The Effect of Maternal Catecholamines on the Caliber of Gravid Uterine Microvessels

Scott Segal, MD
Steven Y. Wang, MD, PhD

BACKGROUND: Changes in maternal catecholamines that accompany the onset of labor analgesia include a decrease in epinephrine (EPI) but no change in norepinephrine (NE). Because EPI exerts predominantly β-adrenergic, and NE predominantly α-adrenergic effects in circulating concentrations, we hypothesized that these changes could lead to uterine arteriole vasoconstriction.

METHODS: Uterine microvessels (73–120 μm internal diameter, n = 18) were harvested from near-term pregnant Sprague-Dawley rats, isolated and studied in a pressurized no-flow state with video microscopy. Drugs were applied extraluminally to the superfusion reservoir and the steady-state vessel diameter recorded. Dose-response curves were constructed for NE with and without the addition of the α-adrenergic antagonist prazosin, EPI (after 20%–30% preconstriction with the thromboxane analog U46619) with and without the addition of the β-adrenergic antagonist propranolol, and NE in the presence of 10−8 M EPI. Washout experiments modeled the changes in circulating maternal catecholamines observed during onset of analgesia: 10−8 M EPI and 10−8 M of NE (the EC50) were added simultaneously, then washed with NE only, and then with the β-adrenergic agonist terbutaline and NE. The washout protocol was repeated in the presence of propranolol.

RESULTS: NE caused dose-dependent vasoconstriction (P < 0.0001), which was blocked by prazosin (P < 0.0001). EPI, added to U46619-preconstricted microvessels, caused vasodilation at lower concentrations and vasoconstriction at higher doses (P < 0.0001). Propranolol converted this response to monophasic dose-dependent vasoconstriction (P < 0.0001). Pretreatment of nonprecontracted vessels with EPI, 10−8 M, significantly attenuated NE-induced vasoconstriction (P < 0.0001). In washout experiments, removal of EPI with continued presence of NE resulted in vasoconstriction that was reversed by terbutaline. Propranolol blocked the effect of both EPI and terbutaline.

CONCLUSIONS: The results demonstrate that EPI, in concentrations found in the plasma of laboring women, vasodilates uterine resistance vessels and attenuates NE-induced vasoconstriction. This observation may have implications for changes in uterine blood flow that may accompany the onset of labor analgesia in human parturients, as effective analgesia is accompanied by an acute decrease in circulating EPI levels.

(Pain and maternal anxiety dramatically increase maternal catecholamines during labor. Although pregnancy is associated with a generalized decrease in the sensitivity to adrenergic stimulation, adrenergic mechanisms play an important role in determining uterine blood flow, and α-adrenergic sensitivity in the uterine circulation is actually increased in pregnancy.1,2 Even modest elevations in maternal α-adrenergic tone markedly decrease uterine blood flow in experimental animal preparations.3,4 In general, labor epidural analgesia-induced sympathetic blockade is associated with an increase in uterine blood flow in the absence of hypotension or intravascular injection of local anesthetic.5 However, neuraxial analgesia for labor pain also modifies the pattern of elevated catecholamines, causing a decrease in plasma epinephrine (EPI), but no change in plasma norepinephrine (NE).6–8 NE exerts predominantly α-adrenergic effects, and EPI, in the concentrations found in the plasma of laboring women, exerts predominantly β-adrenergic effects.9–9 Therefore, the changes in maternal catecholamines accompanying the onset of pain relief could theoretically lead to relatively unopposed α-adrenergic action of NE, causing uterine artery vasoconstriction. Previously we demonstrated that simulated changes in maternal catecholamines increased myometrial activity in an in vitro gravid rat.

Accepted for publication November 27, 2007.

Steven Y. Wang is currently at Sacramento Anesthesia Medical Group, 3939 J St. #310, Sacramento CA 95819.

Address correspondence and reprint requests to Scott Segal, MD, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women’s Hospital, 75 Francis St., Boston, MA 02115. Address e-mail to bssegal@zeus.bwh.harvard.edu.

