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Mechanical Properties of Rat Middle Cerebral Arteries With and Without Myogenic Tone

The inner diameter and wall thickness of rat middle cerebral arteries (MCAs) were measured in vitro in both a pressure-induced, myogenically-active state and a drug-induced, passive state to quantify active and passive mechanical behavior. Elasticity parameters from the literature (stiffness derived from an exponential pressure-diameter relationship, β, and elasticity in response to an increment in pressure, $E_{inc\text{-}p}$) and a novel elasticity parameter in response to smooth muscle cell (SMC) activation, $E_{inc\text{-}a}$, were calculated. β for all passive MCAs was 9.11±1.07 but could not be calculated for active vessels. The incremental stiffness increased significantly with pressure in passive vessels; $E_{inc\text{-}p}$ (10^6 dynes/cm^2) increased from 5.6±0.5 at 75 mmHg to 14.7±2.4 at 125 mmHg, ($p<0.05$). In active vessels, $E_{inc\text{-}p}$ (10^6 dynes/cm^2) remained relatively constant (5.5 ±2.4 at 75 mmHg and 6.2±1.0 at 125 mmHg). $E_{inc\text{-}a}$ (10^6 dynes/cm^2) increased significantly with pressure (from 15.1±2.3 at 75 mmHg to 49.4±12.6 at 125 mmHg, $p<0.001$), indicating a greater contribution of SMC activity to vessel wall stiffness at higher pressures. [DOI: 10.1115/1.1645525]

The mechanical properties of arteries influence circulation dynamics, vascular development, and progression of arterial diseases through effects on blood flow and pressure, and arterial mass transport [1]. The two general classifications of arteries, elastic and muscular, are based not only on structure but also on function. While the mechanical properties of elastic arteries appear to be dominated by the interactions of collagen and elastin, muscular arteries modify their geometry and apparent mechanical properties by altering the degree of muscular tone [1]. Cerebral arteries are muscular vessels primarily made up of an intima and media with relatively little adventitia; in rats, up to 73% by volume of these vessels are smooth muscle cells (SMCs) [2]. The SMC activity of the cerebral vessels maintains myogenic tone, which is defined as the sustained contraction of SMCs, and myogenic reactivity, which is defined as the ability of the vascular SMCs to contract (relax) in response to increased (decreased) transmural pressure [3]. Myogenic tone and reactivity in cerebral arteries are important factors in cerebral blood flow autoregulation [4–6]. Thus, SMC activity is a critical component of the functional and mechanical properties of these muscular arteries.

The mechanical properties of arteries with pharmacologically-active SMCs have been studied [7–11], but the mechanical behavior of myogenically-active cerebral vessels remains poorly understood. In addition, the methods used to attain SMC activation vary between studies, making comparisons difficult. Goedhard et al. found that pharmacologically-induced SMC contraction in pig thoracic aortas changed the mechanical properties of the arteries [7] but did not indicate whether the level of SMC activation attained was physiological. Hayashi and coworkers used an exponential curve fit to pressure-diameter data to represent the stiffness, β, of human cerebral arteries [8]. However, this parameter is only useful when the curve fit applies. Incremental elastic moduli, which are descriptions of the radial and tangential stiffness in response to small, incremental strains [9], also have been used to characterize non-linear active and passive behavior of arteries. Incremental moduli can be calculated at active and passive states, but the comparison between the two is hampered by the baseline difference in strain. The limitations of these mechanical parameters demonstrate the need for a tissue material property calculation that can account for SMC activity in muscular arteries more directly. Such a calculation would provide insight into the pharmacologically-active state of muscular arteries as well as the myogenic response of cerebral vessels to pressure changes.

This study utilized *in vitro* pressure-diameter data to characterize the myogenically active and pharmacologically-induced passive behavior of rat middle cerebral arteries (MCAs). Rats are used frequently as animal models in studies regarding hypertension and ischemia [2,5,11–14]. To facilitate a comparison with other mechanical property calculations for arteries in nonphysiologically active states, Hayashi's β-parameter [8] and Hudetz's incremental elastic modulus [9] were calculated. In addition, a modified incremental elastic modulus that specifically incorporates the effect of active SMC contractility was developed and calculated. These material properties will provide a basis for understanding mechanical changes that occur with disease and other experimental interventions, and provide insight into myogenic mechanisms of cerebral blood flow autoregulation.

