

Effects of ischemia and myogenic activity on active and passive mechanical properties of rat cerebral arteries

REBECCA J. COULSON,¹ NAOMI C. CHESLER,¹
LISA VITULLO,² AND MARILYN J. CIPOLLA²

¹Department of Mechanical Engineering and ²Department of Neurology,
The University of Vermont, Burlington, Vermont 05405

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Coulson, Rebecca J., Naomi C. Chesler, Lisa Vitullo, and Marilyn J. Cipolla. Effects of ischemia and myogenic activity on active and passive mechanical properties of rat cerebral arteries. *Am J Physiol Heart Circ Physiol* 283: H2268–H2275, 2002. First published August 29, 2002; 10.1152/ajpheart.00542.2002.—Passive (papaverine induced) and active (spontaneous pressure induced) biomechanical properties of ischemic and nonischemic rat middle cerebral arteries (MCAs) were studied under pressurized conditions in vitro. Ischemic (1 h of occlusion), contralateral, and sham-operated control MCAs were isolated from male Wistar rats ($n = 22$) and pressurized using an arteriograph system that allowed control of transmural pressure (TMP) and measurement of lumen diameter and wall thickness. Three mechanical stiffness parameters were computed: overall passive stiffness (β), pressure-dependent modulus changes ($E_{inc,p}$), and smooth muscle cell (SMC) activity-dependent changes ($E_{inc,a}$). The β -value for ischemic vessels was increased compared with sham vessels (13.9 ± 1.7 vs. 9.1 ± 1.4 , $P < 0.05$), indicating possible short-term remodeling due to ischemia. $E_{inc,p}$ increased with pressure in the passive vessels ($P < 0.05$) but remained relatively constant in the active vessels for all vessel types, indicating that pressure-induced SMC contractile activity (i.e., myogenic reactivity) in cerebral arteries leads to the maintenance of a constant elastic modulus within the autoregulatory pressure range. $E_{inc,a}$ increased with pressure for all conditions, signifying that changes in stiffness are influenced by SMC activity and vascular tone.

incremental elastic modulus; middle cerebral artery; vascular smooth muscle; myogenic reactivity

CEREBRAL ARTERIES function to supply oxygen and glucose to the brain, remove waste products, and regulate the ionic environment (17, 20). They also play an important role in the regulation of cerebral blood flow through autoregulation, a well-established phenomenon that is caused by a combination of myogenic, neuronal, and metabolic mechanisms (17, 23). Myogenic reactivity is the ability of the vascular smooth muscle cell (SMC) to contract in response to stretch or to an

increase in transmural pressure (TMP) and to relax in response to a decrease in TMP (21). The myogenic response has been shown to contribute significantly to autoregulation in the cerebral circulation and provides a relatively constant blood flow even with large changes in arterial pressure (10, 27). Underlying the active response to pressure change is myogenic tone, a state of partial constriction of the vessel that provides a starting point from which an artery can modify its diameter and thus control cerebral blood flow (21). Many studies have documented the existence of myogenic tone in rat cerebral arteries and have found that autoregulation can begin at pressures as low as 40 mmHg and end at pressures as high as 180 mmHg (7, 12, 13, 25, 26, 28). Above this range, the vessels simply dilate with increases in pressure, a phenomenon known as forced dilatation (7).

It is this active constriction of arteries within the autoregulatory range that prompts studies of not just passive mechanical properties but also active mechanical properties. Passive properties provide an understanding of the behavior of the acellular extracellular matrix components of arteries (e.g., collagen and elastin); however, to understand how the artery behaves in vivo, it is necessary to understand mechanical properties with SMC activation. In addition, it is desirable to study cerebral vessels in their natural myogenic state rather than during drug-induced active states (e.g., KCl or norepinephrine) because the true autoregulatory efficiency of the myogenic mechanism is significantly decreased when the level of SMC activity increases above that due to intrinsic vascular tone (26). Thus pressure-induced SMC contraction gives a more physiological measure of active arterial mechanics compared with SMC contraction induced by pharmacological doses of vasoactive compounds. Mechanical properties might also be altered if the vessel is diseased or damaged. For example, ischemia has been shown to reduce vascular myogenic tone, which might lead to brain tissue damage during postischemic reperfusion by causing decreased vascular resistance and autoregulatory failure (2, 4–6). Therefore, studying the

Address for reprint requests and other correspondence: M. J. Cipolla, Dept. of Neurology, The Univ. of Vermont, Given Bldg., Rm. C454, 89 Beaumont Ave., Burlington, VT 05405 (E-mail: mcipolla@zoo.uvm.edu).