Copyright © 2008 International Anesthesia Research Society DOI: 10.1213/ane.00013e181617451
uterus model. The present study was designed to test the hypothesis that alterations in catecholamines similar to those that accompany the onset of neuraxial analgesia in labor cause uterine microvascular constriction. We therefore studied the effects of EPI and NE, alone and in combination, in an ex vivo rat uterine microvessel preparation.

METHODS

Tissue Preparation
This study was approved by the Harvard Medical Area Standing Committee on Animals. The methods of microvessel preparation and study have been described in detail previously. Pregnant Sprague-Dawley rats ($n = 6$) were killed at 21 days gestation (term = 22 days) with an intraperitoneal injection of pentobarbital (30 mg/kg). The entire uterus was rapidly excised, and the fetuses and placentas were gently removed. The uterus was then placed in an ice-cold Krebs-Henseleit buffer (composition in mM: Na$^+ 143$, K$^+ 5.9$, Ca$^{2+} 2.6$, Mg$^2+$ 2, Cl$^- 128$, H$_2$PO$_4^-$ 2.2, HCO$_3^-$ 24.9, SO$_4^{2-} 1.2$). Uterine arterioles ($n = 18$) were dissected from the placental surface of the uterine wall with a $10 \times 60$ dissecting microscope. Microvessels, 73–120 $\mu$m in internal diameter and approximately 1 mm in length, were placed in a plastic organ chamber, cannulated at each end with glass micropipettes (30–80 $\mu$m in external diameter) and secured with 10–0 monofilament nylon suture. Oxygenated (95% O$_2$/5% CO$_2$) Krebs-Henseleit buffer at $37^\circ$C and pH 7.40 was continuously circulated through the organ chamber and reservoir containing a total volume of 100 mL. The vessels were pressurized to 50 mm Hg in a no-flow state using a burette manometer filled with Krebs-Henseleit buffer solution, and the pressure was continuously monitored with a pressure transducer. An inverted video microscope (40–200$\times$, Living System Instrumentation, Burlington, VT) was used to project the image of the vessel on a video monitor. An integrated dimension analyzer (Living System) was used to measure internal diameter. Vessels were bathed without intervention for 30 min before initiating the study protocols.

Study Protocols
Drugs were added to the superfusion reservoir to achieve desired final concentrations. Cumulative concentration-response curves were constructed for NE and EPI ($10^{-10}$ to $10^{-5}$ M), by measuring steady-state vessel diameter after each drug addition. Steady-state was determined by inspection of the video image and dimension analyzer and was achieved within 1–2 min after drug addition. The following series of experiments were conducted.

Series 1A and 1B
The responses to NE and EPI were studied individually. EPI concentration-response curves were performed after approximately 20% preconstriction with the thromboxane analog U46619. Responses to NE and EPI were then reassessed in the presence of prazosin, $10^{-7}$ M or propranolol, $10^{-5}$ M, respectively. U46619-induced vasoconstriction was (mean ± sd) 17.0% ± 13.6% in the control experiments and 21.8% ± 12.9% in the propranolol experiments.

Series 2
The ability of EPI to modify the response to NE was tested by treating nonprecontracted vessels with EPI, $10^{-8}$ M, followed by increasing concentrations of NE ($10^{-10}$ to $10^{-7}$ M).

Series 3
Washout experiments were conducted to model the changes in circulating maternal catecholamines observed at the time of onset of neuraxial labor analgesia. Vessels were treated simultaneously with EPI $10^{-8}$ M and NE $10^{-6.5}$ M (approximately the EC$_{50}$) and then washed with fresh superfusion buffer containing only NE $10^{-6.5}$ M. The preparation was then treated with the $\beta_2$-adrenergic agonist terbutaline at $10^{-7}$ M. This protocol was repeated in the presence of propranolol, $10^{-5}$ M.

