. To test this, isometric force displacement was measured in isolated PA rings from proestrus, estrus, metestrus, and diestrus female Sprague-Dawley rats and male Sprague-Dawley rats.

## MATERIALS AND METHODS

### Animals

All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health 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, Ind) weighing 225 to 350 g were allowed *ad libitum* access to food and water up to the time of experimentation. All animals were cared for in a

nonstressful environment for at least 1 week before experimentation. The estrous cycle of the female rats was determined using vaginal smears as described previously by Hubscher et al. (17). Briefly, the estrous cycle of the female rat consists of four phases and lasts 4 to 5 days. The proestrus phase is characterized by high endogenous estrogen levels, whereas the estrus, metestrus, and diestrus phases exhibit low endogenous estrogen levels (17). Female rats were divided into proestrus and estrus, metestrus, or diestrus animals.

### 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, Colo) for isometric force measurement. Care was taken during the entire process to avoid injury to the endothelium. The PA rings were immersed in individual water-jacketed organ chambers containing modified KH solution bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub> 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, Calif).

### Experimental protocol and groups

Before starting the 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 mM 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 μM) after phenylephrine (PE; 1 μM) precontraction. Rings demonstrating less than 200-mg contraction to PE were discarded. In endothelium-intact PA, rings demonstrating less than 50% vasorelaxation to acetylcholine were discarded. After washout of acetylcholine, PA rings were allowed to equilibrate. After equilibration, PA rings were precontracted with PE. After PE precontraction, hypoxia was induced by changing the bubbled gas to 95% N<sub>2</sub>/5% CO<sub>2</sub>, producing a P<sub>O<sub>2</sub></sub> of 30 to 35 mmHg. Each experiment

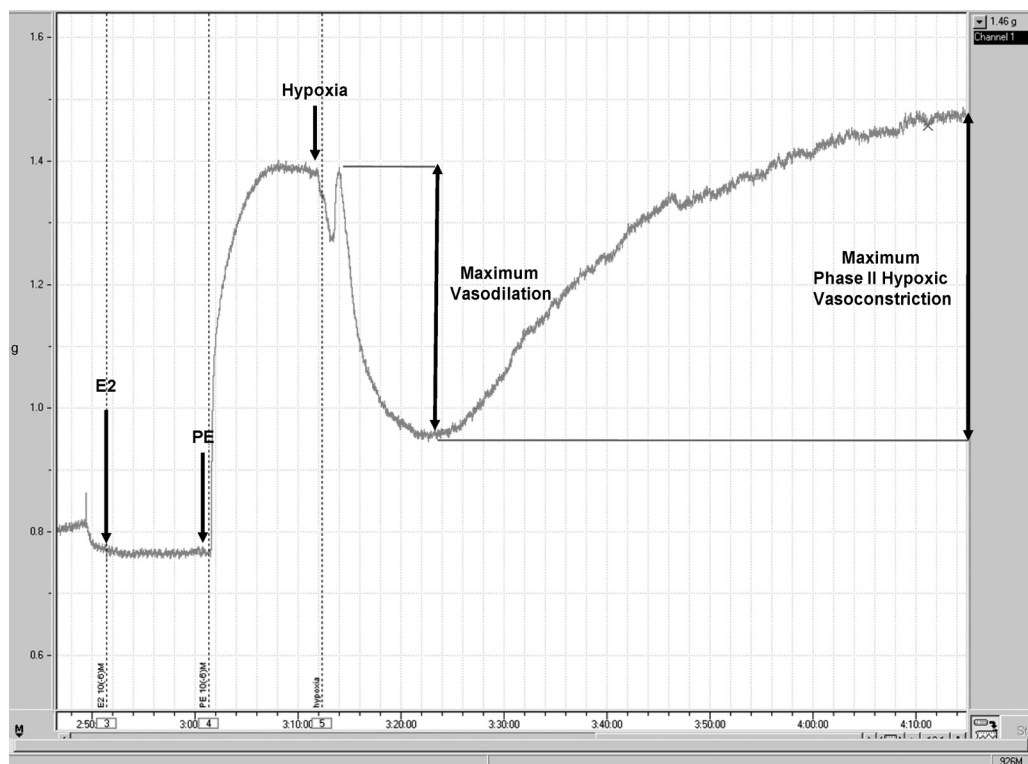


FIG. 1. Representative pressure tracing of hypoxic vasoconstriction in isolated pulmonary arteries. Force in grams is depicted on the y axis. Time in hours and minutes is represented on the x axis. Pulmonary arteries precontracted using phenylephrine (PE, 1 μM) were exposed to hypoxia (P<sub>O<sub>2</sub></sub> = 30–35 mmHg) for 60 min. Maximum vasorelaxation 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. E2, 17β-estradiol.