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mechanical properties of vessels in disease states to determine whether they are able to adequately take part in controlling local blood flow may be important when considering therapeutic treatments such as thrombolysis (4).

Hayashi et al. (14) used a stiffness parameter (β) and an incremental elastic modulus in their study of human intracranial and extracranial cerebral arteries. These parameters have been shown to be adequate measures of arterial stiffness and elasticity for many different types of arteries, including human cerebral arteries, pig thoracic aortas, and rabbit carotid arteries (11, 14–16, 24). In this study, we examined the mechanical properties of rat middle cerebral arteries (MCAs) in their active (pressure activated) and passive (papaverine induced) states. These arteries, like other large cerebral arteries, operate in a state of partial constriction or tone (2, 4–6, 17), which contributes significantly to cerebrovascular resistance, and respond myogenically to changes in TMP (2, 4–6, 17, 18). Therefore, the influence of myogenic tone on the mechanical properties of cerebral arteries was determined. In addition, data from MCAs exposed to ischemia and reperfusion were analyzed to determine whether 1 h of ischemia with 24 h of reperfusion has an effect on the mechanical properties of the vessel wall. Parameters calculated from the MCA pressure-diameter data include β , an incremental elastic modulus ($E_{inc,p}$), and a modified incremental modulus ($E_{inc,a}$) that accounts for SMC activation at different levels of TMP.

MATERIALS AND METHODS

MCA occlusion model. All procedures were approved by the Institutional Animal Care and Use Committee. Ischemia was

produced by filament occlusion of the right MCA in male Wistar rats (weight 280–300 g) (2, 5, 22). The animals were anesthetized via an inhalation mask with halothane and oxygen. Body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ with a heating pad and monitored with a rectal probe. The right carotid bifurcation was exposed, and the external carotid artery was coagulated distal to the bifurcation. After temporary ligation of the common carotid artery, a 5-0 nylon suture coated with silicon was inserted into the carotid artery through the external carotid artery stump and advanced through the internal carotid to occlude the origin of the MCA. The filament was carefully inserted just to the bifurcation of the MCA and anterior cerebral artery so as to avoid any mechanical disruption of the MCA (5). Laser-Doppler flowmetry was used to confirm successful occlusion and reperfusion. After 1 h of occlusion, the suture was removed to allow reperfusion, and the animal was allowed to recover. Animals were euthanized by anesthesia and decapitated 24 h after the suture was removed. Sham-operated control animals underwent anesthesia and a midline neck incision but no occlusion.

Preparation of arteries and pressurized arteriograph system. The MCA from both the right (occluded) and left (contralateral to ischemia) side of the brain was dissected, cleared of extraneous connective tissue, and placed in the arteriograph chamber. Dissected arteries were mounted on two glass microcannulas suspended above an optical window within the arteriograph chamber, perfused with physiological saline solution (PSS), and secured with two strands of nylon thread on both the proximal and distal cannulas. The distal cannula was closed off to flow, and a static TMP was applied to the vessels.

The arteriograph bath (Living Systems Instrumentation; Burlington, VT) consisted of two 20-ml fluid chambers with inlet and outlet ports for suffusion of PSS and drugs. PSS was continually recirculated and pumped through a heat exchanger to warm it to 37°C before it entered the arteriograph bath and was aerated with a gas mixture of 5% CO_2 -10% O_2 -85% N_2 to maintain a constant pH of 7.4 ± 0.05 . A servo system, which consisted of an in-line pressure transducer,