Statistical Analysis
All vessel diameters were normalized to resting equilibrated diameter before experimental protocols and are expressed and analyzed as percent change from baseline. Each protocol was performed in multiple vessels ($n = 6$ per protocol) and mean responses ± sd are shown in the figures. Effects of dose and drug on vessel diameter were analyzed by analysis of variance (ANOVA) for repeated measures. Results for individual doses compared with baseline were subsequently analyzed by one sample t-test using a hypothesized mean of 0% change (individual drugs), or two-group t-test (between drugs) with P values considered after Bonferroni correction for multiple comparisons as appropriate. $P < 0.05$ was considered significant.

RESULTS
Mean baseline vessel diameters did not vary between experimental protocols (ANOVA, $P = 0.14$).

Series 1A and 1B
The responses of microvessels to EPI and NE are shown in Figure 1. NE caused a dose-dependent constriction of arterioles (Fig. 1A). The individual responses to $10^{-8}$ to $10^{-5}$ were statistically different from baseline. The selective $\alpha_1$-adrenergic blocker prazosin markedly attenuated this vasoconstriction. EPI caused a biphasic response in U46619-preconstricted microvessels, causing vasodilation at lower concentrations and vasoconstriction at higher doses (Fig. 1B). The individual responses at $10^{-9}$ to $10^{-5}$ were significantly different from baseline. The $\beta_2$-adrenergic blocker propranolol converted this response to monophasic dose-dependent vasoconstriction.
Series 2

Pretreatment of nonprecontracted vessels with EPI, $10^{-8}$ M, significantly attenuated NE-induced vasoconstriction (Fig. 2).

Figure 1. Response of uterine microvessels to norepinephrine and epinephrine. (A) Norepinephrine caused dose-dependent vasoconstriction, which was blocked by the α-adrenergic antagonist, prazosin ($P < 0.0001$). * $P < 0.05$ for difference in norepinephrine response (without prazosin) from baseline. (B) Epinephrine: vessels were preconstricted by 20%–30% with U46619. Epinephrine caused a biphasic response, with modest vasodilation at lower concentrations, and vasoconstriction at higher concentrations. Propranolol converted this response to a monophasic vasoconstriction ($P < 0.0001$ for comparison between control and propranolol groups). * $P < 0.05$ for difference in epinephrine response (without propranolol) compared with baseline. Symbols indicate mean ± sd. NE = norepinephrine; EPI = epinephrine.

Figure 2. Effect of epinephrine, $10^{-8}$ M, in response to norepinephrine. Epinephrine significantly attenuated the vasoconstriction seen with norepinephrine alone ($P < 0.0001$). Symbols indicate mean ± sd. NE = norepinephrine; EPI = epinephrine.

Figure 3. Washout experiments. Treatment of vessels with epinephrine $10^{-8}$ M and the norepinephrine $10^{-6.5}$ M (approximate EC$_{50}$), followed by washout with buffer containing only norepinephrine, caused significant vasoconstriction ($P < 0.05$). Treatment with the selective β$_2$-adrenergic agonist terbutaline restored vessels to a diameter indistinguishable from prewash values ($P = 0.96$). * $P < 0.05$ for diameter compared with prewash value. Propranolol blocked the response to both epinephrine and terbutaline ($P = 0.0051$). Bars are mean ± sd. NE = norepinephrine; EPI = epinephrine.

Series 3

Figure 3 shows the results of the washout experiments. Removal of EPI ($10^{-8}$ M) with continued presence of NE ($10^{-6.5}$ M) resulted in significant vasoconstriction. Addition of terbutaline ($10^{-7}$ M) restored vessels to a diameter indistinguishable from that in the presence of EPI before washout. Pretreatment with propranolol before the washout
experiment completely blocked the effect of both EPI and terbutaline.

DISCUSSION

The results demonstrate that EPI, in concentrations found in the plasma of laboring women, vasodilates uterine resistance vessels and attenuates NE-induced vasoconstriction. This effect seems to be mediated by β-adrenergic receptors, because the β-adrenergic antagonist propranolol blocked the vasodilating effects of low concentrations of EPI. In addition, the effect is mimicked by the selective β₂ agonist, terbutaline. NE seems to exert a predominantly vasoconstrictive effect via α₁-adrenergic receptors because the α₁-adrenergic antagonist, prazosin, blocks the response.