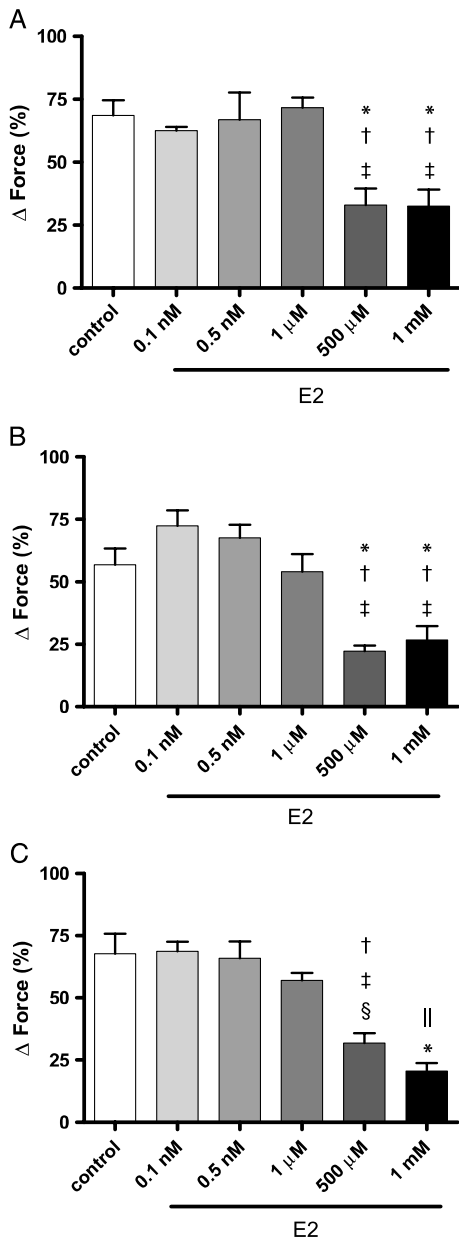


FIG. 2. Rapid effects of E2 on phenylephrine (PE)-induced vasoconstriction in PA rings from males (A), proestrus females (B), and estrus, metestrus, and diestrus females (C). The PE (1  $\mu$ M) was added to the organ bath 10 min after addition of estradiol. Values are normalized within each group to their respective resting tension. The 0.1-nM, 0.5-nM, and 1- $\mu$ M doses did not affect PE-induced vasoconstriction in any of the groups. In contrast, the 500- $\mu$ M and 1-mM doses significantly attenuated PE-induced vasoconstriction in all groups. A, \* $P$  < 0.05 vs. 0.5 nM;  $\dagger P$  < 0.01 vs. 0.1 nM and vs. control;  $\ddagger P$  < 0.001 vs. 1  $\mu$ M. B, \* $P$  < 0.05 vs. 1  $\mu$ M;  $\dagger P$  < 0.01 vs. control;  $\ddagger P$  < 0.001 vs. 0.1 nM and 0.5 nM. C, \* $P$  < 0.0001 vs. 0.1 nM, 0.5 nM, 1  $\mu$ M, and 4control;  $\dagger P$  < 0.0001 vs. 0.1 nM;  $\ddagger P$  < 0.01 vs. 1  $\mu$ M;  $\S P$  < 0.01 vs. 0.5 nM and control;  $\parallel P$  = 0.05 vs. 500  $\mu$ M.

was terminated after 60 min of hypoxia, and rings were immediately flash-frozen in liquid nitrogen for future experiments.

Water-soluble 17 $\beta$ -estradiol (E2, cyclodextrin-estradiol) in different concentrations (0.1 nM, 0.5 nM, 1  $\mu$ M, 500  $\mu$ M, and 1 mM) was added to the organ bath 10 min before the second administration of PE and 20 min before induction of hypoxia. The 0.1-nM dose represents the physiologically maximally achievable estradiol level in cycling rodents (18). The 1-mM dose was based on previous experiments during normoxia by English et al. (7).

#### Hypoxic pulmonary vasoconstriction

To measure the effect of hypoxia on PA, we gassed PE-precontracted PA rings with 95% N<sub>2</sub>/5% CO<sub>2</sub> for 60 min. This produced a P<sub>O<sub>2</sub></sub> of 30 to 35

mmHg in the organ bath, which was measured with a blood-gas analyzer (Synthesis 20; Instrumentation Laboratory, Lexington, Mass). 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 vasorelaxation and a late phase II (occurring 10–15 min after hypoxia exposure) contraction (Fig. 1). Because of 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 PE precontraction. Maximum vasorelaxation was measured as the difference between PE precontraction and the lowest force

