

Influence of Constriction, Wall Tension, Smooth Muscle Activation and Cellular Deformation on Rat Resistance Artery Vasodilator Reactivity

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Key Words

Acetylcholine • Endothelium • Sodium nitroprusside • Tone

Abstract

This study investigated how vasoconstriction (tone), wall tension, smooth muscle activation, and vascular wall deformation influence resistance artery vasodilator reactivity. Resistance arteries, from two different regional circulations (splanchnic, uterine) and from pregnant and non-pregnant rats, were cannulated and pressurized, or mounted on a wire myograph under isometric conditions prior to being exposed to both endothelium-dependent (acetylcholine, ACh) and -independent (sodium nitroprusside, SNP) vasodilator agonists. A consistent pattern of reduced vasodilator sensitivity was noted as a function of extent of preconstriction for both agonists noted in pressurized arteries. A similar pattern regarding activation was noted in wire-mounted arteries in response to SNP but not ACh. Wall tension proved to be a major determinant of vascular smooth muscle vasodilator reactivity and its normalization reversed this pattern, as more constricted vessels were *more* sensitive to ACh

relaxation without any change in SNP sensitivity, suggesting that endothelial deformation secondary to vasoconstriction augments its vasodilator output. To our knowledge, this is the first study to dissect out the complex interplay between biophysical forces impinging on VSM (pressure, wall tension), the ambient level of tone (vasoconstriction, smooth muscle cell activation), and consequences of cellular (particularly endothelial) deformation secondary to constriction in determining resistance artery vasodilatory reactivity.

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Introduction

Biophysical forces that normally impinge on the vascular wall (wall tension, shear stress) exert significant physiological effects through the process of mechanotransduction [1]. For example, many studies have investigated the interaction between flow-induced shear stress and vascular reactivity, especially with regard to endothelial secretion of nitric oxide [2-5].

The effects of transmural pressure are also well-characterized vis-a-vis myogenic reactivity, in which wall

tension secondary to transmural pressure induces vasoconstriction through a combination of mechanisms that include vascular smooth muscle (VSM) membrane depolarization, calcium entry, and the activation of enzymes that modulate calcium sensitivity, such as Rho kinase and protein kinase C [1, 6-8]. In spite of considerable research effort, the cellular elements involved in sensing and mediating flow- or pressure-mediated mechano-transduction have not been defined. Candidate structures include the actin cytoskeleton [9-11], intermediate filaments [11, 12], focal adhesions [13, 14], *caveolae* [15, 16], myoendothelial junctions [17] and intracellular-matrix linkages through transmembrane molecules such as integrins [1, 10, 18-21]. The cytoskeleton in particular is viewed as an important scaffold for enzymes and regulatory molecules whose activity may be affected, or even regulated by the physical state of the cell which, in turn, determines the spatial relationship between functional components such as cytoskeleton, contractile filaments, enzymes, ion channels and intracellular organelles [22-23].

Although many studies on isolated, cultured cells grown on an elastic matrix have shown that cyclic stretch leads to activation of signal transduction pathways associated with contractility and secretion of vasoactive molecules [24-26], little consideration has been given to the effects of cellular deformation resulting from vasoconstriction on vascular behavior. More than 20 years ago, Greensmith and Duling [27] described morphological changes in small vessels as they underwent constriction, and noted the significant inward buckling of the intimal surface associated with the formation of numerous "endothelial ridges" that altered luminal profile and resulted in a reorientation of VSM contractile filaments from the circumferential to the radial direction. These ridges induced pronounced deformation of the endothelium and were quite prominent in constricted arteries, ranging from 5-10 microns in height and extending several hundred microns along vessel length. This is an important physiological issue since small vessels normally possess intrinsic myogenic tone *in vivo*, and the level of tone may, in turn, be altered by physical stimuli such as pressure. Chronic hypertension is an excellent example of this, as it illustrates how an alteration in a physical stimulus (blood pressure) leads to a change in the vascular response (tone and reactivity) which, in turn, leads to vascular wall remodeling [14, 28-30].

Our hypothesis in this study was that vasodilator reactivity – as defined by pharmacologic sensitivity and efficacy – is significantly influenced by the level of arterial

constriction and/or the extent of VSM activation. Two secondary hypotheses were that: (1) any observed differences in arterial reactivity could be related to changes in wall tension and/or vascular smooth muscle activation, and (2) endothelial deformation in and of itself is an important determinant of its vasodilatory influence.

By obtaining dimensional measurements of pressurized, cannulated vessels (lumen diameter and wall thickness) from two regional circulations (splanchnic and uterine), and by varying the level of tone and transmural pressure, we were able to evaluate the contribution of circumferential wall tension to any observed differences in reactivity. To segregate the effects of VSM activation from cellular deformation, and of VSM force from wall tension, separate experiments were conducted using one vessel type (mesenteric second order resistance arteries) mounted in an isometric (wire myograph) rather than isobaric (pressure arteriograph) preparation.

In addition, we used vessels from both pregnant and non-pregnant animals, since pregnancy is known to produce significant structural and functional changes in uterine and mesenteric resistance arteries. We reasoned that documentation of similar responses in vessels from both pregnant and non-pregnant animals (such as was obtained) strengthens the conclusion that the observed changes in vasodilator responsiveness as a function of vessel tone reflect fundamental properties of both endothelial and vascular smooth muscle cells, rather than behavior specific to vessel type or physiological state.

The results illustrate the complex interplay between wall tension, smooth muscle activation and endothelial deformation as complementary and significant determinants of arterial vasodilatory reactivity.