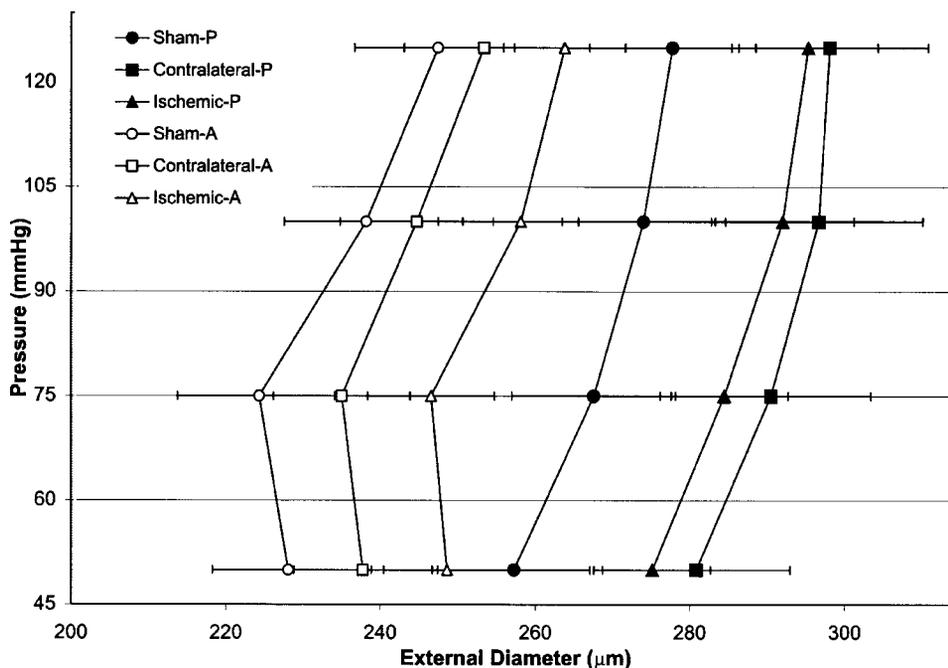


Fig. 1. Pressure-diameter relationship for sham-operated control ($n = 9$), contralateral ($n = 6$), and ischemic ($n = 9$) vessels in active and passive states within the myogenic pressure range of 50–125 mmHg. A, active smooth muscle cells (SMCs); P, passive SMCs (papaverine treated). Values are means \pm SE.

miniature peristaltic pump, and controller connected to the proximal cannula, was used to measure and control TMP. The entire chamber that contained the mounted arteries was placed on an inverted microscope with an attached videocamera and monitor to allow viewing and electronic measurement of vessel dimensions. Lumen diameter and wall thickness were measured ($\pm 1.0 \mu\text{m}$) by the video scan line, which detects the optical contrast of the vessel walls on the video monitor and generates a voltage ramp within the video dimension analyzer that is proportional to diameter (30). The output of the video dimension analyzer and pressure controller were sent to an IBM-compatible computer by means of a serial data-acquisition system (DATAQ) for visualization of dynamic responses of diameter and TMP.

Experimental protocol. Mounted and pressurized arteries were equilibrated at a TMP of 50 mmHg for 1 h. Pressure was

then increased stepwise in increments of 25 mmHg from 50 to 125 mmHg, and arterial diameter and wall thickness were recorded at each TMP once stable (~ 10 min). Active diameters were only determined within the myogenic range of 50–125 mmHg (17). In previous studies, these arteries have been shown to operate at 60–80% of systemic arterial pressure (17, 18). Once active data were obtained, papaverine (0.1 mmol/l), a compound that causes SMC relaxation, was added to the bath. This was used to obtain fully relaxed diameters and wall thickness measurements at each pressure from 0 to 200 mmHg.

Arteries studied were from two types of animals: sham-operated control ($n = 7$) and ischemic ($n = 15$). The three types of arteries studied were nonischemic sham, contralateral to ischemic, and ischemic. Calculations and statistical analysis were performed if complete data sets were available