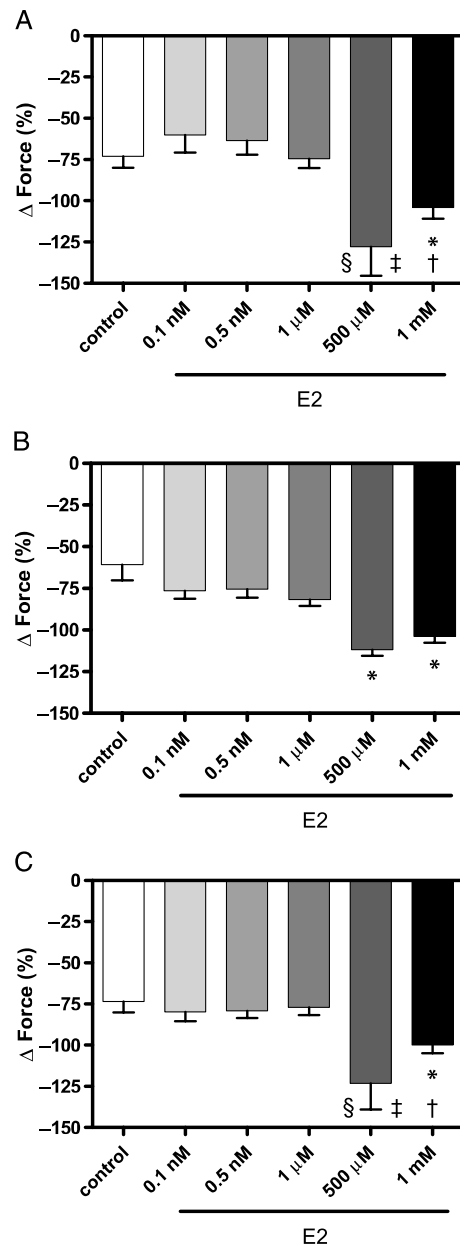


FIG. 3. Rapid effects of E2 on vasorelaxation during hypoxia in PA rings from males (A), proestrus females (B), and estrus, metestrus, and diestrus females (C). Estrogen was added to the organ bath 20 min before induction of hypoxia. Force displacement during hypoxia is expressed as percent change from the amount of PE precontraction. Estradiol in 500  $\mu$ M and 1 mM concentrations significantly potentiated vasorelaxation in all three groups. Lower estrogen doses did not cause any significant changes. A, \* $P$  < 0.05 vs. control;  $\dagger P$  < 0.01 vs. 0.1 nM, 0.5 nM, and 1  $\mu$ M;  $\ddagger P$  < 0.05 vs. control, 0.1 nM, and 0.5 nM;  $\S P$  < 0.01 vs. 1  $\mu$ M. B, \* $P$  < 0.01 vs. 0.1 nM, 0.5 nM, 1  $\mu$ M, and control. C, \* $P$  < 0.05 vs. 0.1 nM;  $\dagger P$  < 0.01 vs. 0.5 nM, 1  $\mu$ M, and control;  $\ddagger P$  < 0.05 vs. 0.1 nM and 0.5 nM;  $\S P$  < 0.01 vs. 1  $\mu$ M and control.