Materials and Methods

Animals and experimental design

Non-pregnant (NP) and late pregnant (day 20/22; LP) 12-14 wk old female Sprague-Dawley rats were purchased from Charles River Canada and housed at the University of Vermont Animal Facility for studies on pressurized vessels. Adult (12-14 wk old) female Sprague-Dawley rats were purchased from Charles River Canada and housed at the University of Alberta Animal Facility for studies with wire-mounted mesenteric vessels (experiment 2, see below). The Institutional Animal Care and Use Committee approved the experimental protocols for use of the animals at each institution, and the provided guidelines were followed. Animals were provided with feed and water *ad libitum* and given three days to acclimate to their surroundings. On the day of an experiment, animals were injected with pentobarbital (50 mg/kg)

intraperitoneally to induce a surgical plane of anesthesia. When no longer responsive to a hard pinch to the feet, rats were decapitated with a small animal guillotine. The uterus and mesentery were dissected out and placed in cold (4°C) HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid)-buffered saline solution.

In vitro vessel methodologies

Pressurized (isobaric) vessel preparation. Mesenteric arteries (100-200 μm unstressed diameter), and uterine arcuate arteries (120-200 μm) were dissected from surrounding tissue and cut in half to produce two functioning vessel segments. We used similarly-sized resistance arteries from two different regional circulations (splanchnic and uterine), and from pregnant (LP) and non-pregnant (NP) animals to evaluate whether observed differences were generalizable in a physiological sense; i.e. the occurrence of a similar pattern of reactivity regardless of vessel type and physiological state would suggest this to be a fundamental physiological property. Arterial segments were transferred to a dual chamber arteriograph (Living Systems Instrumentation, Burlington, VT). Each chamber contained two glass cannulae and had its own independent superfusion system for drug delivery. All arteries were cannulated, and intraluminal pressure set to 50 mmHg prior to a 45-60 min equilibration at 37°C using a servo-controlled pump and in-line pressure transducer (Living Systems Instrumentation). Following cannulation, the arteriograph was set on a mobile stage attached to an inverted microscope (Zeiss SR, Carl Zeiss, Thornwood, NY) with an attached monochromatic video camera that was connected to a video dimension analyzer and a television monitor as described in earlier publications [31, 32].

Wire-mounted (isometric) vessel preparation. Mesenteric arteries were isolated and cleaned of all surrounding adipose and connective tissues, and mounted on two 25 μm wires attached to a wire myograph (DMT, Aarhus, Denmark) to allow isometric tension recordings. Vessels were normalized through a series of stepwise increases in diameter to determine their optimal resting tension, set to $0.8 \times \text{IC}_{100}$ (the internal circumference equivalent to a transmural pressure of 100 mmHg). Following a 30-minute equilibration period, endothelial integrity was confirmed by assessing relaxation with methylcholine.

Arterial imaging. Cannulated, pressurized (50 mmHg) mesenteric arterial segments from NP rats were constricted to 20% (low tone) or 60% (high tone) prior to fixation in 4% paraformaldehyde for 3-4 h. After rinsing in cacodylate buffer (Iado Research, Williston, USA), the tissues were post-fixed in 1% osmium tetroxide for 2 hours at 4°C. The tissues were then rinsed in cacodylate buffer, dehydration through graded ethanols, cleared in propylene oxide and embedded in Spurr's epoxy resin. Semithin sections (1 μm) were cut with glass knives on a Reichert ultracut microtome and stained with toluidine blue (Electron Microscopy Sciences, Fort, Washington PA). Images were obtained using an Olympus BX50 light microscope (at 40x magnification) coupled to a CCD camera.

Experimental protocols

Experiment 1 (pressurized mesenteric and uterine vessels from non-pregnant and late pregnant animals) - effects of level of preconstriction (tone) on SNP and ACh vasodilator reactivity. All uterine or mesenteric vessels were given increasing concentrations of phenylephrine (Phe) to achieve low vs. high levels of constriction; specifically, one vessel was constricted 20-40% from its original diameter (defined as 'low tone') while the second vessel was constricted 60-80% from its original diameter ('high tone'). Axial length was adjusted as needed to remove any buckling resulting from changes in tone or pressure.

Once the desired levels of constriction were achieved, vessels were allowed 20 minutes to ensure that the level of preconstriction (lumen diameter) was stable. Incremental concentrations of SNP or ACh (endothelium-independent and -dependent vasodilators, respectively) were then administered until a maximal response was observed (defined as no further dilation with additional drug). Complete relaxation was then achieved using a mixture of papaverine (10^{-4} M) and diltiazem (10^{-5} M), and lumen diameters recorded.

The vasodilator efficacy of each drug was calculated as the percent dilation achieved by a vessel at the supramaximal concentration of vasodilator relative to full relaxation obtained in the presence of papaverine and diltiazem. Sensitivity was determined by best fit standard curve analysis (SigmaPlot), which allowed calculation of the concentration of vasodilator required to produce 50% of the maximal agonist response (EC_{50}).

Once data were obtained, regression analyses were conducted to relate vessel lumen diameter or tone to sensitivity (EC_{50} value) to wall tension (T, which is defined as the product of pressure (P) and radius (r) by the LaPlace equation, $T=Pr$). In the first series of experiments, pressure was kept constant at 50 mmHg in all high- and low-tone vessels; hence, tension was directly proportional to diameter. The strength of relationship between tension and pharmacologic sensitivity was expressed by the correlation coefficient (r^2 value), which indicates how much of the variability in sensitivity could be attributed to the dependent variable (e.g. lumen diameter/wall tension, or level of tone).