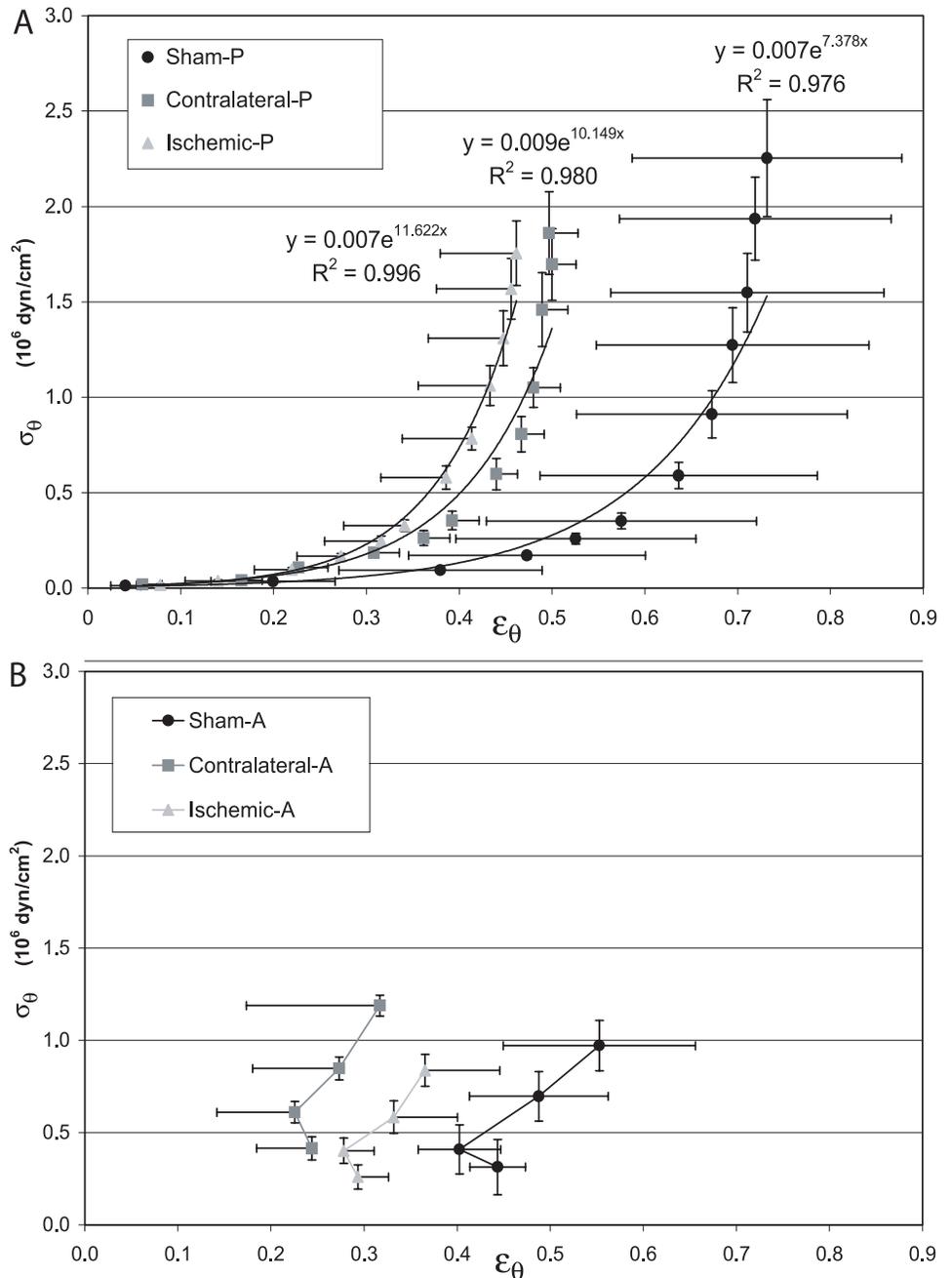


Fig. 2. Stress-strain diagrams for passive (A) and active (B) sham-operated control, contralateral, and ischemic vessels. Circumferential stress (σ_θ) and strain (ϵ_θ) are both calculated at the outer radius. Passive values are reported for the pressure range of 5–200 mmHg, and active values are reported for the pressure range of 50–125 mmHg. Exponential curves for passive vessels are also shown with the corresponding equations and R^2 values. Values are means \pm SE.

(i.e., if no diameter or wall thickness values were missing or indeterminate).

Mechanical tissue property calculations For the purpose of this study, the rat MCA is considered to be a thick-walled cylindrical tube with orthotropic elasticity. The wall material is assumed to be incompressible, homogeneous, and globally nonlinear, but incrementally linear between neighboring states of deformation.

Because a thin wall assumption (ratio of wall thickness to internal radius < 0.1) is not valid for these vessels, Laplace's equation is not an accurate calculation for determination of wall stress. Therefore, the circumferential stress (σ_θ) was calculated at the inner and outer radius using the following equation developed for a hollow cylinder submitted to uniform pressure (29)

$$\sigma_\theta(r) = \frac{r_i^2 p_i}{r_e^2 - r_i^2} \left(1 + \frac{r_e^2}{r^2} \right)$$

where r is the radius, r_i is the internal radius, r_e is the external radius, and p_i is the internal pressure, respectively. Circumferential strain (ϵ_θ) was calculated at the internal and external radius using

$$\epsilon_\theta(r) = \frac{r - r_0}{r_0}$$

where r_0 is the radius at zero pressure.

β was calculated for the passive vessels using the pressure-diameter data obtained over the entire pressure range (0–200 mmHg). A regression was performed for each vessel using the following equation (15)

$$\ln \left(\frac{p}{p_s} \right) = \beta \left(\frac{d}{d_s} - 1 \right)$$

where p is the internal pressure, p_s is a reference pressure chosen in the physiological pressure range, d is the external diameter, and d_s is the external diameter of the vessel at the reference pressure. The reference pressure was chosen to be 75 mmHg because it lies within the physiological pressure range and gave a good fit to the equation (mean $R^2 > 0.90$).