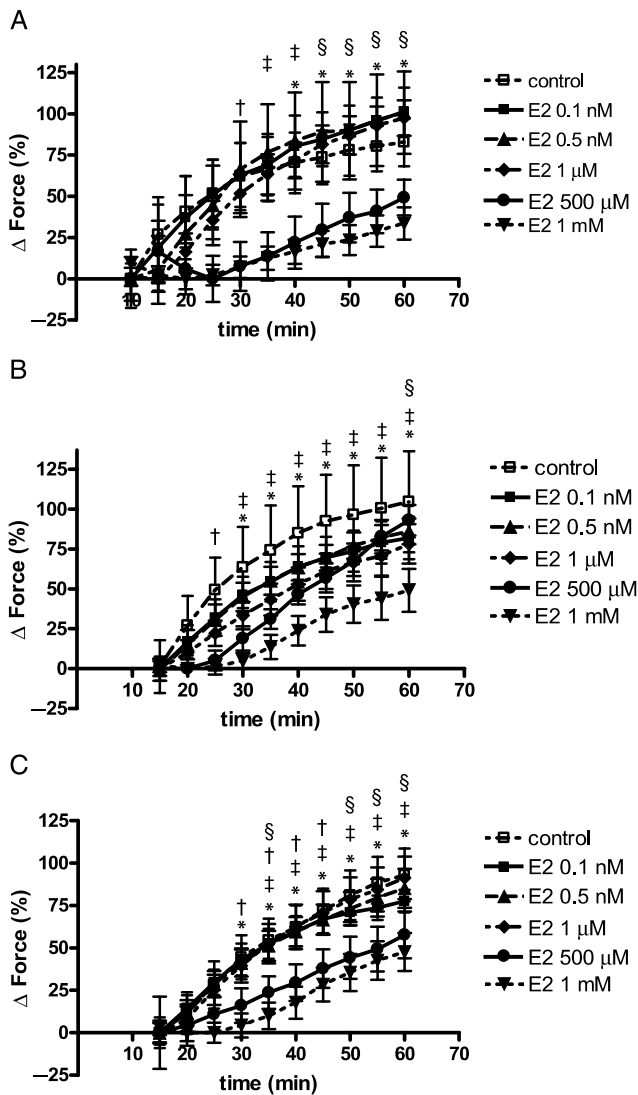


FIG. 6. Time line of phase II HPV for males (A), proestrus females (B), and estrus, metestrus, and diestrus females (C). Because the vasodilatory phase of hypoxia was prolonged in the highest-dose estradiol groups, the onset of phase II HPV was delayed and occurred after 20 min. The highest estradiol concentration attenuated HPV in PA rings from males, proestrus females, and estrus, metestrus, or diestrus females. In contrast, 500  $\mu$ M of estradiol did not significantly attenuate HPV in PA rings from proestrus animals. A, \* $P$  < 0.05 for 1 mM vs. 0.1 nM; † $P$  < 0.05 for 1 mM vs. 1  $\mu$ M; ‡ $P$  < 0.01 for 1 mM vs. 1  $\mu$ M; § $P$  < 0.001 for 1 mM vs. 1  $\mu$ M. B, \* $P$  < 0.05 for 1 mM vs. 0.5 nM; † $P$  < 0.01 for 1 mM vs. 1  $\mu$ M; ‡ $P$  < 0.001 for 1 mM vs. 1  $\mu$ M; § $P$  < 0.05 for 1 mM vs. 500  $\mu$ M. C, \* $P$  < 0.05 for 1 mM vs. 0.5 nM; † $P$  < 0.05 for 1 mM vs. 0.1 nM; ‡ $P$  < 0.05 for 1 mM vs. 1  $\mu$ M; § $P$  < 0.05 for 1 mM vs. control.

occurred in less than 10 min. No significant decreases in resting tone were noted in rings from male or proestrus animals.

**E2 rapidly attenuates PE-induced vasoconstriction**

Exogenous E2, when given in 0.1-nM, 0.5-nM, and 1- $\mu$ M doses did not significantly affect PE-induced vasoconstriction in PA rings from male, proestrus female, or estrus, metestrus, and diestrus females (Fig. 2). In contrast to these findings, the 500- $\mu$ M and 1-mM doses significantly attenuated PE-induced vasoconstriction in all three groups. There was a trend toward more pronounced effects of the 1-mM dose in the estrus, metestrus, or diestrus females ( $P = 0.05$ ). The increase in force after 500  $\mu$ M of E2 was  $33.08\% \pm 6.56\%$  in male PA

rings,  $22.24\% \pm 2.22\%$  in proestrus PA rings, and  $31.77\% \pm 4.0\%$  in estrus, metestrus, or diestrus rings. For the 1-mM dose, the increase in force was  $32.54\% \pm 6.63\%$  in male PA rings,  $26.74\% \pm 5.53\%$  in proestrus PA rings, and  $20.54\% \pm 3.28\%$  in estrus, metestrus, or diestrus rings. This effect occurred within 10 min of administration.

**E2 potentiates vasorelaxation during hypoxia**

The 0.1-nM, 0.5-nM, and 1- $\mu$ M doses of E2 did not significantly alter vasorelaxation after the onset of hypoxia (Fig. 3). However, when given as a 500- $\mu$ M or 1-mM dose, E2 markedly enhanced vasorelaxation in all three groups. This was reflected by a significant decrease in force (500  $\mu$ M: males,  $-127\% \pm 17.5\%$ ; proestrus females,  $-111.75\% \pm 3.85\%$ ; estrus, metestrus, and diestrus females,  $-123.14\% \pm 15.77\%$ ; 1 mM: males,  $-104.1\% \pm 6.68\%$ ; proestrus females,  $-103.75\% \pm 3.93\%$ ; estrus, metestrus, and diestrus females,  $-100.0\% \pm 4.92\%$ ). In addition, 1 mM of E2 also delayed the

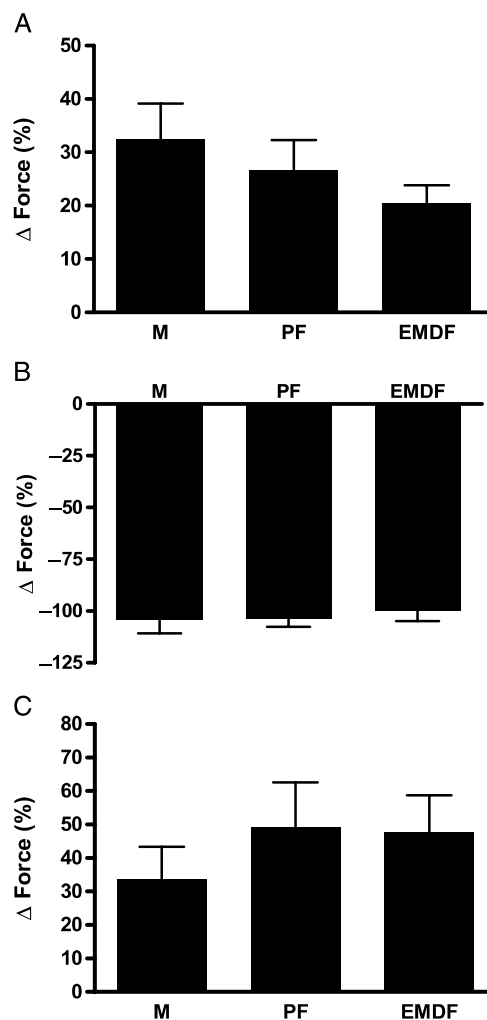


FIG. 7. Comparison of the effects of 1 mM of E2 between PA rings from male (M), proestrus female (PF) and estrus, metestrus or diestrus female (EMDF) animals. In all three groups, the administration of 1 mM of E2 attenuated PE-induced vasoconstriction (A) and phase II of HPV to a similar degree (C), reflected by similar amounts of generated force ( $P =$  not significant for M versus PF, M versus EMDF, and PF versus EMDF). In addition, this dose potentiated vasorelaxation during hypoxia to a similar degree (B), as reflected by similar decreases in force ( $P =$  not significant for M versus PF, M versus EMDF, and PF versus EMDF).

