Published in final edited form as:
Reprod Sci. 2009 November ; 16(11): 1072–1081. doi:10.1177/1933719109340927.

Local Uteroplacental Influences are Responsible for the Induction of Uterine Artery Myogenic Tone during Rat Pregnancy

Natalia I. Gokina, PhD, **Olga Y. Kuzina, MA**, **Robert Fuller, MD, PhD**, and **George Osol, PhD**
Department of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, University of Vermont, Burlington, VT 05405

Abstract

Uterine artery constrictor responses to elevation of intraluminal pressure (myogenic tone) are considerably enhanced in late pregnant rats, although the underlying causes remain unknown. A single uterine horn ligation model was used to differentiate local from systemic influences, and to test the hypothesis that that factors associated with the site of placentation, rather than systemic hormonal changes, are primarily involved in the induction of this adaptive process. Radial uterine arteries were dissected from the gravid and non-gravid uterine horns of late pregnant rats, cannulated and pressurized. Changes in arterial diameter and smooth muscle $[Ca^{2+}]_i$ in response to the elevation of intraluminal pressure were studied using intact and endothelium-denuded arteries loaded with the ratiometric Ca^{2+}-sensitive dye fura-2. Elevations of pressure from 10 to 60 and 100 mm Hg resulted in passive arterial distention of arteries from non-gravid horns with a minor change in $[Ca^{2+}]_i$. In contrast, arteries from gravid horns developed myogenic tone associated with a significant elevation in $[Ca^{2+}]_i$. Synchronous oscillations in $[Ca^{2+}]_i$ and lumen diameter were frequently observed in vessels from gravid horns. Endothelial denudation augmented tone in the gravid horn but did not uncover myogenic tone in vessels from the non-gravid horn. In summary, pregnancy-associated uterine artery myogenic behavior is due to an upregulation of calcium-handling mechanisms, occurs independently of the endothelium, and is induced by local uteroplacental influences.

Keywords

one-horn pregnant rat; smooth muscle Ca^{2+}; fura-2

INTRODUCTION

Normal human and mammalian pregnancy is a state of a marked cardiovascular adaptation that includes changes in blood pressure, heart rate, stroke volume, and vascular reactivity. Perhaps the most dramatic changes occur in the uterine circulation, and are manifested by vessel growth and remodeling, as well as significant changes in vasomotor function.[1–11]

Vasoconstriction, in response to an elevation in intraluminal pressure, is a fundamental property of resistance blood vessels and is termed myogenic tone. This response to pressure or stretch is intrinsic to vascular smooth muscle cells (SMCs) and a well-established physiological phenomenon.[12–16] Pressure-induced tone contributes to vascular regional resistance of small arteries and arterioles and serves to protect capillaries from excessive blood pressure.[13] Elevation of SMC cytoplasmic $[Ca^{2+}]_i$ (due to Ca^{2+} influx into cells and Ca^{2+}

Address for correspondence: Natalia I. Gokina, PhD, Department of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, University of Vermont, 89 Beaumont Avenue, Given Building, C-213A, Burlington, VT 05405, Phone: (802) 656-1205, Fax: (802) 656-8771, Natalia.Gokina@uvm.edu.

release from internal stores) as well as Ca^{2+} sensitization of the contractile process are two major mechanisms of pressure-induced vasoconstriction.[14–21]

In late gestation, pressure-induced tone of vessels from mesenteric and renal circulations of rodents is significantly reduced, most often due to augmented endothelial vasodilatory influences.[10, 22, 23] Pressure-induced vasoconstriction has been described in uterine arteries from pregnant animals, and it is most evident in smaller radial arteries.[6, 9, 10, 21, 24–26] Myometrial arteries from pregnant women can also develop myogenic tone in response to pressure elevation[27]. Studies from several different laboratories, including our own, demonstrated that myogenic tone of small uterine arteries of the rat is significantly enhanced in late gestation.[6, 21, 26] Although we recently reported diminished activity of SMC delayed rectifier potassium channels (which results in enhanced depolarization) to be an underlying mechanism for enhanced tone during gestation, the factor or factors that induce this adaptation are not known.[21]

Pregnancy is also associated with significant expansive growth of uterine vessels, and our recent study demonstrated unequivocally that local (uteroplacental) rather than systemic (endocrine) influences play the dominant role in the structural remodeling of both large and small uterine arteries.[3, 11, 28, 29] In this study, we hypothesized that changes in arterial structure (remodeling) and function (reactivity) may be linked, such that local uteroplacental factors associated with vessel enlargement would also induce phenotypic changes in the cells within the vascular wall, e.g. myogenic tone and reactivity in SMCs. This may be due to direct (altered cellular handling of calcium) or indirect (increased wall tension due to an increase in lumen diameter leading to depolarization) effects of remodeling on the cells of the vascular wall. The alternative hypothesis is that changes in myogenic behavior result from the altered humoral (endocrine) milieu of pregnancy and would therefore not be expected to be site-specific.

The objective of this study was to differentiate between systemic and local influences in effecting myogenic tone by using a surgical ligation model in which implantation is restricted to one of two rat uterine horns. If differences were apparent (and they were), secondary objectives were to: (1) determine whether augmented myogenic tone is due to increased elevation in smooth muscle $[Ca^{2+}]_i$; and (2) define the role of the endothelium in the differential myogenic behavior of uterine arteries from the nongravid versus gravid uterine horn.

Our data demonstrate that late pregnancy is associated with a striking enhancement of uterine artery myogenic reactivity that is due to local uteroplacental influences, occurs independently of the endothelium, and can be attributed to an upregulation of SMC calcium handling mechanisms that lead to oscillatory elevations in SMC cytosolic $[Ca^{2+}]_i$.