To determine the inherent wall elastic properties, the incremental elastic modulus developed for orthotropic, incompressible arteries was calculated using the following equation (19)

$$E_{inc,p} = \frac{\Delta p}{\Delta r_i} \frac{2r_i r_e^2}{r_e^2 - r_i^2} + \frac{2p r_e^2}{r_e^2 - r_i^2}$$

where Δp is the incremental change in transmural pressure, Δr_i is the corresponding change in inner radius, and r_i , r_e , and p are the internal radius, external radius, and pressure at the beginning of the increment, respectively.

A modified incremental modulus was used to determine the effect of SMC activity with increasing pressure because SMC activity alters the mechanical properties of the arteries

$$E_{inc,a} = \frac{\Delta \sigma_\theta}{\Delta r_i} \frac{2r_i r_e^2}{r_e^2 - r_i^2} + \frac{2\sigma_\theta r_e^2}{r_e^2 - r_i^2}$$

where $\Delta \sigma_\theta$ is the change in circumferential stress (calculated at the internal radius) from passive to active, Δr_i is the change in internal radius from passive to active, and the remaining terms are taken at the passive vessel state.

Statistical analysis. A repeated-measures ANOVA was performed on the $E_{inc,p}$ and $E_{inc,a}$ data using a program (BMDP 5V) that is capable of handling data containing missing values for paired data. ANOVA was used to examine

significant differences among groups for β -values, with a post hoc Bonferroni test for multiple comparisons.

RESULTS

The active, spontaneous constriction of the vessel at 75 mmHg was apparent in all vessel types (Fig. 1), indicative of myogenic behavior. The passive response, on the other hand, showed a steady increase in diameter with increasing pressure (Fig. 1). Also, the vessel diameters were noticeably smaller in the active vessels, indicating the presence of myogenic tone.

Circumferential stress-strain relationships were plotted for the active and passive vessels over the entire range of pressures (Fig. 2, A and B). Passive vessels showed the “J”-shaped curve typically seen in arteries; an exponential fit gave a good R^2 value for all passive vessel types (mean $R^2 = 0.99$). In contrast, the active vessels did not fit an exponential and displayed an irregularly shaped curve due to the activity of the smooth muscle.

The overall stiffness parameter β , calculated for passive vessels, was largest in the ischemic vessels (13.9 ± 1.7) and smallest in the shams (9.1 ± 1.1) with $P < 0.05$ (Fig. 3). Contralateral vessels had β -values (10.2 ± 0.4) that were not significantly different from sham or ischemic vessels. β was only calculated for the passive vessels because the active vessels did not have an exponential pressure-diameter curve and thus gave a poor fit to the equation (mean $R^2 < 0.55$).

$E_{inc,p}$ increased with increasing pressure for all passive vessels (Fig. 4A) but remained relatively constant in the active vessels (Fig. 4B). Passive values were also significantly higher than active values at 125 mmHg ($P < 0.05$). Passive incremental modulus values (10^6 dyn/cm²) at 75 mmHg were 5.6 ± 1.2 , 5.3 ± 0.4 , and 6.0 ± 1.0 compared with 14.7 ± 3.2 , 35.5 ± 8.1 , and 23.4 ± 5.1 at 125 mmHg for sham, contralateral, and ischemic vessels, respectively. Active modulus values (10^6 dyn/cm²) at 75 mmHg were 6.2 ± 2.7 , 4.5 ± 1.3 , and 5.7 ± 1.6 compared with 6.9 ± 1.6 , 5.6 ± 0.9 ,

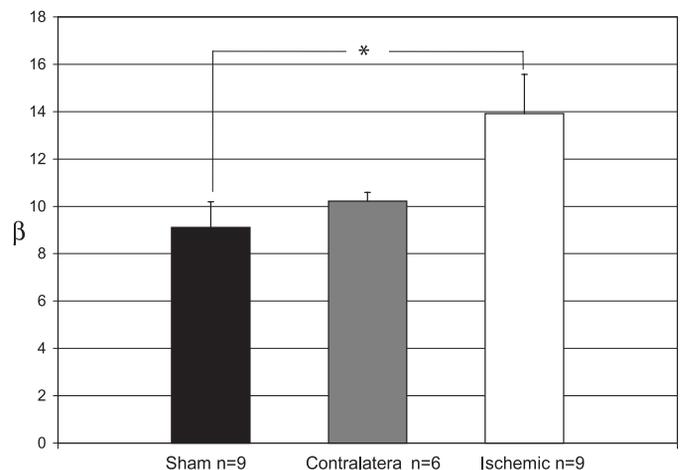


Fig. 3. Averaged values of the stiffness parameter (β) for passive sham-operated control, ischemic, and contralateral vessels. Values are means \pm SE. * $P < 0.05$.