Experiment 2 (wire-mounted mesenteric vessels, SNP and ACh) - effects of varying smooth muscle force (activation) without changing wall tension or cellular (vascular wall) deformation on SNP and ACh reactivity. Although pressurized vessels are under more physiological conditions than wire-mounted arteries, as they maintain a cylindrical shape and can change diameter, the wire technique is isometric, which permits the imposition of a fixed amount of stretch (wall tension), and thereby vary only smooth muscle activation (force production). The extent of cellular deformation is also similar, since all vessels are equally pre-stretched.

Mesenteric vessels were constricted with Phe to approximately 35% or 70% of their maximal tension (determined by an initial cumulative concentration-response curve to Phe, 0.1 to 30 μM) and allowed to stabilize for 10 minutes. Following preconstriction, a concentration-response to SNP (0.001 to 100 μM) or ACh (0.001 to 30 μM) was then obtained in vessels with

high ($\approx 70\%$) vs. low ($\approx 35\%$) pre-activation to determine both sensitivity and efficacy using the standard pharmacological approach for isometric wire-mounted vessel preparations.

Experiment 3 (pressurized mesenteric vessels) - effects of varying wall tension on SNP reactivity in vessels with similar levels of tone: Two vessel segments were pressurized to 50 mmHg, and constricted with Phe to induce $\approx 60\%$ tone. Pressure was then increased from 50 to 100 mmHg in one vessel to double wall tension with a similar degree of precontraction (tone). By slowly changing pressure (~ 1 mmHg/sec), vessel diameter remained unchanged due to myogenic activation. This procedure allowed us to directly evaluate of the effect of wall tension on vasodilator reactivity to SNP without any change in the level of constriction (tone).

Experiment 4 (pressurized mesenteric vessels) - effects of cellular (vascular smooth muscle, endothelial) deformation at different levels of constriction and similar wall tension on SNP and ACh reactivity: These experiments were aimed at dissociating the level of cellular deformation from wall tension by inducing different levels of constriction (low vs. high) in one pair of vessels, as above, and then normalizing the wall tension by increasing the intraluminal pressure in the highly constricted artery until the wall tension was similar to that of the less constricted artery. In practice, wall tension values of approximately 6,000 mN/mm (intermediate between the high and low tension values achieved in Experiment 3) were achieved in all vessels studied. This was achieved without changing the lumen diameter or tone in the more constricted vessels since vessels were able to maintain their diameter, presumably due to myogenic activation. Once diameter was stable, vessel responses to SNP and ACh were evaluated.

Drugs and solutions

The HEPES physiologic salt solution contained the following (in mmol/L): sodium chloride 141.8, potassium chloride 4.7, magnesium sulfate 1.7, calcium chloride 2.8, potassium phosphate 1.2, HEPES 10.0, EDTA 0.5, and dextrose 5.0. The solution was prepared in deionized water and titrated with sodium hydroxide to a physiologic pH of 7.4. L-phenylephrine hydrochloride, sodium nitroprusside and acetylcholine hydrochloride (Sigma, St Louis, MO) were administered from stock solutions prepared daily in deionized water. All chemicals were purchased from Fisher Scientific (Fair Lawn, NJ) and Sigma unless otherwise specified.

Calculations and statistics

All data are expressed as means \pm SE. SNP and ACh efficacies are presented as a percentage of complete vessel dilation obtained in relaxing solution, while sensitivities are presented relative to the maximal extent of dilation obtained in response to each agonist. Sensitivity was determined by constructing a semi-logarithmic concentration response curve for each vessel, and then extrapolating to the concentration that produced 50% of the maximum effect to calculate the EC_{50} . Data are shown as the average of the individual EC_{50} values \pm SE. The results were analyzed with Students t-test (paired or unpaired, as appropriate); p values of <0.05 were considered statistically significant.

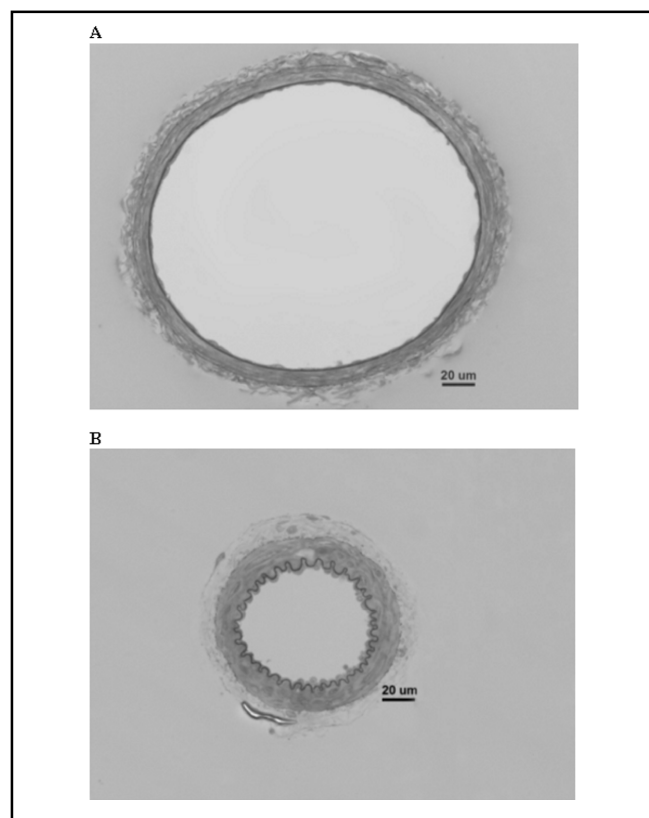


Fig. 1. A cross-sectional view of vessels with low vs. high constriction (tone). Cross-sections of pressurized (50 mmHg) mesenteric arterial segments from non-pregnant rat representative of those with low vs. high tone. The actual level of constriction in panel A was 20%, while in panel B was 60%. Histological procedure is described in Methods. Scale bar indicates 20 μ m.