METHODS

All experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85–23, Revised 1996), and the experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Animals and Preparation of Arteries

In this study, we utilized a surgical ligation model in which the implantation was restricted to one of two rat uterine horns.[28] Fifteen virgin female rats had one-sided uterine horn ligation surgery, accomplished by tying off the oviduct with non-resorbable suture at 8 weeks of age by Charles River Laboratories. Animals were received at our facility at 9 weeks of age with staples approximating a cranial-caudal incision in the right flank. Staples were removed 7 days post-op and the rats were acclimated for at least one week before breeding.

Surgically ligated rats were bred overnight in isolated pairs using metabolic cages at the University of Vermont Small Animal Facility. If a seminal plug was observed on the following morning, that day was designated day 1 of pregnancy. Pregnancy was confirmed by veterinary technicians by observation and/or palpation on days 11–16. Post-mortem evaluation confirmed an empty right uterine horn and a silk ligature in the upper portion of the oviduct.

Single-horn late pregnant rats were used on day 20/22 of pregnancy at 13 to17 weeks of age. They were anesthetized by an intraperitoneal injection of Nembutal (50mg/kg) and killed by decapitation. The abdominal wall was transected and the entire uterus and uterine vasculature were rapidly removed and pinned in a dissecting dish filled with aerated cold physiological salt solution (PSS: see *Solutions and drugs* for composition).

Second-order uterine radial arteries were identified within the mesometrial arcade and dissected from the nongravid and gravid horns. Only uteroplacental arteries (radial arteries feeding the placenta) were dissected from the vasculature of pregnant uteri. Arterial segments were cannulated from both ends in the arteriograph and continuously superfused at 3 ml/min with aerated (10% O_2, 5% CO_2, and 85% N_2) PSS at 37°C. Initial intraluminal pressure was set at 10 mm Hg using a servo-null pressure system (Living Systems Instrumentation, Burlington, VT). All experiments were performed under no intraluminal flow conditions.

In a separate set of experiments, the endothelium was removed by infusing air into one end of a cannulated vessel for 5 to 6 minutes, followed by gentle and brief (5 seconds) perfusion with regular PSS before pressurization of the artery. The effectiveness of this denudation procedure was confirmed by the preservation of normal constrictor reactivity to phenylephrine, combined with the absence of a dilatory response to acetylcholine.

Measurement of Smooth Muscle Cell $[Ca^{2+}]_i$ in Pressurized Arteries

After an equilibration period of 20 min at 37°C at 10 mm Hg and measurement of background fluorescence, SMCs within the wall of arteries pressurized at 10 mm Hg were selectively loaded with 5 μmol/L fura-2. An arterial segment was incubated extraluminally in fura-2 AM loading solution at room temperature in the dark for 60 min under no-perfusion conditions. Extraluminal incubation of pressurized arteries with fura-2 AM solution does not result in the loading of endothelial cells with fura 2 most likely due to the presence of elastic lamina in the wall of uterine arteries preventing the diffusion of fura 2 from smooth muscle to the endothelial layer. Fura-2-loaded arteries were then continuously superfused at 3 mL/minute with aerated PSS at 37°C. Ratiometric measurements of fura-2 fluorescence were performed using a photomultiplier system (IonOptix Inc, Milton, MA). Background-corrected ratios of 510 nm emission were obtained at a sampling rate of 5 Hz from arteries alternately excited at 340 and 380 nm. Lumen diameter was simultaneously monitored using the SoftEdge Acquisition Subsystem (IonOptix). All experimental protocols were started following an additional 15 to 20 minutes equilibration period at 10 mm Hg to allow intracellular de-esterification of fura-2 AM.

After the equilibration period, corresponding levels of $[Ca^{2+}]_i$ and diameters were recorded for 5 minutes at 10 mm Hg, and intraluminal pressure was then elevated to 60 and 100 mm Hg. Changes in arterial diameter and levels of SMC $[Ca^{2+}]_i$ were monitored until a stabilization of myogenic constriction occurred (typically 10 minutes for each level of pressure). Papaverine (100 μmol/L) and diltiazem (10 μmol/L) were applied at the end of each experiment, and the arterial diameter was recorded at 10, 60, and 100 mm Hg from the maximally dilated artery. The degree of myogenic tone at any given level of intraluminal pressure was expressed as the percentage reduction from a fully relaxed diameter.

Solutions and Drugs

The physiological salt solution (PSS) contained: 119 mmol/L NaCl, 4.7 mmol/L KCl, 24.0 mmol/L $NaHCO_3$, 1.2 mmol/L KH_2PO_4, 1.6 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 0.023 mmol/L EDTA, and 11.0 mmol/L glucose, pH = 7.4. For the fura-2 calibration procedure, we used a solution of the following composition: 140 mmol/L KCl, 20 mmol/L NaCl, 5 mmol/L HEPES, 5 mmol/L EGTA, 1 mmol/L $MgCl_2$, 5 μmol/L nigericin and 10 μmol/L ionomycin, pH = 7.1.

All chemicals were purchased from Sigma Chemical Co (St. Louis, MO) with the exception of ionomycin and nigericin, which were obtained from Calbiochem (La Jolla, CA). Fura-2 AM and pluronic acid were purchased from Invitrogen (Carlsbad, CA). Fura-2 AM was dissolved in dehydrated DMSO as a 1 mmol/L stock solution, frozen in small aliquots and used within one week of preparation. Papaverine was dissolved in deionized water and used the same day only. Diltiazem was prepared as a 10 mmol/L stock solution in deionized water and kept refrigerated until use (1–2 weeks). Ionomycin and nigericin were prepared as 10 mmol/L stock solutions in methanol and kept at −20°C until use.

