Contribution of vasomotion to vascular resistance: a comparison of arteries from virgin and pregnant rats

ROBERT J. GRATTON, ROBIN E. GANDLEY, JOHN F. McCARTHY, WALTER K. MICHALUK, BRYAN K. SLINKER, AND MARGARET K. MCLAUGHLIN. Contribution of vasomotion to vascular resistance: a comparison of arteries from virgin and pregnant rats. J. Appl. Physiol. 85(6): 2255–2260, 1998.—Intrinsic oscillatory activity, or vasomotion, within the microcirculation has many potential functions, including modulation of vascular resistance. Alterations in oscillatory activity during pregnancy may contribute to the marked reduction in vascular resistance. The purpose of this study was 1) to mathematically model the oscillatory changes in vessel diameter and determine the effect on vascular resistance and 2) to characterize the vasomotion in resistance arteries of pregnant and nonpregnant (virgin) rats. Mesenteric arteries were isolated from Sprague-Dawley rats and studied in a pressurized arteriograph. Mathematical modeling demonstrated that the resistance in a vessel with vasomotion was greater than that in a static vessel with the same mean radius. During constriction with the α1-adrenergic agonist phenylephrine, the amplitude of oscillation was less in the arteries from pregnant rats. We conclude that vasomotor activity may provide a mechanism to regulate vascular resistance and blood flow independent of static changes in arterial diameter. During pregnancy the decrease in vasomotor activity in resistance arteries may contribute to the reduction in peripheral vascular resistance.

THE REDUCTION IN PERIPHERAL vascular resistance is one of the earliest physiological changes in pregnancy and is critical to pregnancy outcome. Previous in vitro studies of vascular behavior during pregnancy have demonstrated a decrease in the sensitivity to vasoconstrictors (4, 5, 14, 21, 29) and an increase in the relaxation response to endothelial-dependent and -independent agonists (13, 24). Both of these changes likely contribute to vascular relaxation and the reduced resistance characteristic of pregnancy. Another aspect of resistance vessel biology that has received little attention during pregnancy is the oscillatory behavior of these vessels.

Vasomotion refers to the spontaneous or induced oscillation of blood vessel diameter that occurs independent of cardiac or respiratory cycles, and the propagation of pulse pressure. Vasomotion has been observed in vivo for many years (2, 18, 30) and appears to be an inherent property of the vasculature (8). It has also been observed in isolated preparations from different vascular beds (1, 7, 8, 19, 20, 27). The cellular mechanisms that give rise to vasomotion are thought to involve fluctuations in membrane potential and intracellular calcium concentrations within the vascular smooth muscle cells (8–10).

The physiological significance of vasomotion within resistance vessels is unknown. However, Funk et al. (7) have suggested that vasomotor behavior in resistance vessels may reduce effective vascular resistance. Several observations, including alterations in vascular behavior during pregnancy, appear contradictory to this theory. First, from studies of isolated resistance arteries from the mesenteric circulation of rats, Meyer et al. (16) have reported that during pregnancy (a low-resistance state) vasomotion was reduced. Second, in preeclampsia (a hypertensive and high-resistance state in human pregnancy) isolated arteries exhibit spontaneous and “exaggerated” vasomotion (6, 23). In addition, vasomotion is increased in animal models of increased vascular resistance and hypertension (16, 17). Because augmented vasomotion is associated with high-resistance states, and conversely, reduced vasomotion with low-resistance conditions, we hypothesized that oscillation in vascular diameter actually increases effective vascular resistance and therefore a reduction in vasomotion during pregnancy may contribute to the decrease in vascular resistance. The purposes of this study were 1) to mathematically model the oscillatory changes in vessel diameter and to determine the effect on vascular resistance and 2) to characterize the vasomotion in resistance arteries from pregnant and nonpregnant rats.

METHODS

Animal model. Virgin Sprague-Dawley rats were housed and bred in the animal facility at Magee-Women’s Research Institute. Two or three cycling female rats were housed with a male for 24 h, and the presence of sperm in vaginal smears confirmed day 1 of pregnancy. Pregnant rats were studied at days 17–20 of gestation. Age-matched nonpregnant (virgin) rats were used as control animals. The animal protocol was approved by the Magee-Women’s Research Institute Institutional Animal Care and Use Committee.