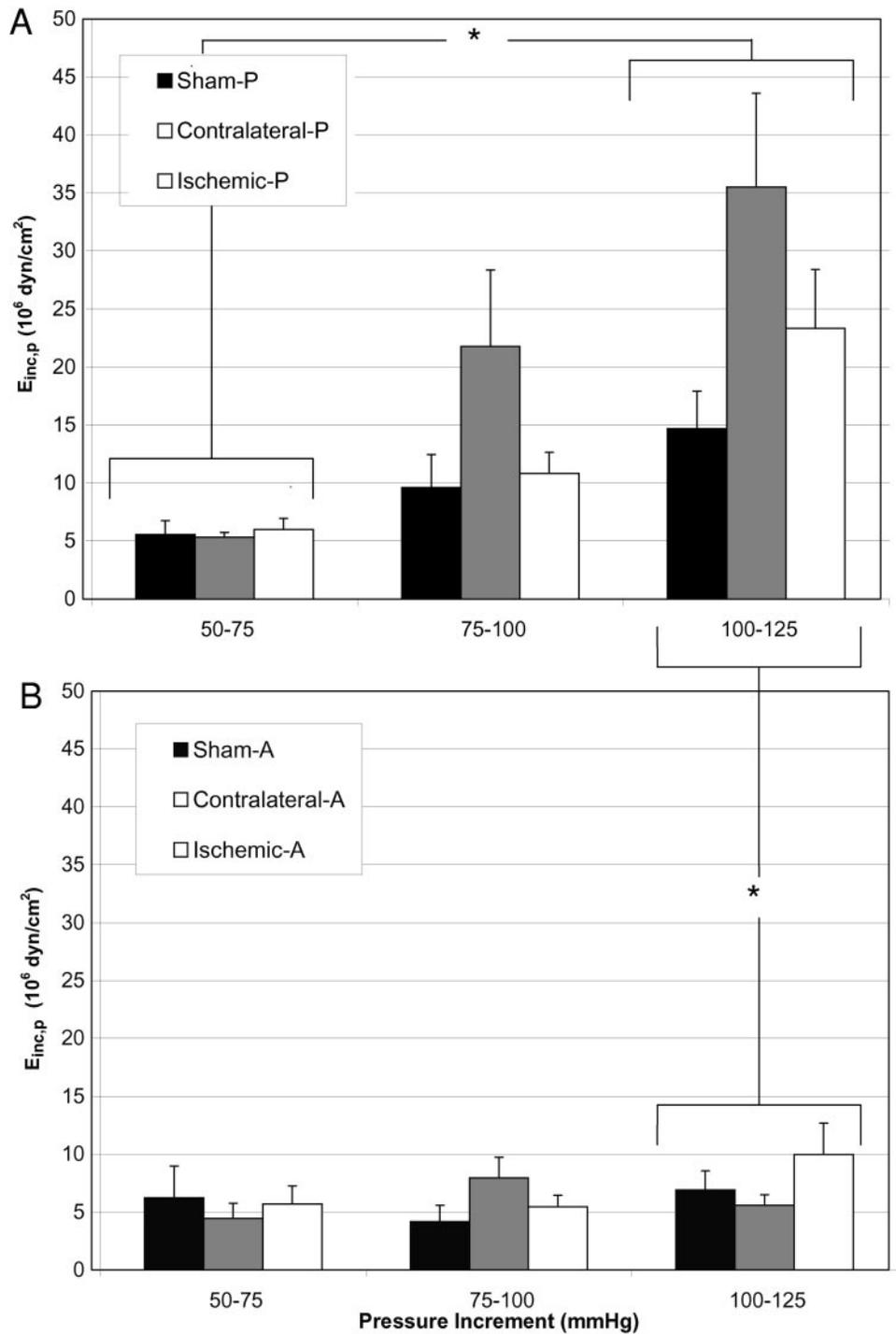


Fig. 4. Incremental elastic modulus ($E_{inc,p}$) for passive (A) and active (B) vessels at three pressure increments. Values are means \pm SE. * $P < 0.05$.