Calculations and Statistical Analysis

SMC $[Ca^{2+}]_i$ was calculated using the following equation[30]: $[Ca^{2+}]_i = K_d\beta\ (R - R_{min})/(R_{max} - R)$, where R is the experimentally measured ratio (340/380 nm) of fluorescence intensities, R_{min} is a ratio in the absence of $[Ca^{2+}]_i$ and R_{max} is a ratio at Ca^{2+}-saturated fura 2 conditions, β is a ratio of the fluorescence intensities at 380 nm excitation wavelength at R_{min} and R_{max}. R_{min}, R_{max} and β were determined by an in situ calibration procedure from the arteries treated with nigericin (5 μmol/L) and ionomycin (10 μmol/L). Calibration was performed on a group of vessels loaded extraluminally with fura-2 (n = 4). These values were then pooled and used to convert the ratio values into a $[Ca^{2+}]_i$. The K_d (the dissociation constant for fura-2) was 282 nM, as determined by using *in situ* titration of Ca^{2+} in fura-2 loaded small arteries.[31]

Arterial diameter, pressure and ratio values were simultaneously recorded using an IonOptix data acquisition program and imported into Sigma Plot and Sigma Stat programs for graphical representation, calculations, and statistical analysis.

Data are expressed as means ± SEM, where each *n* = number of arterial segments studied. Only one artery was used from nongravid or gravid uteri of a late pregnant rat. A paired or unpaired Student's *t*-test or two way repeated measures analysis of variance (ANOVA) were used to determine the significance of differences between sets of data, with $P < .05$ considered significant.

RESULTS

Effects of Gestation and Surgical Restriction of Placentation on Vessel Dimensions, and on the Development of Myogenic Tone in Uterine Radial Arteries

In single-horn pregnant rats, late pregnancy was associated with significant expansive remodeling of the uterine vasculature in the gravid versus nongravid horn, as described in an earlier study,[28] and shown in the photograph in Figure 1A. The passive diameters of cannulated radial arteries (measured at 60 mm Hg in the presence of 10 μmol/L diltiazem and 100 μmol/L papaverine) were 77% larger in gravid (223 ± 14 μm, n = 12) versus nongravid horns (126 ± 6 μm, n = 13, Figure 1B).

A stepwise elevation of intraluminal pressure from 10 to 60, and from 60 to 100 mm Hg resulted in very minor elevations in SMC $[Ca^{2+}]_i$ and little or no vasoconstriction of uterine radial arteries from nongravid horns (Figure 2A). On average, the rise of SMC $[Ca^{2+}]_i$ above the

basal $[Ca^{2+}]_i$ levels was 11 ± 4 nmol/L and 19 ± 7 nmol/L at 60 and 100 mm Hg, respectively (n = 8, Figure 4).

At the same time, these vessels demonstrated a significant elevation in SMC $[Ca^{2+}]_i$ (by 200 ± 32 nmol/L) and constriction (to 61 ± 4% of their fully relaxed diameters at 60 mm Hg) in response to phenylephrine. Subsequent application of acetylcholine resulted in a significant reduction in SMC $[Ca^{2+}]_i$ (by 150 ± 31 nmol/L) to nearly basal levels that were associated with an almost complete vasodilation of 92 ± 4 % (n = 6), as illustrated for one vessel in Figure 2B.

In contrast to vessels from nongravid horns, uteroplacental radial arteries from gravid horns constricted robustly to elevations in pressure (19 ± 3 % and 23 ± 3 % at 60 and 100 mm Hg, respectively). The development of myogenic tone was preceded by a significant increase in SMC $[Ca^{2+}]_i$ consisting of a slow $[Ca^{2+}]_i$ rise with superimposed fast calcium oscillations that were associated with rhythmic vasoconstrictions (Figure 3A). The averaged increases in cytosolic $[Ca^{2+}]_i$ above the basal levels were 151 ± 31 nmol/L and 236 ± 42 nmol/L at 60 and 100 mm Hg, respectively (Figure 4). Application of ACh resulted in full dilatation, demonstrating endothelial integrity of the artery (Figure 3B).

Effect of Arterial Denudation on Myogenic Tone, and its Dependence on Extracellular Calcium

Pregnancy is a state of augmented basal production of endothelium-derived dilator factors like nitric oxide (NO) and prostacyclin.[2, 4, 5] Therefore, a potential increase in basal vasodilatory effects from the uterine artery endothelium might prevent the development of myogenic tone in arteries from the non-gravid uterine horn. To test this suggestion, we studied pressure-induced responses in uterine arteries after endothelial denudation. The effectiveness of endothelial removal was confirmed by an abolition of dilator responses to ACh.

Denuded arteries from nonpregnant horns showed minimal changes in SMC $[Ca^{2+}]_i$ in response to pressure elevations from 10 to 60 mm Hg (5 ± 3 nmol/L), and then to 100 mm Hg (14 ± 12 nmol/L, n = 5; Figure 5A), and developed little or no myogenic tone (2 ± 1 and 6 ± 2 % at 60 and 100 mm Hg, respectively; n = 10, Figure 5). Myogenic tone was well maintained in denuded arteries from gravid horns, averaging 28 ± 7 % and 41 ± 7 % at 60 and 100 mm Hg, respectively (n = 5). These constrictor responses were associated with significant increases in SMC $[Ca^{2+}]_i$ above basal levels of 160 ± 40 and 236 ± 35 nmol/L at 60 and 100 mm Hg, respectively (n = 5, Figure 5). Denudation was associated with a tendency toward increased myogenic tone that reached statistical significance at 100 mm Hg. The frequency of Ca^{2+} oscillations was also higher in denuded vs. intact vessels (45 ± 6 osc/min versus 30 ± 3 osc/min at 100 mm Hg, n = 5 and n = 7 respectively; $P < .05$), although the average increment in SMC $[Ca^{2+}]_i$ was not significantly different.