Vessel preparation. Rats were euthanized after an intraperitoneal injection of methohexital sodium (50 mg/kg body wt). A section of mesentery 5–10 cm distal to the pylorus was rapidly removed and placed in ice-cold physiological saline solution. Resistance-sized mesenteric arteries (~250–300 μm relaxed diameter) were dissected from the surrounding tissue, transferred to an isobaric arteriograph, and mounted on glass microcannulas (Living Systems, Burlington, VT) in a buffer-filled chamber. The proximal microcannula was at-
attached to a solid-state pressure transducer and a servo-controlled peristaltic pump, which maintained the desired intraluminal pressure. The pressure transducer signal was received by a digital voltmeter with a readout precision of 0.1 mmHg. The distal microcannula was occluded to prevent flow.

The arteriograph was placed on an inverted microscope stage. A video camera attached to the microscope provided an image of the artery on a TV monitor. An electronic-dimension-analyzing system processed a selected vidicon line derived from the artery walls. Changes in signal voltage resulted from the line passing through the optically dense walls and less dense lumen. The voltages were converted to wall thickness and luminal diameter dimensions, available both as digital and analog readouts. Before each experiment, the signals were calibrated with a stage micrometer to be proportional to the absolute arterial dimensions in micrometers. The precision of the diameter measurements was within 1%, and the measurements were updated every 33 ms (16).

Solution and drugs. The bathing solution was a HEPES-buffered saline solution (pH 7.4 ± 0.5) that contained (in mmol/l) 142 sodium chloride, 4.7 potassium chloride, 1.17 magnesium sulfate, 1.56 calcium chloride, 1.18 potassium phosphate, 10 HEPES, and 5.5 glucose. Stock solutions of phenylephrine (L-phenylephrine hydrochloride, Sigma Chemical) were prepared in HEPES-buffered saline solution at a concentration of 10 mmol/l for each experiment. Appropriate dilutions of stock solutions were made with HEPES-buffered saline solution.

Experimental design. Vasomotor behavior was compared in single arteries isolated from late pregnant (n = 6) and control nonpregnant (n = 6) rats during constriction of the α1-adrenergic agonist phenylephrine. Arterial segments were equilibrated in HEPES-physiological saline solution at a temperature of 37°C and an intraluminal pressure of 60 mmHg for 1 h. Thirty minutes before phenylephrine constriction, a conditioning stretch was performed (intraluminal pressure increased from 60 to 100 mmHg slowly over 30 s, then returned to 60 mmHg). Vessels were exposed to cumulative concentrations of phenylephrine (1 × 10⁻⁶ to 1 × 10⁻⁴ M), and, after 5 min of equilibration at each dose, the internal diameter was measured. During the oscillating periods for each artery, the minimum arterial diameter and the oscillation amplitude and frequency were recorded continuously over 10 min on both a strip-chart and a video recorder for analysis.

Data analysis. The mean amplitude of oscillation, the initial radius, and the radius at maximal constriction (±SE) observed in arteries from pregnant and nonpregnant rats were compared by using an unpaired Student’s t-test. The frequency of oscillation was distributed nonparametrically and was compared by using a Mann-Whitney rank-sum test. The amplitude of vasomotion with increasing concentrations of phenylephrine was compared with a repeated-measures ANOVA. In the ANOVA, vasomotion amplitude was considered the dependent variable, and pregnancy status and phenylephrine dose were independent variables. The Bonferroni t-test was used to determine differences at specific concentrations. Multiple-regression analysis with an incremental F-test was used to determine whether there was a difference in the relationship between vasomotion amplitude and percent constriction. This analysis controlled for group differences in sensitivity to phenylephrine.

RESULTS

All arteries isolated from pregnant and nonpregnant rats exhibited continuous vasomotor activity when constricted with phenylephrine. In one vessel from a pregnant rat, this occurred only at maximal constriction. Spontaneous oscillation was not observed in any arteries. Figure 1 shows a strip-chart recording from a representative experiment. The oscillation shown is the regular, sinusoidal oscillation in diameter of arteries from a pregnant and nonpregnant rat after partial constriction with phenylephrine (1 × 10⁻⁶ M).