Materials and Methods

Experimental Procedures. MCAs were obtained from five male Wistar rats (weight 280–300 g). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont. Animals were euthanized by anesthesia and decapitation. The left and right MCAs were dissected from the brain, cleared of extraneous connective tissue and placed in an arteriograph chamber (Living Systems Instrumentation, Burlington, VT). The mounted vessel was suspended above an optical window within the arteriograph chamber, perfused with physiologic 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 transmural pressure was applied to the vessels, as described previously [14].

The arteriograph bath consisted of a 20 mL fluid chamber with inlet and outlet ports for suffusion of PSS and drugs. Bath PSS was continually recirculated and pumped through a heat exchanger to warm it to normal body temperature (37°C) before it

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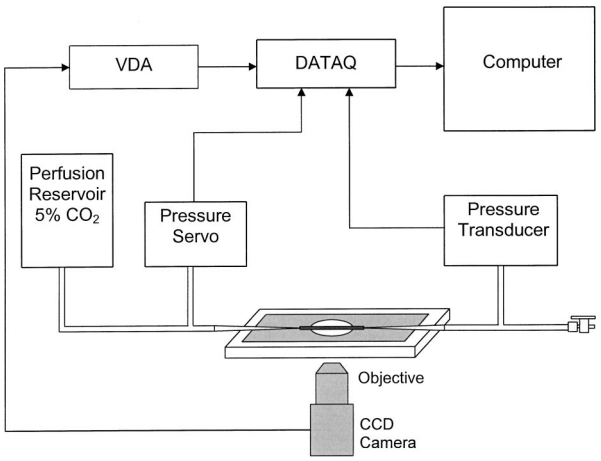

Fig. 1 Experimental setup. The vessel was sutured onto glass cannulas above an optically clear window in the arteriograph chamber. Through this window, the vessel image is magnified by an inverted microscope objective and digitized by a CCD camera. Vessel diameter and wall thicknesses (right and left) were measured from one scan line of this digitized image by a video dimension analyzer (VDA). Perfusate pressure was controlled by a servo-mechanism under computer control through a data acquisition and control system (DATAQ). Perfusate and bath temperature were maintained at 37°C by closed loop control of heating elements (not shown).

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 $\pm$0.05. A servo-system (Living Systems Instrumentation), which consisted of an in-line pressure transducer, miniature peristaltic pump, and controller connected to the proximal cannula, was used to measure and control transmural pressure. The entire arteriograph system that contained the mounted arteries was placed on an inverted microscope with an attached videocamera to allow viewing and electronic measurement of vessel dimensions [14,15]. Lumen diameter and wall thickness were quantified ($\pm$1.0 μm) by the video scan line of a video dimension analyzer, which detected the optical contrast of the translucent vessel with the opaque vessel walls on the video monitor and generated a voltage ramp, the amplitude of which is proportional to diameter. The video scan line was positioned at the axial midpoint of the vessel, and focused on the central plane to obtain maximum contrast at the walls. 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 diameter and transmural pressure (Fig. 1).

Vessels were tested according to previously established protocols [14]. Briefly, mounted and pressurized arteries were equilibrated at a pressure of 50 mmHg for 1 hour to recover from the excision and regain normal myogenic tone. Vessels that did not develop tone were discarded from the study; those with tone have been shown to have stable and reproducible responses to pressure [14]. Pressure was then increased stepwise in increments of 25 mmHg within the autoregulatory range from 50 to 125 mmHg. The step increase in pressure was achieved in one second; arterial diameter and wall thickness were recorded once stable ($\sim$10 minutes) at each pressure. In this way, short time-constant viscoelastic effects were ignored and long time-constant viscoelastic effects were accounted for by taking approximately isochronal measurements.