In arteries from gravid horns, both pressure-induced $[Ca^{2+}]_i$ oscillatory activity and sustained $[Ca^{2+}]_i$ elevations were effectively abolished by diltiazem, an inhibitor of L-type Ca^{2+} channels (Figure 6A and B); treatment with diltiazem also abolished the myogenic tone (Figure 6C), confirming its dependence on the influx of extracellular calcium, as reported in other vessel types.[13, 21, 31]

DISCUSSION

This study, using a rat model, clearly demonstrates that the development of myogenic behavior in uterine arteries from pregnant animals is the result of local rather than systemic influences. These have not been identified, and could be direct (paracrine actions of uteroplacental signals, for example), indirect (e.g., increased wall tension within the vascular wall secondary to expansive remodeling), or a combination thereof. The paracrine molecular signaling concept

brings up the question of route of delivery – that is, how do secreted signals affect the behavior of arteries that are remote from, and upstream of their location? Venoarterial exchange is one possibility. Its existence in vivo has been documented in the utero-ovarian circulation as a mechanism of luteolysis in a number of species, and molecules secreted from the placenta, or from the paraplacental and myometrial tissues would be present in highest concentrations in the venous outflow. However, the importance of this mechanism has not been established in the setting of gestational remodeling.[11]

More likely, placentation and vasodilation lead to increased flow and expansive remodeling secondary to elevated shear stress in maternal upstream arteries such as the ones used for this study. The state of the cells within the remodeled wall is not known, but the pattern of gestational uterine vascular remodeling is generally one of increased diameter with an unchanged wall thickness.[11] As noted in results, the passive lumen diameters of radial arteries taken from parallel anatomical locations in either horn were significantly (>75%) wider on the pregnant side. By the law of LaPlace (tension = pressure × radius), wall tension and stress would both increase, as might intravascular pressure due to a reduction in upstream resistance. The physical force of increased circumferential stretch would result in vascular SMC depolarization and calcium entry.[14, 17, 19, 31, 32] In support of this concept, we recently found that vessels from pregnant animals treated with an inhibitor of endothelial nitric oxide synthase (eNOS) do not remodel and do not exhibit myogenic tone (unpublished observation).

Pregnancy is normally associated with some reduction in systemic blood pressure, therefore, the actual change in wall tension and stress would be dependent upon both factors (intravascular pressure and radius). The development of tone would result in a smaller lumen and a thicker wall, and thus likely reduce wall tension, as well as wall, media and cell stress. In a previous study, we found that different levels of tone in individual vessels within the cerebral circulation could be related to the normalization of media stress.[33] Although a similar mechanism may operate in the uterine circulation, there are no published data to support or refute this concept, and it is therefore purely hypothetical.

Like vessels from nonpregnant animals, similar radial arteries taken from nonpregnant horns did not constrict in response to pressure elevation. The functional integrity of these vessels was confirmed by normal reactivity to phenylephrine and acetylcholine, associated with expected changes in SMC $[Ca^{2+}]_i$ (Figure 2B). Because pregnancy is a state of increased endothelial vasodilatory influence, increased production of relaxing factors by endothelial cells in the non-gravid horn due to systemic hormonal influences could have been responsible for the absence of myogenic tone in these vessels. The fact that endothelial denudation did not potentiate their pressure-induced responses (Figure 6), effectively eliminates this possibility, and again suggests that changes in physical forces, or vascular SMC phenotype secondary to remodeling are responsible.

Endothelial denudation of arteries from pregnant horns resulted in a significant enhancement of myogenic tone in response to pressure elevation to 100 mm Hg. Although removal of endothelium negates both its inhibitory and excitatory effects on underlying SMCs, the inhibitory influence prevails in late pregnancy and moderates myogenic tone of uteroplacental arteries due to release of vasodilator factors. Nitric oxide may be a contributing factor as in our previous study myogenic tone of uterine arteries from late pregnant rats was significantly enhanced after blockade of NO production with N-nitro-arginine (L-NNA).[24] The averaged $[Ca^{2+}]_i$ increment associated with myogenic tone was not significantly different between intact and denuded vessels. These data suggest that tonic release of endothelium-derived vasodilator (s) may inhibit the Ca^{2+} sensitivity of the contractile process and in this way affect the myogenic tone of uterine arteries in late pregnancy. In addition, an increase in frequency of $[Ca^{2+}]_i$

oscillations without change in average $[Ca^{2+}]_i$ levels (such as seen in this study) may also contribute to enhanced myogenic tone after arterial denudation.

The results also indicate that the striking difference in myogenic behavior of uterine arteries from gravid versus nongravid horns is due to an augmented SMC $[Ca^{2+}]_i$ response. The typical pattern of pressure-induced $[Ca^{2+}]_i$ responses in myogenic vessels consisted of Ca^{2+} oscillations superimposed upon a sustained elevation in $[Ca^{2+}]_i$, and vasoconstriction. Both pressure-induced calcium activity and myogenic tone were abolished by diltiazem, implicating Ca^{2+} influx through L-type Ca^{2+} channels as the primary pathway for Ca^{2+} entry being responsible for the development of myogenic tone. Therefore, the mechanisms underlying enhanced myogenic tone in uterine arteries on the site of placentation are similar to those determined in our previous study for uterine arteries from regular late pregnant rats.[21]

Earlier, we demonstrated that late pregnancy is associated with a decreased function of voltage-gated potassium channels (K_v) in smooth muscle cells from uteroplacental arteries, which results in enhanced pressure-induced depolarization, calcium entry and SMC excitability.[21] Decreased K_v channel function not only underlies a pregnancy-enhanced myogenic tone but also predicts an increase in constrictor reactivity of uterine arteries to depolarizing agonists. Indeed, previous studies have demonstrated that uterine arteries from pregnant rats became more responsive to adrenergic stimulation.[7]

The remarkable growth of the uterine vasculature in late pregnancy is well documented and is due to both hypertrophy and hyperplasia of smooth muscle cells. [29, 34, 35] It is well known that cellular growth is associated with augmented Ca^{2+} cell signaling that in part is due to depolarization of SMCs through mechanisms linked to a suppression of K_v channel expression and function.[36–39] Other mechanisms - such as enhanced Ca^{2+} entry secondary to upregulation of TRP channel expression - may also be involved.[40]

The present study confirmed previously published findings that in late pregnancy uterine arteries supplying the hemochorial placenta of rodents can develop a sustained myogenic tone in response to pressure elevation.[6, 21, 24, 26, 27, 41] It is known that the maternal compartment of the hemochorial placenta operates under relatively low levels of pressure (10 – 20 mm Hg) as is necessary for avoiding compression of fetal villi and maintaining an adequate maternal-fetal exchange.[3] The development of myogenic tone may therefore contribute to an increase in uterine vascular resistance and serve as an adaptive protective mechanism that maintains normal pressure in the intraplacental compartment.