occurrence of maximal vasorelaxation, therefore prolonging the vasodilatory phase of hypoxia (Fig. 4). Whereas maximal vasorelaxation occurred after 10 to 15 min in the control group and in the 0.1-nM, 0.5-nM, and 1- $\mu$ M groups, respectively, this occurred after 20 min in all three 1-mM groups. Although there was some delay in the 500- $\mu$ M groups, this effect was not as pronounced as in the 1-mM groups.

### ***E2 rapidly attenuates acute HPV***

High concentrations of E2 (1 mM) attenuated phase II of HPV in PA rings from males, proestrus females, and estrus, metestrus, or diestrus females (Fig. 5). The 500- $\mu$ M dose attenuated phase II of HPV in PA rings from males and estrus, metestrus, and diestrus, but not proestrus females. In addition, the attenuation of HPV from 500  $\mu$ M in males and non-proestrus females was less pronounced than that of 1 mM (increase in force with 500  $\mu$ M in males,  $48.51\% \pm 10.65\%$ ; proestrus females,  $93.1\% \pm 9.61\%$ ; estrus, metestrus, and diestrus females,  $57.69\% \pm 13.73\%$ ; increase in force with 1 mM: males,  $33.59\% \pm 9.8\%$ ; proestrus females,  $49.13\% \pm 13.41\%$ ; estrus, metestrus, and diestrus females,  $47.59\% \pm 11.21\%$ ). Similar to the other experiments, no significant effects were seen with lower estradiol doses. Because the vasodilatory phase of hypoxia was prolonged in all the three highest-dose estradiol groups, the onset of phase II HPV was delayed as well (Fig. 6). This effect was independent of sex or estrous cycle.

### ***Nongenomic E2 effects are independent of sex and estrous cycle***

17 $\beta$ -Estradiol (1 mM) attenuated PE- and hypoxia-induced vasoconstriction to a similar degree in all three groups, irrespective of the sex and the phase of the estrous cycle (Fig. 7). Similarly, the potentiation of vasorelaxation during hypoxia was of the same magnitude in all groups, irrespective of sex and estrous cycle.

## **DISCUSSION**

The results of this study demonstrate that high doses of E2 (500,000–1,000,000 greater than the maximum physiological concentration in rodents) acutely attenuate PA vasoreactivity through a rapid and therefore most likely nongenomic mechanism. This attenuation was seen after administration of the vasoconstrictive agent PE and during acute hypoxia. In addition, we were able to show that the magnitude of these effects is not dependent on endogenous estrogen levels. The E2 dose that affected vasoreactivity the most (1 mM) mediated this irrespective of the sex or the phase of the estrous cycle. In other words, a high E2 dose made PA rings from male and female animals “look the same.” Furthermore, we demonstrated that the highest estradiol dose also caused a small, but statistically significant, vasorelaxation in PA rings from estrus, metestrus, and diestrus females.

These results are in concordance with our previous study (5), in which we were able to show that endogenous estrogen attenuated PA vasoreactivity and acute HPV. The current data expand our knowledge by demonstrating that pharmacological doses of E2 have an immediate and acute effect on the pul-

monary vasculature. However, this effect is dose-dependent and only seen with high doses (500  $\mu$ M and 1 mM). Although fluctuations in endogenous estrogen affect PA vasoreactivity (5), these sex differences seem to be largely abolished by high doses of exogenous estrogen.

Estradiol has well-documented genomic effects on the pulmonary vasculature (6, 13, 19, 20). There is also evidence that anti-inflammatory mechanisms contribute to the vasodilatory properties of estrogens. Pulmonary artery vasoreactivity is influenced by the integrity of the endothelial cell layer and the inflammatory state of the vasculature (21). Hypoxic pulmonary vasoconstriction is associated with smooth muscle cell contraction, inflammation, generation of reactive oxygen species, and cytokine release (11, 22). Interestingly, estrogen has been shown to decrease inflammation and to stabilize cellular integrity in other organ systems (23). Because PA vasoreactivity and HPV were rapidly affected in our model, any genomic estrogen effects can most likely be excluded. Nongenomic estrogen receptor- $\alpha$ -mediated increases in nitric oxide (NO) production and increases in prostacyclin release have been demonstrated in tissue culture experiments (24, 25). Antioxidant effects have been attributed to estrogen in the setting of myocardial ischemia-reperfusion injury (26). It is conceivable that these mechanisms play a role in the hypoxic pulmonary vasculature as well, but further research is needed to determine if this is truly the case.