Once the final active data were obtained at 125 mmHg, papaverine (0.01 mmol/L), a compound that causes SMC relaxation, was added to the bath. Vessel dilation occurred in $\sim$3 minutes. After $\sim$5 minutes, the passive vessel was inflated to 200 mmHg, deflated to 0 mmHg and re-inflated to 200 mmHg at a rate of approximately 5 mmHg/s to precondition the vessel to the pressure levels of interest for passive mechanics. Passive measurements then were taken during deflation for 25, 10 and 5 mmHg steps in the sequence: 200, 175, 150, 125, 100, 75, 50, 40, 30, 20, 10, 5 and 0 mmHg. Again, step changes in pressure occurred in one second; diameter and wall thickness values were recorded once stable ($\sim$2 minutes). Preliminary experiments demonstrated less than 2% change in passive diameter values from the second to the fourth inflation/deflation preconditioning cycles from 0 to 200 mmHg. Also, no changes occurred between 2 minutes and 10 minutes at any pressure level.

Modeling and Calculations. For modeling purposes, the rat MCA was assumed to be an incompressible, homogeneous, orthotropic, thick-walled, nonlinearly elastic cylindrical tube [9,16,17]. The thin wall assumption was found to be invalid for these vessels since the mean ratio of internal radius to wall thickness was not greater than 10 at 150 mmHg (7.4$\pm$0.4 for the passive vessels).

The Cauchy stress in the circumferential direction at the internal radius r_i was calculated as:

$$\sigma_\theta = \frac{p(r_i^2 + r_e^2)}{r_e^2 - r_i^2} \tag{1}$$

where r_e, and p are the external radius and transmural pressure, respectively [18]. The equation for radial stress reduced to $\sigma_r = -p$ at the inner surface [18]. Circumferential Almansi strain for large deformations was calculated at the internal radius using:

$$e_\theta = \frac{1}{2}\left(1 - \frac{1}{\lambda_\theta^2}\right) \tag{2}$$

[16] where the circumferential stretch ratio $\lambda_\theta = 2\pi r_i / 2\pi r_{i_0}$ and r_{i_0} is the internal radius at zero transmural pressure. Radial strain at r_i was calculated similarly using:

$$e_r = \frac{1}{2}\left(1 - \frac{1}{\lambda_r^2}\right) \tag{3}$$

where the radial stretch ratio $\lambda_r = 1/\lambda_\theta$ based upon the assumption of incompressibility ($\lambda_r \lambda_\theta \lambda_z = 1$) and zero axial strain ($\lambda_z = 1$).

The stress-strain behavior of vessels in active and passive states characterizes their behavior under the applied loading conditions but does not provide a single parameter with which comparisons to other states and conditions are easily made. Hayashi's β parameter, on the other hand, does provide a single measure of tissue behavior with which healthy and disease states can be compared. Since it is essentially a curve-fitting technique, no material property assumptions are required. The logarithmic transformation of the pressure-diameter data and linear regression to find β was performed for each vessel using the following equation [8]:

$$\ln\left(\frac{p_i}{p_s}\right) = \beta\left(\frac{r_e}{r_{e_s}} - 1\right) \tag{4}$$

where p_s is a reference pressure chosen in the physiological pressure range and r_{e_s} is the external radius of the vessel at the reference pressure. A reference pressure of 75 mm Hg was chosen because it lies within the physiologic pressure range and gave a good fit to the data (mean $R^2 > 0.90$). Note, β can only be reasonably calculated for vessels that exhibit an exponential pressure-diameter curve. The pressure-diameter data of active vessels in the myogenic range, for example, were poorly fit by this equation.

The incremental elastic modulus, by contrast, is well-suited to nonexponential and nonlinear stress-strain behavior and to modeling data obtained with the experimental protocol used in this study. In particular, Hudetz developed an elastic modulus formulation for orthotropic, incompressible arteries with nonlinear behavior pressurized at a fixed length using the mechanics of incremental deformations [9]. The step-wise increments in pressure to

which vessels were subjected in this study in both the active and passive states were precisely incremental deformations. A brief review of the derivation for this incremental modulus will provide useful background for a novel modification we performed that models the effect of SMC activity.