A significant enhancement of uterine artery myogenic tone and adrenergic sensitivity in late pregnancy might also be essential for minimizing blood loss during parturition. Vessels used in this study were taken from 20 day pregnant rats, that is approximately 48 hours prior to term. Although vascular adaptations associated with the last stage of pregnancy have not been characterized to any extent, the induction of myogenic tone and heightened adrenergic sensitivity could both act to limit uteroplacental blood flow and possible hemorrhage during parturition.

In summary, late pregnancy is associated with a striking enhancement of uterine artery reactivity to intraluminal pressure due to an up-regulation of cellular mechanisms that lead to an oscillating elevation in SMC cytosolic $[Ca^{2+}]_i$. These adaptive changes in myogenic behavior are governed by local uteroplacental influences, may be linked to the process of structural remodeling, and are induced by the modulation of intrinsic calcium handling properties of vascular smooth muscle cells independently of endothelial effects.

Acknowledgments

This work was supported by NIH HL073895 and HL079253 to George Osol, and HL067250 and HL088245 to Natalia Gokina.

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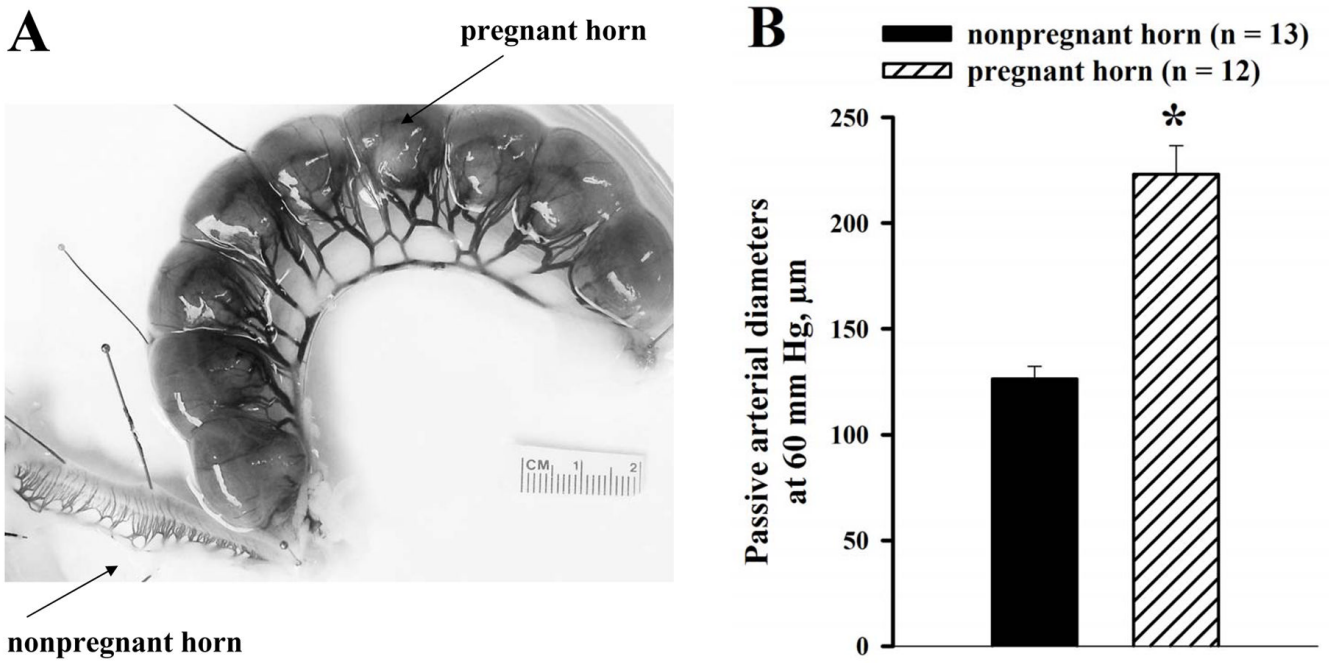

Figure 1.

A, Photograph showing the uterus of a late pregnant (20 day) rat that underwent surgical ligation of one uterine horn several weeks prior to breeding. Most of the picture is taken up by the pregnant horn that contained 9 pups. The nonpregnant horn can be seen in the lower left corner, along with the mesometrial arcade containing arteries and veins that perfuse the uterine corpus. Note difference in the size of the vasculature due to the predominance of local influences on remodeling, as previously described.[28] B, Summary graph demonstrating a significant difference in the passive diameters of uterine radial arteries from nonpregnant versus pregnant horns of late pregnant rats. All vessels were pressurized to 60 mm Hg and maximally dilated with 10 μmol/L diltiazem and 100 μmol/L papaverine. * Significant difference at $P < .05$ (unpaired Student's t-test); numbers in parentheses indicate the number of arteries tested.