In addition to estradiol's effects on PA vasoreactivity, it has been shown that various estradiol metabolites affect vascular tone and vascular remodeling as well. A line of evidence suggests that several estradiol effects are mediated through a number of downstream metabolites that exert anti-inflammatory and antiproliferative effects (4). For example, the estradiol metabolite 2-methoxyestradiol attenuated monocrotaline-induced pulmonary hypertension in rats. Interestingly, ovariectomized rats developed more severe monocrotaline-induced pulmonary hypertension than normal females, and this was attenuated by treatment with 2-methoxyestradiol (4). However, the estrogen metabolite was given on a chronic basis, and its acute effects could therefore not be investigated.

One strength of our model is that it allows the investigation of immediate (and therefore most likely nongenomic) estrogen effects on PA vasoreactivity. For this reason, estrogen was given 10 min before administration of PE and 20 min before induction of acute hypoxia. Because the conversion of estradiol to its metabolites requires several steps (4), it is unlikely that the effects of estradiol metabolites significantly contributed to the effects observed in our model. Although English et al. (7) investigated nongenomic effects of various sex hormones including E2 on PA tone under normoxic conditions, to our knowledge, there are no studies investigating the rapid actions of estradiol in the setting of hypoxia or after exposure to vasoconstrictor agents. Similar to other studies, the previous investigations on hypoxic vasoconstriction in isolated sheep lungs by Wetzel et al. (12) and Gordon et al. (13) focused on chronic (and therefore genomic) estrogen effects.

The potential therapeutic implications of the rapid and nongenomic effects of estrogen have been demonstrated in several settings, including trauma-hemorrhage, shock, sepsis,

and acute lung injury (14–16). The beneficial properties of estrogen and its metabolites may explain why, in the setting of chronic hypoxia, females have been noted to exhibit less severe pulmonary hypertension than their male counterparts (2). It is tempting to speculate that the various genomic and nongenomic effects of estrogen may contribute to this sex difference in disease severity. However, in contrast to chronic hypoxic pulmonary hypertension, the idiopathic form of pulmonary arterial hypertension is much more common in females (1). This seems to be in contradiction to the previously described vasoprotective properties of estrogen. One may speculate that a defect in estrogen receptor pathways or intracellular signaling may contribute to this paradox.

Although chronic long-term estrogen therapy may be associated with significant side effects in females (27), an acute one-time dose or a short course 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 or after lung transplantation (11). In both circumstances, and in patients with the acute respiratory distress syndrome, HPV can lead to severe pulmonary hypertension with right ventricular decompensation (11). A potent pulmonary vasodilator can be lifesaving in those settings. Because the currently available treatments are expensive and sometimes associated with significant side effects or tachyphylaxis (11), a drug that mimics the acute vasoactive effects of E2 may be of clinical benefit.

An acute high dose of estradiol is unlikely to cause serious toxicity other than nonspecific gastrointestinal side effects (28). However, because estrogens have the potential to cause significant neural, cardiovascular, endocrine, or metabolic side effects, it would be of special interest to better understand the signaling mechanisms through which estradiol exerts its protective effects, so that targeted nonhormonal mechanisms can be identified and then be applied in patients. The development of new drugs mimicking some or all of the rapid vasomotor effects of estrogen may also be of benefit for the treatment of the vasoconstriction and vascular remodeling associated with pulmonary arterial hypertension. The work by Tofovic et al. (4) provides further evidence along those lines. In this context, it is also of interest that the administration of E2 or the estrogen receptor- $\beta$  agonist diarylpropiolnitrile has been shown to attenuate lung injury after trauma-hemorrhage in male rodents (29).

In addition, our findings of E2-induced attenuation of vasoconstriction after stimulation with PE may be of importance for the treatment of shock, in which there is concern that vasopressors can induce or worsen pulmonary hypertension. Although many factors contribute to the morbidity and mortality associated with trauma, shock, and sepsis (30–33), decreased vasopressor-induced pulmonary hypertension may contribute to the improved survival observed in females in these conditions (9, 34, 35). This may be another clinical scenario in which estradiol or an estradiol-like drug may be of benefit.

We acknowledge that 500  $\mu$ M and 1 mM of E2 are relatively high doses. However, these concentrations were based on

previous experiments by English et al. (7), in which the effects of E2 were clearly dose related. As pointed out by English and colleagues (7), the dose needed to affect vasoreactivity in large vessels like the main pulmonary arteries seems to be higher than the dose needed to affect medium-sized or small arteries. This may be caused by the fact that the vessels of the precapillary segment of the pulmonary vascular bed (which contribute to most of the pulmonary vascular resistance), in contrast to the large vessels, are only partially muscularized (22). In addition, differences exist between proximal and distal smooth muscle cells with regard to their electrophysiologic properties, differentiation, and growth factor response (22). English et al. (7) also observed that the pulmonary vessels were less responsive to the effects of E2 and other steroid hormones than the coronary vasculature, therefore requiring higher doses. Thus, vessel size and type seem to play important roles. In addition, it is well recognized that discrepancies exist between the concentrations of agents that are required to produce effects *in vivo* and *in vitro*. For example, this has been shown for the potassium-channel opener cromakalim, which exerts vasodilatory effects *in vivo* at a lower dose than *in vitro* (36, 37). Furthermore, it has been demonstrated that sex hormones may actively be adsorbed in the capillaries, so that the active sex hormone concentration at the capillary membrane active site may be significantly higher than that estimated by serum samples (38). Therefore, it is conceivable that *in vivo* smaller estradiol doses than the ones used *in vitro* may be sufficient to exert similar effects. Interestingly, dose-dependent mechanisms have also been described for estradiol metabolites, where low doses have been shown to induce proliferation of cultured vascular endothelial cells, whereas higher concentrations ( $\geq 100$  nM) inhibit the proliferation of these cells (39). However, our data suggest that pharmacological doses are most likely necessary to achieve this effect, although it may be difficult to achieve estrogen concentrations as high as the ones used in our experiments in intact animals or patients.