The equation of equilibrium for an incompressible elastic tube under axisymmetric plane strain conditions with incremental stresses is given as:

$$\frac{\partial T_r}{\partial r}+\frac{\mathbf{T}_r-\mathbf{T}_\theta}{r}=0 \tag{5}$$

where $\mathbf{T}_r$ and $\mathbf{T}_\theta$ are the radial and tangential components of the incremental Kirchoff-Piola stress tensor. Using the linear-incremental correspondence principle along with the equation of equilibrium and incremental constitutive equations, Hudetz's first expression for incremental modulus was:

$$E_{inc}=\frac{\Delta p'}{\Delta r_i}\frac{2r_i r_e^2}{r_e^2-r_i^2} \tag{6}$$

where $\Delta p'$ is an incremental Kirchoff-Piola stress. Because it cannot be measured directly, this stress term is replaced using the following approximate conversion between Cauchy stress and Kirchoff-Piola stress:

$$\mathbf{T}_r=\mathbf{t}_r+t_{0r}\frac{u_r}{r} \tag{7}$$

where $\mathbf{t}_r$ is an incremental Cauchy stress in the radial direction, t_{0r} is the initial radial Cauchy stress, u_r is the radial displacement, and r is the radius [9]. At $r=r_i$, $\mathbf{t}_r$ is the incremental change in pressure and t_{0r} is the pressure at the beginning of the increment, which gives the following relationship:

$$\Delta p'=\Delta p+p\frac{\Delta r_i}{r_i} \tag{8}$$

where Δp is the incremental change in transmural pressure, and Δr_i is the corresponding change in internal radius over the increment. Substitution of equation (8) into equation (6) gives the elastic modulus developed by Hudetz to characterize vessel nonlinear mechanical properties under isometric conditions. In particular, this modulus quantifies the vessel response to a step change in pressure. We denote this by annotating the traditional incremental modulus nomenclature, E_{inc}, with the subscript "p" for pressure. This parameter was calculated for both active and passive vessels according to:

$$E_{inc\text{-}p}=\frac{\Delta p}{\Delta r_i}\frac{2r_i r_e^2}{r_e^2-r_i^2}+\frac{2pr_e^2}{r_e^2-r_i^2} \tag{9}$$

where all nonincremental (non-Δ) parameters are taken at the beginning of the increment. In this equation, the second term is a correction term that reflects the influence of the initial pressure, p, on the modulus [9].

Based on this derivation and set of assumptions, we developed a new incremental modulus that describes the incremental elastic modulus with SMC activity. In particular, the radial stress (i.e., pressure) was replaced with the circumferential stress (σ_θ, from equation (1)), where now the increment is taken from the passive to the active state at a fixed pressure. That is, equation (8) was modified to:

$$\Delta p'=\Delta\sigma_\theta+\sigma_\theta\frac{\Delta r_i}{r_i} \tag{10}$$

where $\Delta\sigma_\theta$ is the change in circumferential stress (calculated at the internal radius) from passive to active, σ_θ is the stress calculated with SMCs passive, and Δr_i is the change in internal radius from passive to active. Then, substitution of equation (10) into equation (6) gives the increment in modulus with SMC activity denoted by the subscript "a" for activation:

$$E_{inc\text{-}a}=\frac{\Delta\sigma_\theta}{\Delta r_i}\frac{2r_i r_e^2}{r_e^2-r_i^2}+\frac{2\sigma_\theta r_e^2}{r_e^2-r_i^2} \tag{11}$$

where again all nonincremental (non-Δ) parameters are taken at the beginning of the increment (i.e., at the passive state). Analogous to equation (9), the second term above is a correction that reflects the condition of the vessel at the initial state of the increment.

By directly substituting $\Delta\sigma_\theta$ for Δp in equation (8), we developed a stiffness measure that is analogous to the radial incremental modulus but is not strictly in the radial direction. To instead calculate a true change in radial stiffness resulting from a change in SMC activity, one could equivalently calculate the change in pressure (radial stress) that resulted from the change in activity (from Eq. 1) and then substitute this pressure increment into equation (8). The result would be only slightly different from equation (11) such that:

$$E'_{inc\text{-}a}=\frac{\Delta\sigma_\theta}{\Delta r_i}\frac{2r_i r_e^2}{r_e^2+r_i^2}+\frac{2\sigma_\theta r_e^2}{r_e^2+r_i^2} \tag{12}$$

For the purposes of this study, we used equation (11) to quantify incremental stiffness with SMC activity.