10.0 \pm 2.7 at 125 mmHg for sham, contralateral, and ischemic vessels, respectively.

$E_{inc,a}$ also increased for all vessel types with increasing pressure, indicating that the SMC activity increased with pressure (Fig. 5). Statistical analysis revealed a highly significant change in $E_{inc,a}$ over the four pressures ($P < 0.001$). Values (10^6 dyn/cm^2) at 50 mmHg were 7.3 \pm 1.2, 10.7 \pm 2.2, and 6.0 \pm 0.9 and increased to 49.4 \pm 12.6, 30.4 \pm 4.4, and 36.6 \pm 9.7 at 125 mmHg for sham, contralateral, and ischemic vessels, respectively.

DISCUSSION

In this study, we used mechanical tissue property measurements to study normal and ischemic MCAs in passive and active states. Our results indicate that ischemic vessels were stiffer than healthy vessels, which may be due to ischemia-induced short-term remodeling. The overall stiffness parameter β was significantly higher in the ischemic vessels than the sham-operated controls. The contralateral vessel stiffness, although not significantly different than that in

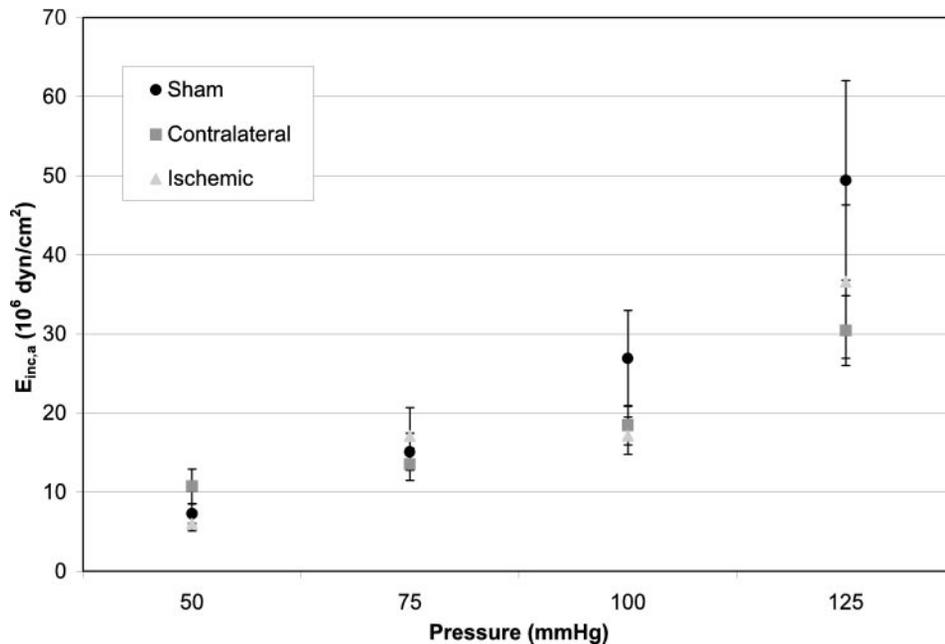


Fig. 5. Activation modulus ($E_{inc,a}$) for sham-operated control, contralateral, and ischemic vessels. The increase in $E_{inc,a}$ over the 4 pressures is highly significant ($P < 0.001$). Values are means \pm SE.

the sham or ischemic vessels, tended to be more similar to the sham vessels, indicating that the passive vessel stiffness changes were limited to the ischemic vessels. This change in tissue mechanical behavior of the ischemic arteries suggests that some remodeling may be occurring in the passive arterial wall elements (e.g., increased collagen deposition).

The passive stress-strain relationship of blood vessels is nonlinear due to the composite action of collagen and elastin found in both the internal elastic lamina and media. The initial shallow slope is due to the deformation of the highly extensible elastin fibers, and the steepest portion is due to the recruitment of the stiffer collagen fibers (20). The results of the stress-strain curves for the passive vessels mirrored the results of the β -calculations. As seen in Fig. 2A, the sham vessels showed the most distensibility. That is, for any given circumferential stress, the sham vessels showed the greatest strain. The exponential fits to the data also indicate that the ischemic vessels were the stiffest; here, the highest exponential multiplier signifies the fastest increase and thus a steeper slope. Because neither the β -calculation nor an exponential fit to the stress-strain curve can be used to determine the mechanical properties of the active vessels, comparisons between the active and passive overall stiffness were not possible.