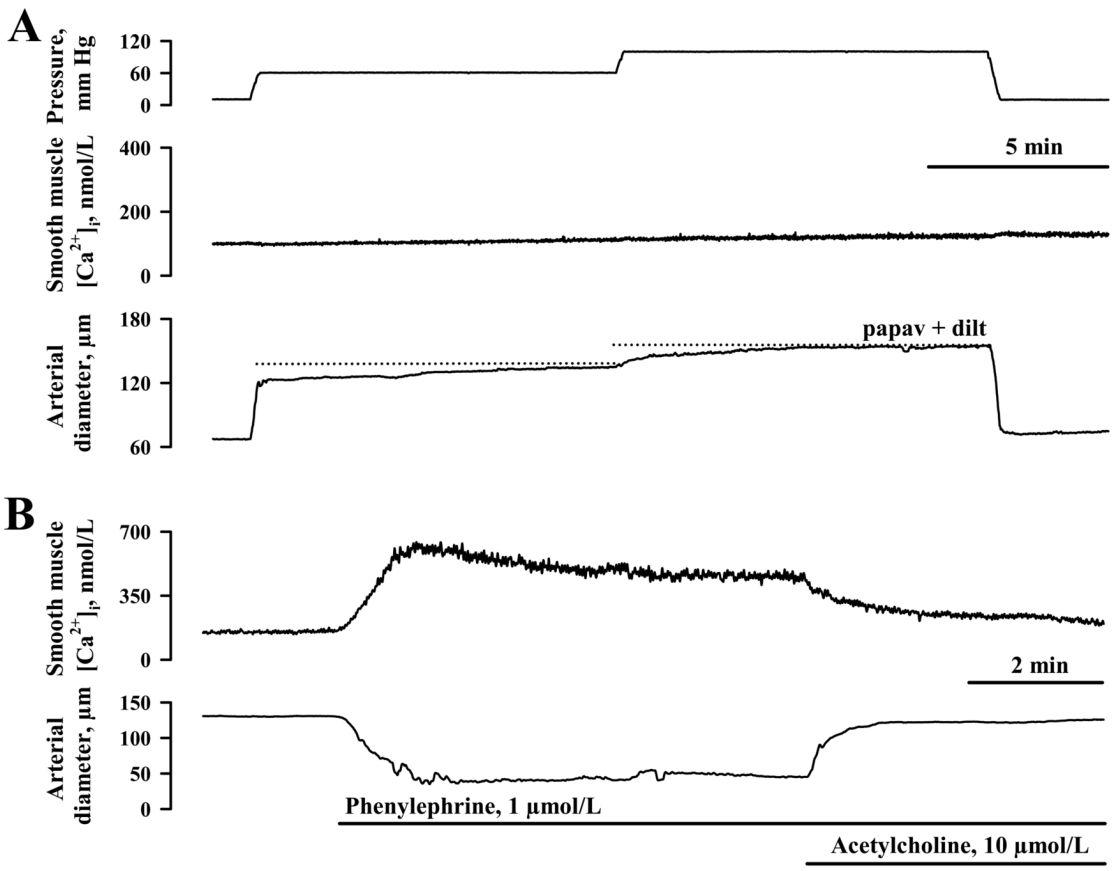

Figure 2.

A, Representative tracings showing the effects of stepwise elevation in intraluminal pressure from 10 to 60 and then to 100 mm Hg on smooth muscle cell (SMC) $[Ca^{2+}]_i$ and the diameter of a uterine radial artery from the non-pregnant horn of a late pregnant rat. The dotted lines indicate the maximal diameter of the same artery in the presence of 100 µmol/L papaverine and 10 µmol/L diltiazem. B, Smooth muscle and endothelial integrity of the same artery is evidenced by the presence of constrictor and dilator responses to phenylephrine and ACh, respectively, along with changes in SMC $[Ca^{2+}]_i$.

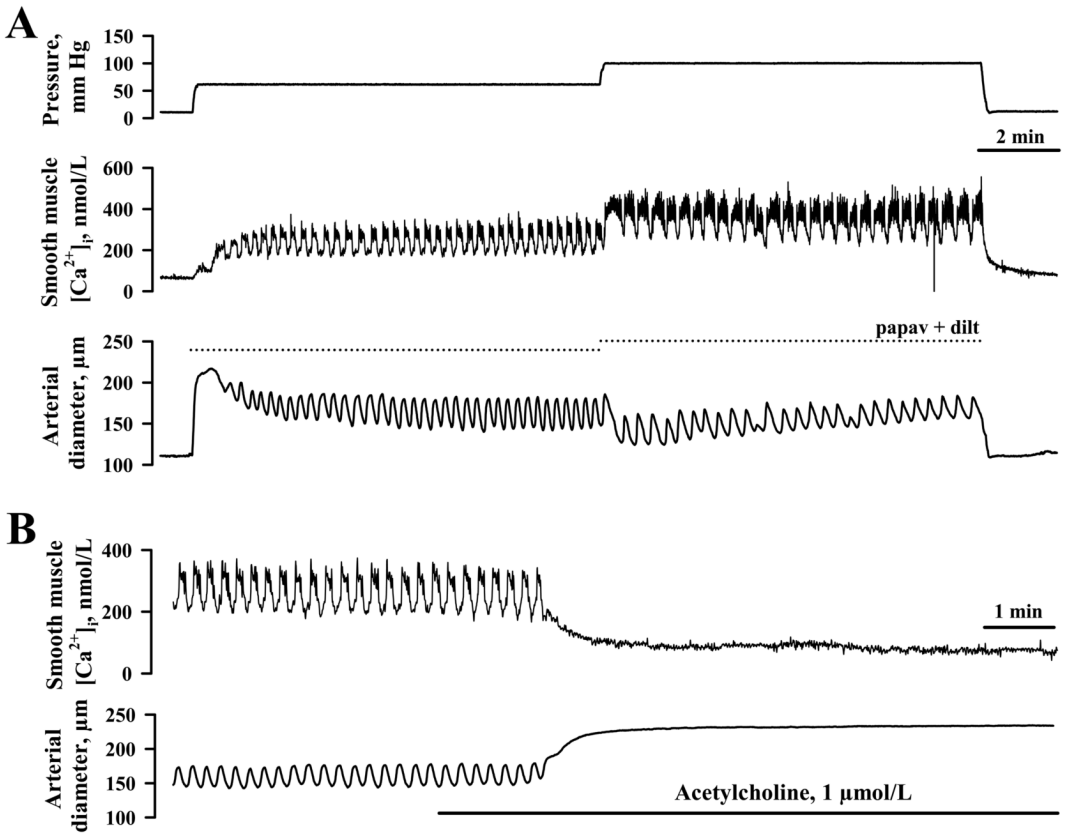

Figure 3.