Results

Low vs. high levels of constriction (tone) in pressurized vessel

Vessels with “low tone” had a mean constriction of $30 \pm 1.3\%$ ($n = 24$; range = 20–41%). Vessels with “high tone” had a mean constriction of $68 \pm 1.1\%$ ($n = 24$; range = 60–79%). A cross-sectional view of vessels with low vs. high tone is shown in Fig. 1. Tone was similar in both vessel types (mesenteric and uterine) for each level, and high vs. low tone levels were significant ($p < 0.05$) within each vessel type studied (Fig. 2).

Effects of pre-constriction on SNP and ACh vasodilator reactivity in mesenteric and uterine arteries (Experiment 1): Although the actual EC_{50} values differed with vessel type, significantly higher vasodilator concentrations were consistently required to produce an equivalent extent of dilation in vessels with high tone (Tab. 1).

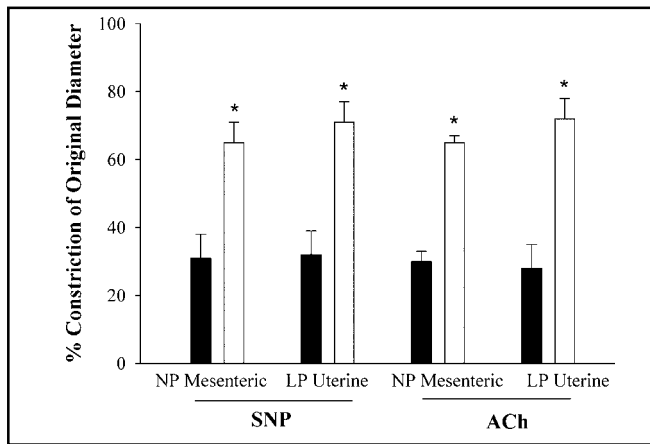


Fig. 2. High vs. low levels of constriction (tone). Low (black bars) vs. high (grey bars) levels of constriction/tone expressed as % of the original diameter in non-pregnant (NP) mesenteric and late pregnant (LP) uterine vessels prior to the SNP or ACh experiments. Data are expressed as mean \pm SE, $n = 6$, * $p < 0.05$.

Specifically, mesenteric vessels with high tone had an SNP EC_{50} 7-fold greater than those with low tone ($p < 0.05$). LP uterine arcuate arteries displayed a similar trend, as high tone vessels had EC_{50} values that were ≈ 15 -fold greater than those with low tone ($p < 0.05$).

The effect of constriction (level of tone) on endothelial vasodilator influence was tested via the administration of ACh, which leads to the release of several vasodilator molecules including nitric oxide, prostacyclin and EDHF. The proportion of each may vary with vessel type, and is known to be affected by pregnancy [33]. Mesenteric high tone vessels were 3.4-fold less sensitive to ACh than low tone vessels ($p < 0.05$); similarly, uterine arteries with high tone were 5-fold less sensitive to ACh than the arteries with low tone ($p < 0.05$).

Graphs summarizing sensitivity of pressurized mesenteric and uterine vessels in response to SNP and ACh are shown in Fig. 3 and Fig. 4, respectively. There were also significant differences in three out of the four groups in efficacy (Tab. 2). At maximal drug concentrations, dilation in all vessels with low tone was virtually complete ($\geq 98\%$), while vessels with high tone dilated $>94\%$ to ACh, but less to SNP, averaging 82 and 67%, respectively, for LP uterine and NP mesenteric arteries ($p < 0.05$ vs. low tone counterpart in each vessel type).

A strong correlation between vasodilator sensitivity (EC_{50} value) and wall tension or tone was evident for all vessel types and for both SNP and ACh stimulation, with coefficients of determination (r^2 values) ranging from 0.42 to 0.82 for sensitivity vs. wall tension or tone. The r^2 values indicate that wall tension accounts for

Agonist	Vessel Type	EC_{50} (μM) Low Tone	EC_{50} (μM) High Tone	Fold Difference	P Value
SNP	NP Mesenteric	0.01 ± 0.003	0.07 ± 0.015	7	0.02
	LP Uterine	0.04 ± 0.010	0.61 ± 0.129	15	0.01
ACh	NP Mesenteric	0.05 ± 0.023	0.17 ± 0.036	3.4	0.01
	LP Uterine	0.02 ± 0.005	0.10 ± 0.011	5	0.01

Table 1. Summary of SNP and ACh sensitivity (EC_{50} values) for pressurized mesenteric and uterine arteries preconstricted to high vs. low tone with Phe. Data are expressed as mean \pm SE, $n = 6$ for each experimental result; total vessels: $n = 48$.