To analyze the oscillatory activity, an artery with vasomotion was considered to have a time-dependent oscillation in radius (Fig. 2). Because the oscillation pattern was sinusoidal, the radius at any point was described by the harmonic equation \( r(t) = r_0 + b \sin \theta(t) \). Parameters that we have used to characterize the vasomotion include 1) the mean radius \( r_0 \) and 2) the amplitude of oscillation \( b \). The magnitude of oscillation can also be normalized by dividing \( b \) by \( r_0 \) to obtain an amplitude that is independent of vessel size and represents the amplitude as a fraction of the mean radius. To mathematically model the effect of vasomotion on vascular resistance, and to compare the}
tor activity in this study, we have used this "normalized" amplitude $(b/r_0)$.

From Poiseuille's law, the vascular resistance in a static vessel of radius $r_0$ is proportional to $1/r_0^4$. If the vessel now oscillates about the radius $r_0$ with an amplitude $b$, the resistance as a function of time, represented as $R_{vm}(t)$, is proportional to $(1/(r_0 + b \sin \theta))^4$. To calculate the average resistance in an oscillating vessel, the integral of this equation was calculated (see APPENDIX). To compare the resistance in an oscillating vessel (i.e., $R_{vm}$) to that in a static vessel ($R_s$), $R_{vm}$ was expressed relative to $R_s$ in a vessel with the same mean radius ($R_{vm}/R_s$). Figure 3 shows this relative resistance and demonstrates that $R_{vm} > R_s$ in a vessel of the same mean radius. In addition, as the amplitude of oscillation increases, the resistance increases in an exponential fashion.

The second purpose of this study was to characterize the vasomotion in arteries from pregnant and nonpregnant rats. As illustrated in Fig. 1, the amplitude of oscillation appeared reduced in arteries isolated from pregnant rats. The mean amplitude of oscillation (±SE), which was the average of all observed oscillatory activity during all doses of phenylephrine, was significantly less in arteries from pregnant animals (pregnant $b/r_0 = 0.08 ± 0.04$, nonpregnant $b/r_0 = 0.29 ± 0.03$, $P = 0.02$). The frequency of oscillation was not statistically different between the two groups (pregnant 0.24 ± 0.02, nonpregnant 0.23 ± 0.01 Hz, $P = 0.37$) and did not change with increasing contraction.

The amplitude of vasomotion at increasing phenylephrine concentrations is shown in Fig. 4. In arteries from both groups, the amplitude of oscillation increased with increasing phenylephrine concentration, but the amplitude of oscillation was much less in arteries from pregnant animals compared with those from nonpregnant animals. Vasomotion was induced only at higher phenylephrine concentrations in vessels from pregnant animals. For a given adrenergic stimulation, the vasmotor response was blunted in arteries from pregnant animals. We then evaluated whether the group differences in amplitude of oscillation were a function of differences in the degree of constriction at given phenylephrine concentrations rather than in pregnancy status. The initial arterial radius after equilibration (pregnant $r = 140.8 ± 6.8$ (SE) µm, nonpregnant $r = 148.5 ± 5.5$ µm, $P = 0.40$) and at maximum constriction (pregnant $r = 62.8 ± 9.8$ µm, nonpregnant $r = 59.3 ± 9.8$ µm, $P = 0.80$) were similar in both groups. In these experiments there was no difference in the phenylephrine sensitivity between groups. Because previous studies in our laboratory using isometric measures have demonstrated a decreased sensitivity to phenylephrine in late gestation (5), we have expressed the vasomotion amplitude as a function of percent constriction (Fig. 5) to further control for this possibility. This graph demonstrates that the amplitude and onset of vasomotion were blunted in resistance vessels from pregnant animals even after controlling for any potential difference in phenylephrine sensitivity.