In conclusion, we demonstrated that high doses of estradiol rapidly decrease PA tone after stimulation with a pharmacological agent (PE) or hypoxia. Whether this occurs through nongenomic mechanisms will need to be determined in future experiments, so that targeted nonhormonal mechanisms can be identified and applied in patients, therefore eliminating the pharmacodynamic problems that may be associated with high estradiol doses.

## ACKNOWLEDGMENT

The authors thank Irina Petrache, MD, for valuable criticism and insights.

## REFERENCES

- McLaughlin VV, McGoon MD: Pulmonary arterial hypertension. *Circulation* 114:1417–1431, 2006.
- Rabinovitch M, Gamble WJ, Miettinen OS, Reid L: Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. *Am J Physiol* 240:H62–H72, 1981.
- Resta TC, Kanagy NL, Walker BR: Estradiol-induced attenuation of pulmonary hypertension is not associated with altered eNOS expression. *Am J Physiol Lung Cell Mol Physiol* 280:L88–L97, 2001.
- Tofovic SP, Zhang X, Jackson EK, Dacic S, Petrussevska G: 2-Methoxyestradiol mediates the protective effects of estradiol in monocrotaline-induced pulmonary hypertension. *Vascul Pharmacol* 45:358–367, 2006.

5. Lahm T, Patel KM, Crisostomo PR, Markel TA, Wang M, Herring C, Meldrum DR: Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle. *Am J Physiol Endocrinol Metab* 293:E865–E871, 2007.
6. Earley S, Resta TC: Estradiol attenuates hypoxia-induced pulmonary endothelin-1 gene expression. *Am J Physiol Lung Cell Mol Physiol* 283:L86–L93, 2002.
7. English KM, Jones RD, Jones TH, Morice AH, Channer KS: Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Horm Metab Res* 33:645–652, 2001.
8. Crisostomo PR, Wang M, Herring CM, Morrell ED, Seshadri P, Meldrum KK, Meldrum DR: Sex dimorphisms in activated mesenchymal stem cell function. *Shock* 26:571–574, 2006.
9. Choudhry MA, Schwacha MG, Hubbard WJ, Kerby JD, Rue LW, Bland KI, Chaudry IH: Gender differences in acute response to trauma-hemorrhage. *Shock* 24(Suppl 1):101–106, 2005.
10. Meldrum DR: G-protein-coupled receptor 30 mediates estrogen's nongenomic effects after hemorrhagic shock and trauma. *Am J Pathol* 170:1148–1151, 2007.
11. Morrell ED, Tsai BM, Crisostomo PR, Hammoud ZT, Meldrum DR: Experimental therapies for hypoxia-induced pulmonary hypertension during acute lung injury. *Shock* 25:214–226, 2006.
12. Wetzel RC, Zacur HA, Sylvester JT: Effect of puberty and estradiol on hypoxic vasomotor response in isolated sheep lungs. *J Appl Physiol* 56:1199–1203, 1984.
13. Gordon JB, Wetzel RC, McGeady ML, Adkinson NF Jr, Sylvester JT: Effects of indomethacin on estradiol-induced attenuation of hypoxic vasoconstriction in lamb lungs. *J Appl Physiol* 61:2116–2121, 1986.
14. Yokoyama Y, Kuebler JF, Matsutani T, Schwacha MG, Bland KI, Chaudry IH: Mechanism of the salutary effects of 17beta-estradiol following trauma-hemorrhage: direct downregulation of Kupffer cell proinflammatory cytokine production. *Cytokine* 21:91–97, 2003.
15. Suzuki T, Shimizu T, Yu HP, Hsieh YC, Choudhry MA, Chaudry IH: Salutary effects of 17beta-estradiol on T-cell signaling and cytokine production after trauma-hemorrhage are mediated primarily via estrogen receptor- $\alpha$ . *Am J Physiol Cell Physiol* 292:C2103–C2111, 2007.
16. Suzuki T, Yu HP, Hsieh YC, Choudhry MA, Bland KI, Chaudry IH: Estrogen-mediated activation of non-genomic pathway improves macrophages cytokine production following trauma-hemorrhage. *J Cell Physiol* 214:662–672, 2007.
17. Hubscher CH, Brooks DL, Johnson JR: A quantitative method for assessing stages of the rat estrous cycle. *Biotech Histochem* 80:79–87, 2005.
18. Warner M, Gustafsson JA: Nongenomic effects of estrogen: why all the uncertainty? *Steroids* 71:91–95, 2006.