Agonist	Vessel Type	Efficacy (% Max Relaxation)		Value
		Low Tone	High Tone	
SNP	NP Mesenteric	98 ± 1.0	67 ± 3.0	0.01
	LP Uterine	99 ± 0.6	82 ± 7.3	0.03
ACh	NP Mesenteric	99 ± 0.7	96 ± 0.7	0.05
	LP Uterine	99 ± 0.4	94 ± 3.5	0.39

Table 2. SNP and ACh efficacy (% of maximal relaxation obtained in a relaxing solution containing papaverine + diltiazem) of pressurized mesenteric and uterine arteries. Data are expressed as mean \pm SE, $n = 6$ for each experimental result; total vessels: $n = 48$.

Agonist	Activation (% Max)	EC_{50} (μM)	P Value
SNP	34.8 ± 1.8	6.6 ± 1.5	0.05
	72.0 ± 1.8	13.9 ± 3.2	
ACh	36.7 ± 2.0	19.0 ± 7.2	0.56
	71.9 ± 2.9	14.0 ± 3.9	

Table 3. Levels of activation (% max) and SNP and ACh sensitivity (EC_{50} values) for wire-mounted mesenteric arteries (SNP, low constriction: $n = 7$, high constriction: $n = 5$; ACh low constriction: $n = 8$, high constriction: $n = 6$). [Experiment 2]

approximately 40 to 80% of the variability in vasodilator sensitivity. By the Law of LaPlace, the circumferential force that favors dilation (wall tension) is greater in less constricted vessels; thus, less agonist is required to produce an equivalent effect regardless of vessel type or vasodilator. On the other hand, an effect of activation *per se*, rather than wall tension cannot be distinguished using the pressurized vessel methodology (see Discussion); alternatively, changes in cell deformation (VSM shortening) may be contributory as well.

Effects of vascular smooth muscle activation on mesenteric artery SNP and ACh vasodilator reactivity at similar levels of wall tension and wall deformation (Experiment 2). In wire-mounted vessels in which the level of pre-stretch was normalized, the only difference was level of smooth muscle activation, as the isometric nature of the technique prevents VSM cell shortening (deformation). Levels of activation were approximately

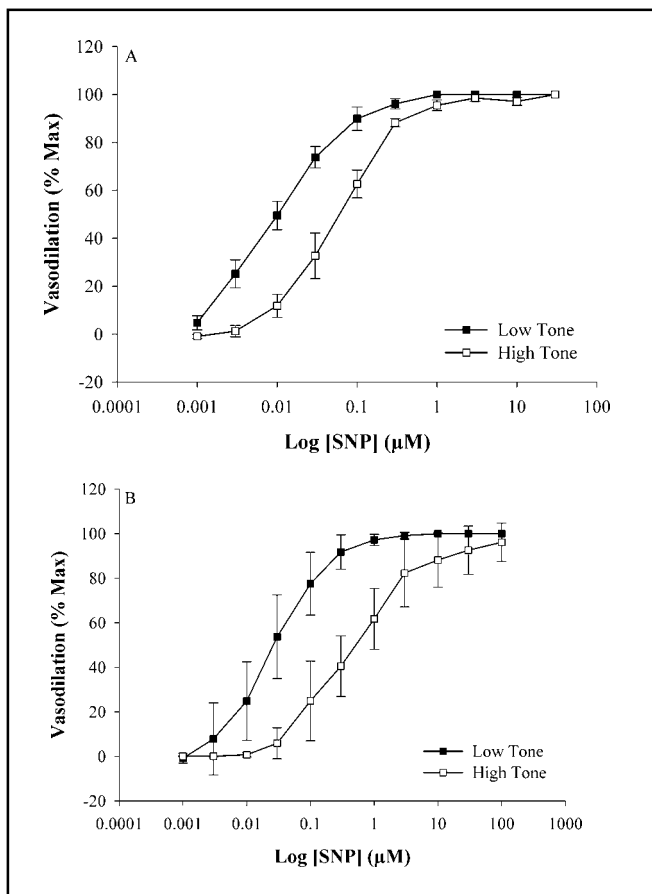


Fig. 3. Mesenteric and uterine artery sensitivity to SNP [Experiment 1]. SNP concentration-response curves of pressurized mesenteric (A) and uterine (B) arteries with high vs low constriction (tone). The vasodilation response is normalized to maximal SNP response (sensitivity). Data are expressed as mean \pm SE, $n = 6$. EC_{50} values were derived for each vessel and used for statistical analysis as described in Methods.

35% (low activation) vs. 70% (high activation) of the force obtained in response to maximal concentrations of Phe as determined by a Phe concentration-response curve prior to the administration of SNP or ACh (Tab. 3).

More activated vessels were significantly less sensitive to SNP relaxation, while there were no differences in sensitivity to ACh, ($p=0.56$, Tab. 3). Differences in SNP relaxation were most pronounced at low concentrations (Fig 5A), while the ACh concentration-response curves were virtually superimposed (Fig 5B).

When individual vessels were considered, a positive correlation between smooth muscle activation and SNP responses was noted, with a coefficient of determination (r^2) of 0.56, indicating that roughly half of the variability in sensitivity could be accounted for by the level of activation. The corresponding r^2 value for activation and