Although we recognize the limitations of extrapolating this in vitro functional assessment to in vivo hemodynamics, we attempted to translate the decrease in vasomotion into differences in effective vascular resistance. On the basis of the equation for resistance in a vessel with vasomotion (see APPENDIX), we calculated from the mean amplitude values the theoretical resistance contributed by the observed vasomotion in arteries from pregnant and nonpregnant rats as presented in Fig. 5. Figure 6 shows the calculated relative resistances due solely to the differences in vasomotor activity ($R_{vm}$ divided by $R_s$ in a vessel with the same mean radius) as a function of percent maximum constriction. This mean calculated resistance due to vasomotion was much less in arteries during pregnancy than in vessels from nonpregnant animals, with differences accentuated at higher phenylephrine concentrations.
compared with nonpregnant rats.

The pregnancy effect persisted when the amplitude of oscillation was evaluated as a function of the degree of constriction, further controlling for any potential small differences in phenylephrine sensitivity at individual doses. The decrease in vasomotion in isolated vessels from pregnant rats, suggest that vasomotion may be functionally important in determining effective vascular resistance. Because augmented vasomotion is associated with high-resistance states, and conversely, reduced vasomotion with low-resistance conditions, we hypothesized that oscillatory activity increased effective vascular resistance.

A previous report modeling vasomotion predicted that the vascular resistance in a vessel with vasomotion is decreased (7). To address this discrepancy, we have mathematically modeled vasomotion for a vessel with a time-dependent variation in radius and calculated the resistance in such a vessel. The results (see APPENDIX, Fig. 3) indicate that a vessel with vasomotion has an increase in effective vascular resistance compared with a static vessel with the same mean radius. In addition, as the amplitude of the vasomotion increases, the resistance increases. We suggest that the previous report (7) contains an improper formulation of the integral used to calculate the resistance in a vessel with vasomotion (see APPENDIX). We believe that oscillation in vessel diameter increases vascular resistance and may provide an additional mechanism beyond static changes in vascular diameter to regulate resistance. Our modeling for the time-averaged resistance agrees with that of a more recent study (22). However, this more extensive analysis of microvascular vasomotion also included a calculation of time-averaged conductance incorporated into the model as a parallel circuit. Increasing amplitude of sinusoidal vasomotion resulted in an increase in the time-averaged resistance and also a paradoxical increase in conductance.

In the present study, oscillatory changes were observed during the graded constriction with phenylephrine in all resistance-sized mesenteric arteries from pregnant and nonpregnant rats. The amplitude of oscillation was significantly decreased in isolated arteries from pregnant rats and occurred only at higher phenylephrine concentrations compared with arteries from nonpregnant rats. We have previously demonstrated, by using isometric measures, decreased sensitivity to phenylephrine in late pregnancy (5), but in these studies using the isobaric arteriograph there was no significant difference in sensitivity to phenylephrine between arteries from pregnant and nonpregnant rats. The pregnancy effect persisted when the amplitude of oscillation was evaluated as a function of the degree of constriction, further controlling for any potential small differences in phenylephrine sensitivity at individual doses. The decrease in vasomotor activity observed in
arteries isolated from pregnant rats is consistent with a previous report that studied similar vessels on a wire myograph system (16). Although we recognize the limitations of extrapolating this in vitro functional assessment to in vivo hemodynamics, the reduction in vasomotion in resistance arteries may, on the basis of our modeling, contribute to the decrease in vascular resistance during pregnancy.

The fundamental mechanism underlying vasomotion is thought to involve fluctuations in vascular smooth muscle membrane potential and in intracellular calcium concentrations (8–10). Studies by Gustafsson et al. (8–10) have suggested that the voltage-operated calcium channels (VOC), the sarcoplasmic reticulum calcium pool, the Na+/K+ pump, and the nitric oxide second messenger cGMP are essential components of the oscillating feedback mechanism. During pregnancy, the vascular smooth muscle is hyperpolarized (16), the open-state probability of the VOC is reduced, and the influx of calcium is likely to be decreased. Studies using the calcium-channel blocker nifedipine and the calcium-channel activator Bay K 8644 have confirmed that VOC function is altered during pregnancy (25, 28). These changes in membrane potential and in VOC function may contribute to the decrease in vasomotion during pregnancy. Conrad et al. (3) have reported evidence for an attenuation of myo-inositol uptake, phosphoinositol turnover, and inositol phosphate production in aortas of pregnant rats. If these alterations also occur in resistance vessels, mobilization of calcium from the sarcoplasmic reticulum may be impaired. Also, in rat pregnancy there is an increase in biosynthesis of nitric oxide and its second messenger, cGMP (15). The contribution of each of these potential mechanisms to the alteration in vasomotor activity observed during pregnancy requires further study.