A, Representative tracings demonstrating the significant elevation in smooth muscle $[Ca^{2+}]_i$ and constriction of a uteroplacental radial artery from the pregnant horn of a late pregnant rat in response to stepwise elevation of intraluminal pressure from 10 to 60 and then to 100 mm Hg. The dotted lines indicate the maximal diameter of the same artery in the presence of 100 μmol/L papaverine and 10 μmol/L diltiazem. B, The endothelial integrity of the artery pressurized to 60 mmHg is confirmed by a marked decrease in smooth muscle $[Ca^{2+}]_i$ and dilation in response to the application of 1 μmol/L ACh.

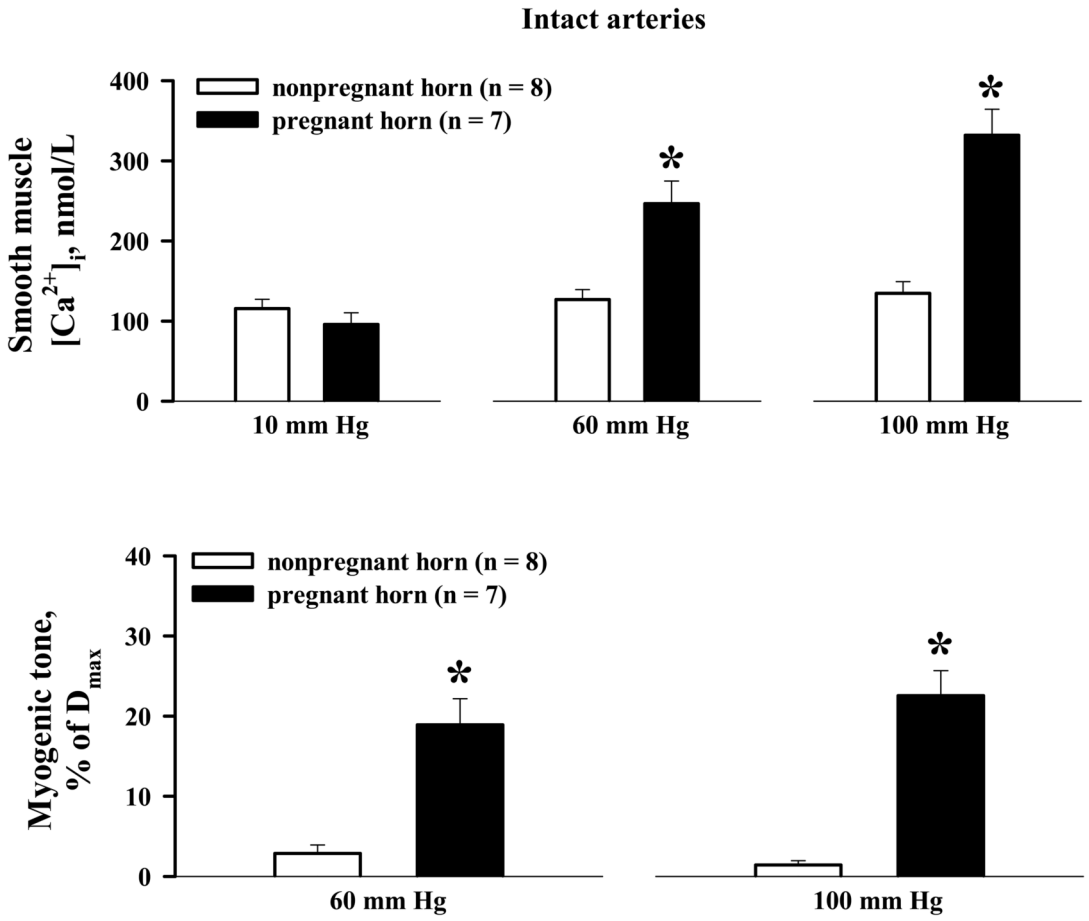

Figure 4.

Summary graphs showing significant differences in pressure-induced SMC $[Ca^{2+}]_i$ responses and associated myogenic tone of uterine arteries from nonpregnant versus pregnant horns of late pregnant rats. No difference was found in the basal levels of SMC $[Ca^{2+}]_i$ in arteries from nonpregnant versus pregnant horns at 10 mm Hg. Myogenic tone is expressed as a percentage of the diameter measured in the presence of 100 μmol/L papaverine and 10 μmol/L diltiazem (D_{max}). *Significantly different at $P < .05$ (two-way repeated measures ANOVA); numbers in parentheses indicate the number of arteries tested.