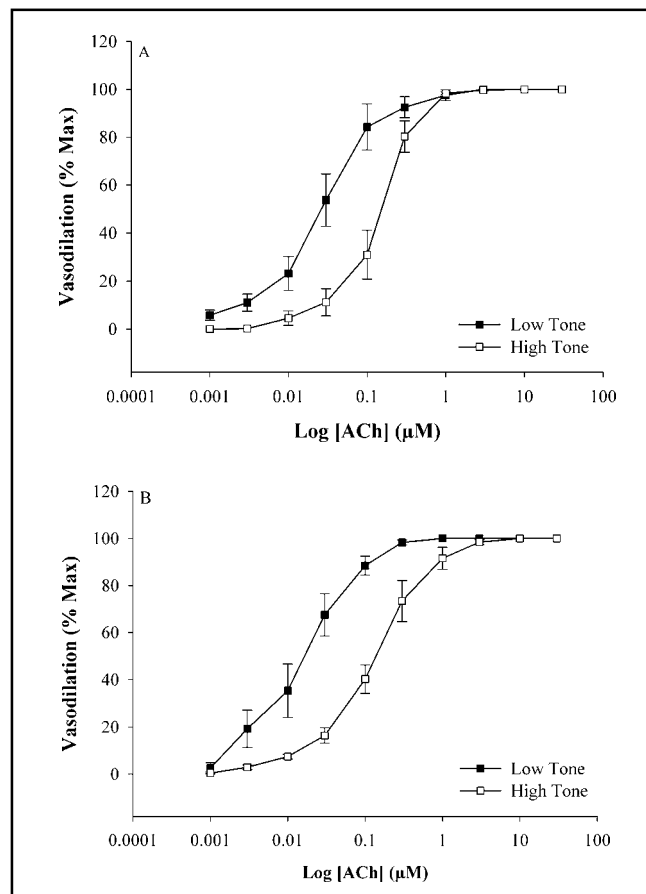


Fig. 4. Mesenteric and uterine artery sensitivity to ACh [Experiment 1]. ACh concentration-response curves of pressurized mesenteric (A) and uterine (B) arteries with high vs low constriction (tone). The vasodilation response is normalized to maximal ACh response (sensitivity). Data are expressed as mean \pm SE, $n = 6$. EC_{50} values were derived for each vessel and used for statistical analysis as described in Methods.

ACh sensitivity was 0.01.

Effects of varying wall tension at similar levels of preconstriction (tone) and wall deformation on mesenteric artery reactivity to sodium nitroprusside (Experiment 3). Given that vessels with varying levels of tone also have different degrees of cellular deformation secondary to constriction, we next conducted a group of experiments using pressurized vessels in which the level of constriction (and, therefore, cellular deformation) was kept constant while varying wall tension. This was accomplished by constricting mesenteric vessels by about 60% and then slowly increasing the transmural pressure in one of the two vessel pairs from 50 to 100 mmHg prior to the administration of SNP. In this case, we were attempting to isolate the influence of wall tension on vascular smooth muscle vasodilator sensitivity.

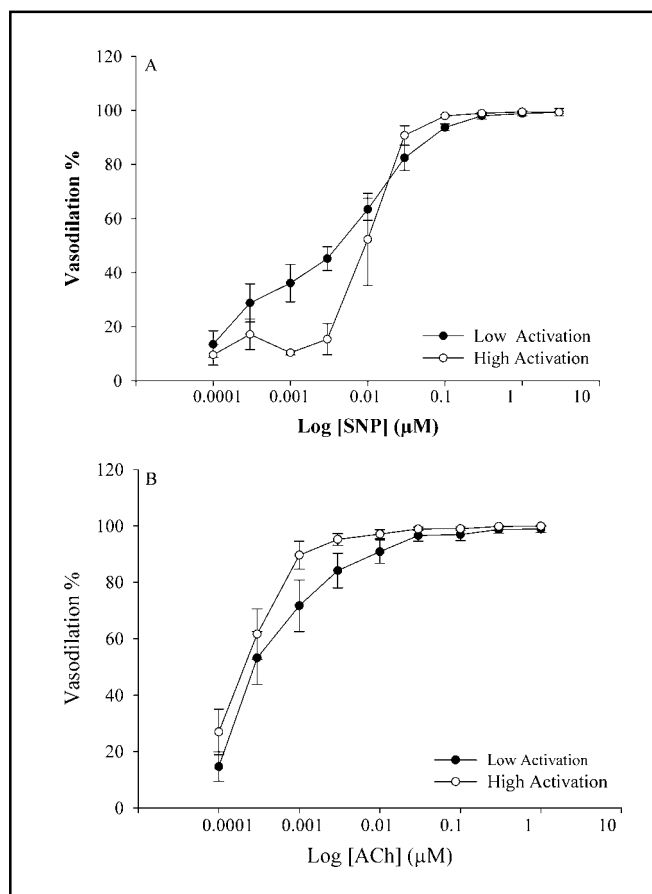


Fig. 5. Sensitivity of wire-mounted mesenteric arteries to SNP and ACh [Experiment 2]. A - SNP concentration-response curves (sensitivity) of wire-mounted mesenteric normalized to maximal vasorelaxation obtained at the highest concentration (35% force: $n = 7$; 70% force: $n = 5$). B - ACh concentration-response curves (sensitivity) of wire-mounted mesenteric arteries normalized to maximal vasodilation obtained at the highest concentration (35% force: $n = 8$; 70% force: $n = 6$). EC_{50} values were derived for each vessel and used for statistical analysis as described in Methods.