In summary, we provide evidence from mathematical modeling that oscillatory behavior in resistance-sized vessels increases vascular resistance relative to a static vessel with the same mean radius. The observed reduction in vasomotion may, therefore, contribute to the decrease in vascular resistance during pregnancy. The precise mechanisms underlying vasomotion are unknown, but the fundamental cellular events must involve oscillation in vascular smooth muscle calcium concentrations. We speculate that the reduction in vasomotion observed during pregnancy may result from the hyperpolarization of vascular smooth muscle cells and a reduction in calcium flux across the vascular smooth muscle membrane. We intend to further investigate this phenomenon by studying vasomotion during pregnancy in response to other vasopressors. We also propose to study these differences in vasomotion in response to flow (in vitro flow-through arteriograph) and in intact in vitro preparations from pregnant and nonpregnant animals. Ultimately, this will lead to studies into the mechanism of the observed differences in vasomotion between arteries from pregnant and nonpregnant animals.

APPENDIX

Mathematical Modeling of the Effect of Vasomotion on Vascular Resistance

A vessel with vasomotion was considered to have a time-varying radius. The pattern of oscillation observed and analyzed on video and strip-chart recording was sinusoidal. The radius was expressed as a function of time, \( r(t) \), by using the harmonic equation \( r(t) = r_0 + b \sin \theta \), where \( r_0 \) is the mean radius, \( b \) is the amplitude of oscillation, and \( \theta \) is the angle of displacement as a function of time. Resistance in a static vessel \( (R_s) \) is calculated by the equation \( 8\pi r_0^4/\pi n^4 \), where \( l \) is length and \( n \) is viscosity. Assuming length and viscosity are constant, resistance \( (R) \) is proportional to \( 1/R^4 \). Resistance in a vessel with a time-varying radius, \( R_{vm}(t) \), is proportional to \( 1/(r_0 + \sin \theta)^4 \). The average resistance in a vessel with vasomotion \( (R_{vm,ave}) \) is calculated as \( 1/2\pi \int 1/(r_0 + \sin \theta)^4 d(\theta) \). The integral was calculated over one oscillation (0–2\( \pi \)), removing frequency as a variable in the modeling. With the use of the computer program Mathematica (Wolfram Research) to perform symbolic computation, this integral computes to \( r_0 (3b^2 + 2r_0^2)/2(2b^2 + r_0^2)^2/2 \). To calculate the resistance relative to that in a static vessel of the same mean radius \( (R_{vm}/R_s) \), we divided by \( 1/r_0^4 \) to obtain \( r_0^3(3b^2 + 2r_0^2)/2(2b^2 + r_0^2)^2/2 \). Vasomotion amplitude as a fractional increase in the mean radius \( (a = b/r_0) \) allows determination of resistance in a vessel with vasomotion, independent of vessel size. The equation for relative resistance in a vessel with vasomotion, where the amplitude is expressed as a fraction of the mean radius is, \( (3a^2 + 2)/(2(1 - a^2))^{0.5} \). Figure 3 is a graphical representation of this relationship.

We have reviewed the modeling of vasomotion in a previously published study (7). Difference in our modeling is primarily the formulation of an integral to calculate the average resistance in a vessel with vasomotion. Following from \( R_{vm}(t) \alpha 1/(r_0 + \sin \theta)^4 \), the correct integral (constants removed) is \( \int 1/(r_0 + \sin \theta)^4 d(\theta) \). The formulation error in modeling by Funk et al. (7) was analogous to \( 1\int (r_0 + \sin \theta)^4 d(\theta) \) and led to the conclusion that vasomotion results in a decrease in effective vascular resistance.

Address for reprint requests: M. K. McLaughlin, Magee-Women’s Research Institute, Dept. of Obstetrics, Gynecology and Reproductive Sciences, Cell Biology and Physiology, 204 Craft St., Pittsburgh, PA 15213.

Received 16 September 1997; accepted in final form 11 August 1998.

REFERENCES