Maternal plasma catecholamines do not increase in normal pregnancy. However, in labor there is a progressive increase in both circulating EPI and NE.¹²,¹³ There is substantial individual variation, with some women experiencing 10-fold increases.¹ EPI levels increase from <50 pg/mL to over 300 pg/mL in some women, while noradrenaline increases from approximately 200 pg/mL to over 1100 pg/mL; these values equate to 10⁻⁸ to 10⁻⁹ M.¹⁴ Neuraxial analgesia consistently reduces circulating EPI, but not NE concentration.⁴⁻⁸ In theory, these catecholamine changes could result in relatively greater influence of NE on uterine microvasculature, and lead to vasoconstriction. Unopposed α-adrenergic tone, albeit in supraphysiologic doses, has been shown to reduce uterine blood flow in other animal models.⁴

Adverse effects of regional analgesia for labor are infrequent, but occasionally fetal heart rate changes accompany the onset of pain relief. This appears somewhat more common after intrathecal opioids administered as part of combined spinal-epidural analgesia for labor.¹⁵ Although the etiology of fetal bradycardia after neuraxial analgesia is unknown, and probably multifactorial, the rapid onset of analgesia and accompanying decrease in plasma EPI, leading to uterine vasoconstriction, may play a role. Other mechanisms may include maternal hypotension, uterine hypertonus, and rapid advancement of labor with compression of the fetal head.¹⁶ Nonetheless, if catecholamine imbalance, as modeled in the present study, is at least partly responsible, then it would be reasonable to consider treatment with a β-adrenergic agonist, such as ephedrine, when fetal bradycardia follows onset of analgesia.

Extrapolating from laboratory experiments to clinical practice should be done with extreme caution. There are several limitations of our study in this regard. First, the concentrations of catecholamines were modeled after measurements in plasma in women in labor. However, circulating catecholamines may not reflect the actual end-organ, resistance vessel, catecholamine concentration in vivo. This is particularly the case for NE, which is released in a paracrine fashion from adrenergic nerves near the vessel. Conversely, this fact may also make it reasonable to study higher concentrations of NE than those encountered in plasma, as was done in this study. The concentration of EPI studied in the combined catecholamine washout experiment, 10⁻⁸ M, represented the concentration exhibiting maximum vasodilation observed in our model. This concentration equals or exceeds the highest reported circulating levels in human parturients. Finally, we cannot ignore differences in rat and human myometrial vessel responses to catecholamines.

Second, this study used vessels in a no-flow state and used vessel diameter as a surrogate measure for effects of vasoconstriction on blood flow. In vivo, blood flow through uterine resistance vessels is the key physiologic variable that determines flow to the placenta and the fetus. Also, catecholamine-induced changes in other vascular beds in the intact animal or human might alter the effect on uterine blood flow observed in isolated uterine vessels. In vivo models in which actual placental flow is measured are necessary to verify that the vessel caliber changes seen in our study are physiologically relevant.

Third, our study used denervated vessels and blood-free perfusion. It is possible that vessels in intact animals are regulated by the autonomic nervous system in a way not modeled by the changes in catecholamines we studied. Other vasoactive substances, such as nitric oxide, thromboxane, and prostacyclin, affect uterine vascular tone and may modify the responses we observed. Furthermore, all drugs in our study were applied extraluminally, and in vivo catecholamines, particularly EPI, may reach the vessel within the lumen. However, similar models successfully mimic other in vivo responses of the uterine, coronary and other organs’ microcirculations to a wide variety of pharmacological stimuli, including nitric oxide, vasopressin, serotonin, acetylcholine, and prostaglandins.¹²,¹⁷–²⁰

Despite these limitations, we believe our results suggest that uterine vasoconstriction may be possible with changes in maternal catecholamines that accompany the onset of effective labor analgesia. Future studies should examine catecholamine changes in intact animal models and in laboring women, and correlate them with uterine microvascular blood flow, fetal heart rate changes and other indices of fetal well-being.

REFERENCES