This experimental protocol resulted in an approximate doubling of wall tension without changing the level of precontraction or circumferential deformation (Tab. 4), as vessel diameter did not change in response to increased transmural pressure due to myogenic activation. Hence, this was an attempt to mimic the conditions experienced by a wire-mounted vessel in a pressurized preparation (a change in VSM force without any change in diameter/VSM shortening/endothelial deformation). Under these conditions, there was a 3.6-fold difference in the SNP EC_{50} values in equally constricted vessels experiencing high vs. low wall tension (Tab. 4). Efficacy values were similar in both groups (80% vs. 74% in high vs. low tension; $p > 0.05$; Tab. 4)

Constriction (%)	Pressure (mmHg)	Wall Tension (mN/mm)	EC_{50} (μ M)	Efficacy
63.0 ± 2	100	8750 ± 270	0.05 ± 0.02	80 ± 6
63.0 ± 2	50	$4063 \pm 285^*$	$0.18 \pm 0.03^*$	74 ± 6

Table 4. Pressurized mesenteric arteries constricted to a similar level of tone but subjected to different wall tensions by increasing transmural pressure show differences in sensitivity (EC_{50}) but not efficacy to SNP. Data are expressed as mean \pm SE, $n = 4$, $^*p < 0.05$. [Experiment 3]

Agonist	Constriction (%)	Pressure (mmHg)	Wall Tension (mN/mm)	EC_{50} (μ M)	Efficacy (%)
SNP	63.0 ± 6.2	56.0 ± 0.8	6028 ± 23	0.05 ± 0.143	94 ± 1.0
	$25.0 \pm 1.3^*$	$34.0 \pm 0.8^*$	$6000 \pm 184^*$	0.04 ± 0.017	100 ± 0.1
ACh	61.0 ± 1.4	57.0 ± 2.0	5966 ± 115	0.20 ± 0.04	93 ± 4.8
	$36.0 \pm 1.6^*$	$37.0 \pm 1.7^*$	6160 ± 128	$0.67 \pm 0.16^*$	91 ± 2.8

Table 5. Pressurized mesenteric arteries at different levels of constriction (high vs. low tone) but similar wall tensions accomplished by varying transmural pressure (mmHg), show differences in sensitivity (EC_{50}) but not efficacy to SNP and ACh. Data are expressed as mean \pm SE, $n = 8$ (for ACh), $n = 4$ (for SNP), $^*p < 0.05$. [Experiment 4]

Effects of equalizing wall tension at different levels of precontraction and wall deformation on mesenteric artery reactivity to SNP and ACh (Experiment 4). An influence of wall tension does not, however, preclude a separate effect of cellular (endothelial and vascular smooth muscle) deformation on its vasodilatory influence. A fourth and final experimental protocol was developed to address this question.

Vessels were constricted to high vs. low levels of tone, as in Experiment 1, and the transmural pressure was then varied until wall tension was similar in both vessels (Tab. 5) prior to the administration of SNP or ACh. To achieve wall tension values intermediate between those measured in Experiment 3, we aimed for a wall tension of approximately 6,000 mN/mm, which resulted in transmural pressures that averaged ≈ 56 vs. ≈ 35 mmHg in the high vs. low tone vessels.

As shown in Tab. 5, vessels with high tone, in which endothelial deformation was greatest, required 3-fold less ACh to achieve half-maximal relaxation, while sensitivity to SNP was equivalent. There were no differences in efficacy between high and low tone vessels ($p > 0.05$).

Together, these results clearly show wall tension and level of activation, rather than shortening, to be the principal determinants of VSM vasodilator sensitivity; conversely, the release of endothelial vasodilator molecules is significantly augmented by its deformation secondary to VSM activation and shortening. The physiological implications of these findings are considered below.

Discussion

As an artery constricts, the vascular wall undergoes considerable three dimensional deformation of both cells and matrix. VSM cells twist, shorten and thicken [34] and the internal elastic lamina and endothelium are buckled inwards to form pronounced axial ridges that encroach upon and deform the normally-cylindrical shape of the lumen in a relaxed vessel (see Fig. 1 and ref 27). The endothelium is also stretched axially as a vessel constricts, and radially compressed, as it is positioned between the activated smooth muscle and intraluminal pressure. While many studies have examined the effects of cyclic stretch using the Flexercell apparatus in which cultured VSM or endothelial cells are seeded onto an elastic substrate on cellular responses [35-37], the effects of compressive deformation on endothelial function have not been experimentally addressed.

The goal of this study was to use available small-artery methodology (pressurized vs. wire-mounted vessels) to understand how wall tension, constriction (tone), VSM activation (force production), and cellular deformation interact and influence resistance artery vasodilatory reactivity. SNP and ACh were chosen because they are relatively specific and commonly-used agonists for VSM vs. endothelial activation, respectively.

The results of the first experimental protocol show that more constricted vessels require more agonist to dilate, as evidenced by the 3- to 15-fold increases in EC_{50} values to both SNP and ACh. This was clear and across-the-board in terms of the similarity in pattern observed in vessels from the gut vs. the uterus, and of those from pregnant and non-pregnant animals. The latter comparison was included to expand the scope to include a unique physiologic state (pregnancy) that is associated with a significantly altered vasodilatory reactivity, as demonstrated by the results of numerous studies. Our assumption was that, if this is a fundamental physiological response, it should be present in all small vessels irrespective of source and physiological condition.

The results support this line of reasoning. The practical implication is that comparison of *in vitro* vasodilator reactivity data from vessels that are unequally pre-constricted is not valid, as the level of constriction introduces significant bias. A corollary is that between-study comparisons in which different levels of pre-constriction were induced also cannot be inferred. Because vessel constriction results in a narrowing of the lumen, unless pressure is increased, wall tension decreases to an extent that is proportional to the level of constriction

by the Law of LaPlace, which states that tension is a product of pressure and radius ($T=Pr$). Everything else being equal, more drug would be required to elicit an equivalent vasodilator response because of the reduced distending force (wall tension). The effect is further amplified by increased wall thickness, which reduces wall stress (defined as tension per unit wall thickness; $\delta = T/\omega$, where T = tension and ω = wall thickness). This is an oversimplified view, however, since at least three other factors deserve consideration.