19. Gonzales RJ, Walker BR, Kanagy NL: 17Beta-estradiol increases nitric oxide-dependent dilation in rat pulmonary arteries and thoracic aorta. *Am J Physiol Lung Cell Mol Physiol* 280:L555–L564, 2001.
20. Lahm T, Crisostomo PR, Markel TA, Wang M, Lillemoe KD, Meldrum DR: The critical role of vascular endothelial growth factor in pulmonary vascular remodeling after lung injury. *Shock* 28:4–14, 2007.
21. Liu SF, Dewar A, Crawley DE, Barnes PJ, Evans TW: Effect of tumor necrosis factor on hypoxic pulmonary vasoconstriction. *J Appl Physiol* 72:1044–1049, 1992.
22. Stenmark KR, Fagan KA, Frid MG: Hypoxia-induced pulmonary vascular remodeling: cellular and molecular mechanisms. *Circ Res* 99:675–691, 2006.
23. Wang M, Crisostomo P, Wairiuko GM, Meldrum DR: Estrogen receptor- $\alpha$  mediates acute myocardial protection in females. *Am J Physiol Heart Circ Physiol* 290:H2204–H2209, 2006.
24. Lantin-Hermoso RL, Rosenfeld CR, Yuhanna IS, German Z, Chen Z, Shaul PW: Estrogen acutely stimulates nitric oxide synthase activity in fetal pulmonary artery endothelium. *Am J Physiol* 273:L119–L126, 1997.
25. Sherman TS, Chambliss KL, Gibson LL, Pace MC, Mendelsohn ME, Pfister SL, Shaul PW: Estrogen acutely activates prostacyclin synthesis in ovine fetal pulmonary artery endothelium. *Am J Respir Cell Mol Biol* 26:610–616, 2002.
26. Murphy E, Steenbergen C: Cardioprotection in females: a role for nitric oxide and altered gene expression. *Heart Fail Rev* 12:293–300, 2007.
27. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, et al.: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* 288:321–333, 2002.
28. Barlow DH: Oestrogen implant overdose. *Lancet* 2:392–393, 1988.
29. Yu HP, Hsieh YC, Suzuki T, Shimizu T, Choudhry MA, Schwacha MG, Chaudry IH: Salutary effects of estrogen receptor-beta agonist on lung injury after trauma-hemorrhage. *Am J Physiol Lung Cell Mol Physiol* 290:L1004–L1009, 2006.
30. Lomas-Neira J, Chung CS, Perl M, Gregory S, Biffi W, Ayala A: Role of alveolar macrophage and migrating neutrophils in hemorrhage-induced priming for ALI subsequent to septic challenge. *Am J Physiol Lung Cell Mol Physiol* 290:L51–L58, 2006.
31. Coopersmith CM, Stromberg PE, Dunne WM, Davis CG, Amiot DM 2nd, Buchman TG, Karl IE, Hotchkiss RS: Inhibition of intestinal epithelial apoptosis and survival in a murine model of pneumonia-induced sepsis. *JAMA* 287:1716–1721, 2002.
32. Wisnoski N, Chung CS, Chen Y, Huang X, Ayala A: The contribution of cd44-cd25+ T-regulatory-cells to immune suppression in sepsis. *Shock* 27:251–257, 2007.
33. Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A: Leukocyte apoptosis and its significance in sepsis and shock. *J Leukoc Biol* 78:325–337, 2005.
34. Schneider CP, Nickel EA, Samy TS, Schwacha MG, Cioffi WG, Bland KI, Chaudry IH: The aromatase inhibitor, 4-hydroxyandrostenedione, restores immune responses following trauma-hemorrhage in males and decreases mortality from subsequent sepsis. *Shock* 14:347–353, 2000.
35. Tsai BM, Wang M, Pitcher JM, Kher A, Brown JW, Meldrum DR: Endothelium-dependent pulmonary artery vasorelaxation is dysfunctional in males but not females after acute lung injury. *Surgery* 138:78–84, 2005.
36. Thomas P, Dixon MS, Winterton SJ, Sheridan DJ: Acute haemodynamic effects of cromakalim in patients with angina pectoris. *Br J Clin Pharmacol* 29:325–331, 1990.
37. Bray K, Quast U: Differences in the K(+) channels opened by cromakalim, acetylcholine and substance P in rat aorta and porcine coronary artery. *Br J Pharmacol* 102:585–594, 1991.
38. Porto CS, Lazari MF, Abreu LC, Bardin CW, Gunsalus GL: Receptors for androgen-binding proteins: internalization and intracellular signalling. *J Steroid Biochem Mol Biol* 53:561–565, 1995.
39. Lippert C, Seeger H, Mueck AO, Lippert TH: The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells. *Life Sci* 67:1653–1658, 2000.