$E_{inc,p}$, on the other hand, was calculated for all active and passive vessels (Fig. 4). This measurement of mechanical tissue behavior increased with pressure for all of the passive vessels but remained relatively constant for the active vessels. This result indicates that the passive vessels were increasingly stiff as pressure increased, whereas the SMC activity induced by pressure (i.e., myogenic reactivity) in the active vessels maintained a constant stiffness of the vessel wall. It is worth noting that at the two highest pressure increments,

$E_{inc,p}$ for the passive contralateral vessels was larger than the other two groups and increased to a greater extent as pressure increased (Fig. 4A). Although there was not a significant difference in $E_{inc,p}$ among sham, contralateral, and ischemic groups at any of the pressure intervals, the greater value for contralateral vessels does suggest there may be a global effect of ischemia that is affecting the mechanical properties of contralateral arteries. Along these lines, we have recently shown an effect of postischemic reperfusion on the myogenic activity of contralateral arteries compared with nonischemic sham controls (2). Together, these results demonstrate that there may be a global effect of ischemia and reperfusion on both the functional (e.g., myogenic tone) and mechanical (e.g., $E_{inc,p}$) properties of cerebral arteries.

Because there appears to be a global effect of ischemia on cerebral artery structure and function, comparison to a nonischemic control becomes important. In this study, we chose to compare results to a sham-operated control, in which the animals underwent anesthesia and midline neck incision but without any occlusion of blood flow. This type of vessel controls for both ischemia and procedures associated with surgery such as anesthesia. However, in this study, the sham arteries were more variable than the ischemic and contralateral arteries, possibly because they were a completely different set of animals, whereas the ischemic and contralateral arteries came from the same animals and therefore had similar variability. This is evident in the stress-strain curves shown in Fig. 2A, which show greater variability in sham vessels compared with the other two groups. Whereas this variability may be why the stress-strain curve for the contralateral vessels are closer to the ischemic vessels but the β -values are closer to the sham group, the

results are the same: that the sham vessels are the most distensible and the least stiff.

It was also observed at higher pressures that active values for $E_{inc,p}$ were lower than the passive values. At first glance, this comparison suggests that SMC activity decreases stiffness compared with passive vessels at the higher pressures. However, the smaller modulus cannot be simply interpreted as a decrease in vessel stiffness with SMC activity. The decrease in diameter with SMC activity shifted the vessel down on the stress-strain curve (Fig. 2). In this region, the passive modulus or stiffness is lower, and the strain is also smaller. So, when the passive and active elastic moduli of vessels were compared at similar strains, the pressure-induced SMC activity actually contributed significant resistance to distension (1, 9).

The activation modulus $E_{inc,a}$, developed to quantify the increase in stiffness with SMC activation, increased with higher pressures, as expected. This measure gives a direct indication of the contribution of the vascular SMC activity to stiffness at different pressures, unlike the somewhat complex comparison between active and passive $E_{inc,p}$ values. These results demonstrate that pressure-induced SMC activity in response to increased TMP (i.e., myogenic reactivity) increased the stiffness of the cerebral artery wall. The increased stiffness at higher pressures could contribute to autoregulation of cerebral blood flow by counteracting the increase in wall tension. Whether the pressure-induced increase in stiffness is due to increased force production (e.g., more actin-myosin interaction) or to active structural remodeling (e.g., SMC actin polymerization), as has been suggested previously (3), is not clear from this study. However, the increased stiffness in response to increased TMP should be considered an important contributor to myogenic reactivity.

Full, three-dimensional constitutive modeling could not be performed based on these data because axial force and strain were not measured. However, vascular SMC activation acts principally in the circumferential direction and has little effect in the longitudinal direction (8). We also assumed that the MCA was radially and axially homogeneous, when in fact it has distinct layers in the radial direction. The active properties are dominated by vascular smooth muscle tone in contrast to the passive properties, which are influenced primarily by collagen and elastin (1). However, layer-specific information is not required to characterize the mechanical behavior of the tissue and our results reflect the bulk behavior of the MCAs.

In conclusion, this study calculated the mechanical properties of healthy and ischemic rat MCAs in both passive and active states. One hour of ischemia followed by 24 h of reperfusion did not appear to cause significant differences in the active mechanical response, but did alter the overall passive stiffness of the vessel. In addition, arterial wall elasticity was strongly influenced by the activity of vascular SMCs, which led to a relatively constant modulus of elasticity over the myogenic pressure range, demonstrating that pres-

sure-induced constriction significantly increased the stiffness of the cerebral arterial wall.

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