The first is that the relationship between tone and smooth muscle activation in terms of crossbridge dynamics is difficult to predict since changes in VSM length, cross-sectional area and volume may alter actin-myosin interactions in ways that are not well understood (e.g. via altered actomyosin motor function and overlap).

Second, there is uncertainty regarding the counterforce produced by compression of intracellular structures; studies in isolated smooth muscle cells have provided evidence for intracellular compression having a spring-like effect that opposes 'sarcomere' shortening (also poorly defined in VSM), and facilitates the restoration of cell length (or, in this case, of arterial diameter) once the contractile stimulus is withdrawn [38]. This internal counterforce is difficult to evaluate experimentally, but the level of activation must balance the sum of pressure-induced circumferential distension and internal (VSM intracellular) compression.

Third, the potential existence of load-bearing but not force-producing elements [latch-bridges, 39] in partially constricted vessels further complicates interpretation, since it uncouples activation from diameter maintenance. Based on these considerations, it is not possible to determine the true level of activation (relative level of VSM force production) in a pressurized vessel with tone. For this reason, isometric wire-mounted experiments (Experiment 2) were carried out in an attempt to evaluate the effects of VSM activation without the complication of cellular shortening/deformation or altered wall tension. The resulting data show that activation is, in and of itself, an important factor in VSM responsiveness to a vasodilatory stimulus, as significantly more SNP was required to produce a proportional decrease in force in a more activated vessel. Relaxation to ACh, however, was unchanged, as might be expected since the endothelium was stretched to an equivalent extent.

It is worth noting that, while the SNP response predicts a blunting of the NO response as well, under these experimental conditions, ACh stimulates the release of multiple vasodilators, so the observed response is the

summation of all endothelial vasodilators (NO, prostacyclin, EDHF, etc.) and, therefore, not specific to NO; precluding direct inference from SNP to ACh data. Thus, the data from the first two experimental series are congruent in showing that wall tension and level of VSM activation both significantly impacted VSM reactivity.

In Experiment 3, vessels were equally constricted (and, therefore, equally deformed), but set at different wall tensions by varying intraluminal pressure. The absence of changes in vessel diameter in spite of a 100% increase in transmural pressure points to enhanced VSM activation as well, presumably via a myogenic mechanism geared to maintaining constriction (diameter) in response to increased pressure. SNP was used to target VSM, and the significantly increased sensitivity to SNP (as reflected in 4-fold lower EC_{50} values) in vessels exposed to a higher wall tension reflect the relative predominance of the wall tension effect over the influence of VSM activation, since an increase in the latter would, based on the result of Experiment 2, have been expected to have the opposite effect (to *increase* EC_{50} values). Considering the 7-fold reduction in high- vs. low-tone mesenteric artery sensitivity to SNP in Experiment 1 and the 2.5-fold increase in sensitivity to a doubling of activation in Experiment 2, the 3.6-fold reduction in high- vs. low-tension vessels in Experiment 3 is quantitatively consistent.

In the fourth and final experimental series, we were interested in determining the specific influence of endothelial deformation on its vasodilatory influence. To accomplish this, pressurized vessels were constricted to high vs. low tone, as in Experiment 1, although here both vasoconstrictor concentration and transmural pressure were adjusted so as to produce similar wall tensions. Under these conditions, more constricted vessels were significantly more sensitive to ACh vasodilation; i.e. without the influence of wall tension, endothelial vasodilatory influence was significantly augmented.

Since ACh releases multiple endothelial vasodilators (nitric oxide, EDHF, prostanoids, endothelin), we only observed a sum effect under these experimental conditions and do not know which is most affected. The fact that the EC_{50} values for SNP were virtually identical confirms the importance of wall tension in regulating vascular smooth muscle dilator reactivity and eliminates the degree of cell shortening as a significant variable.

The most straightforward interpretation is that, under conditions where the distending force is normalized, endothelial deformation – most likely, circumferential and radial compression - produces a significant increase in vasodilator release in response to an identical stimulus. As seen in Fig. 1, the infolding of the internal elastic lamina pushes the endothelium into the luminal compartment and clearly results in a three-dimensional deformation since vessels also lengthen as they constrict. Our results suggest that the influence of the endothelium becomes more potent as the cell deforms secondary to increased vasoconstriction. This effect could be due to any number of factors, e.g. changes in the spatial relationships between cytoskeletal and membrane structures that may alter enzyme or ion channel activity, and thereby affect endothelial secretory activity in favor of increased vasodilatory influence.

In conclusion, our findings indicate that both wall tension and the level of vasoconstriction/activation are important determinants of vascular smooth muscle vasodilator reactivity; conversely, endothelial function does not appear to be appreciably impacted by wall tension, although cellular deformation secondary to vasoconstriction does induces significant changes in vasodilator influence. The results of this study are provocative in their physiological implications since they provide an insight into the dynamic interactions between biophysical forces (pressure, wall tension) and fundamental cellular processes (VSM activation and endothelial deformation) in the determination of vascular reactivity. The real challenge for future studies is to understand how these processes are affected by vascular pathologies such as chronic hypertension (where differences intramural forces such as wall tension induce changes in tone and cellular deformation), or diabetes - a metabolic condition in which hyperglycemia and increased insulin levels induce changes in vascular smooth muscle contractility and endothelial function that may, in turn, alter the physical forces that impinge upon the vascular wall.

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