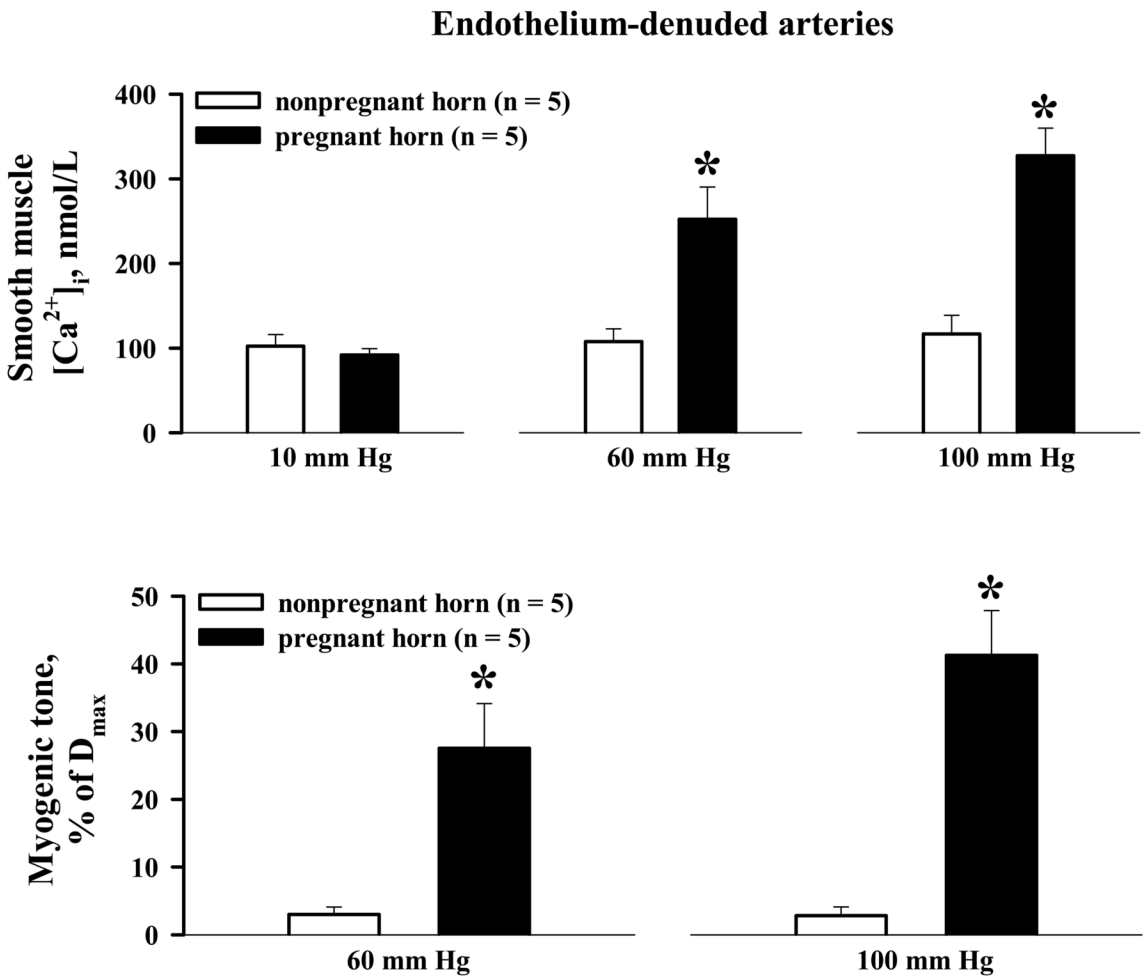

Figure 5.

Summary graphs demonstrating significant differences in pressure-induced smooth muscle $[Ca^{2+}]_i$ responses and myogenic tone in endothelium-denuded uterine arteries from nonpregnant versus pregnant horns of late pregnant rats. * Significantly different at $P < .05$ (two-way repeated measures ANOVA); numbers in parentheses indicate the number of arteries tested.

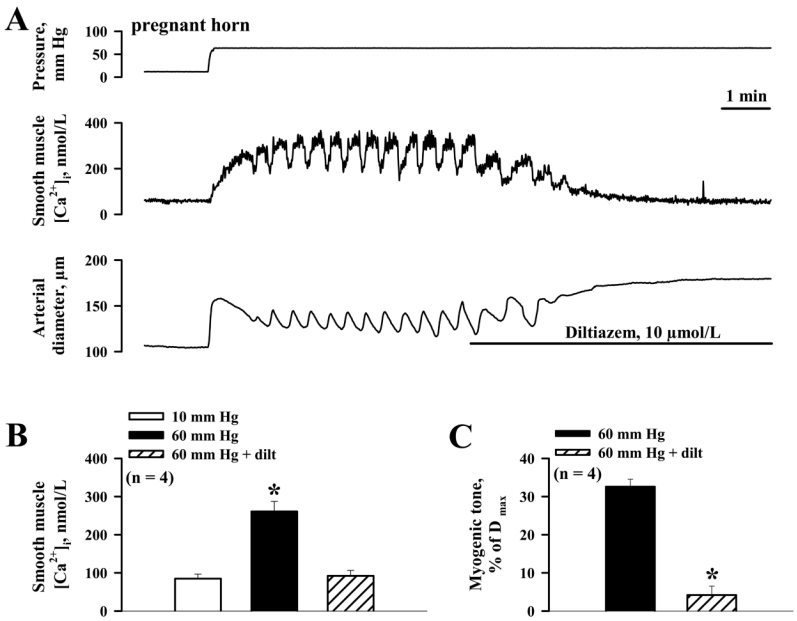

Figure 6.

A, Representative tracings showing inhibition of pressure-induced SMC $[Ca^{2+}]_i$ elevation and myogenic tone by 10 μmol/L diltiazem. B, Summary graph demonstrating the effects of diltiazem on the level of SMC $[Ca^{2+}]_i$ in response to pressure elevation from 10 to 60 mm Hg. *Significantly different from SMC $[Ca^{2+}]$ levels at 10 mm Hg (paired Student's t-test). C, Bar graph summarizing the effects of diltiazem on myogenic tone induced by pressure elevation from 10 to 60 mm Hg. Myogenic tone is expressed as a percentage of the fully dilated diameters (D_{max}) of each artery (in the presence of a combination of papaverine and diltiazem). *Significantly different from myogenic tone before application of diltiazem at $P < 0.05$ (paired Student's t-test). On B and C, Numbers in parentheses indicate the number of arteries tested.