Note, whereas the increment in pressure is actual, the increment in SMC activity is virtual. That is, experimentally, the vessel was subjected to incremental pressure steps in the active and then passive state. The vessel was not held at one pressure and allowed to become myogenically active after being pacified pharmacologically as this calculation might suggest. Instead, the difference between the active and passive hoop stress is transformed to obtain an incremental stiffness with an increment in SMC activity. To illustrate, if the active hoop stress is identical to the passive hoop stress, $E_{inc\text{-}a}$ is zero (at that pressure). Or, if the active hoop stress is larger than the passive hoop stress by some multiplicative factor that is independent of pressure, then $E_{inc\text{-}a}$ will be constant with pressure. Therefore, this activation modulus is a quantitative measure of the effect of SMC activity on vessel stiffness, which can be used to determine the pressure-dependence of myogenic activity in isolated vessels.

Statistical Analysis. A repeated measures analysis of variance (ANOVA) was performed on the $E_{inc\text{-}p}$ and $E_{inc\text{-}a}$ data using a program capable of handling data containing missing values for paired data (BMDP 5V, Statistical Solutions Ltd., Boston, MA). Comparisons between $E_{inc\text{-}p}$ values were made using three different factors: state (active or passive SMCs), side (left or right), and pressure increment (50–75, 75–100, and 100–125 mmHg). Comparisons between $E_{inc\text{-}a}$ values were made using side and pressure (50, 75, 100, and 125 mmHg) effects. Differences between groups were considered significant at $p<0.05$. Model fits were considered highly correlated for $R^2>0.9$.

Results

Complete data were obtained for four right MCAs and five left MCAs from five animals. Statistical analysis indicated that there was no interaction between side, state, and pressure and also no interaction between side and pressure. Since there were no significant differences between the behavior for left and right MCAs, the two sides were grouped together for the remaining analyses ($n=9$).

The average inner diameter and wall thickness at 100 mmHg were 237 ± 39 μm and 18.3 ± 6.2 μm under passive conditions and 206 ± 35 μm and 19.3 ± 5.4 μm under active conditions. The average active and passive stress-strain relationships were calculated for each vessel according to equations (1), (2), and (3) (Fig. 2). The passive vessels exhibited the typical "J"-shaped exponential

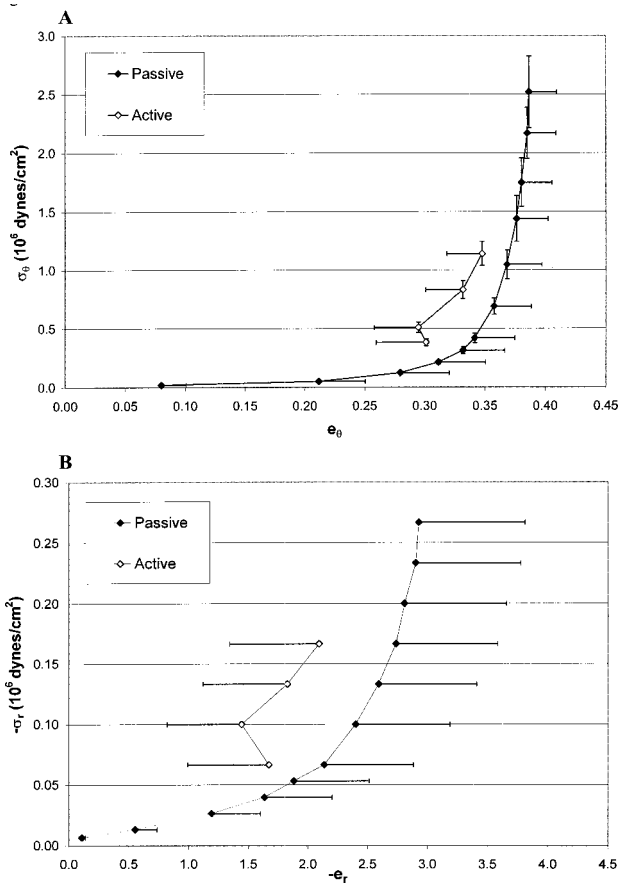

Fig. 2 Circumferential (A) and radial (B) Cauchy stress and Almansi strain for passive and active rat MCAs. Active values are reported for the 50–125 mmHg pressure range (◇). Passive values are reported for the 5–200 mmHg pressure range (◆). Stress and strain were both calculated at the inner radius. The reference state used for the active and passive strain calculations was the passive inner radius at 0 mmHg. Values are mean ±SE.

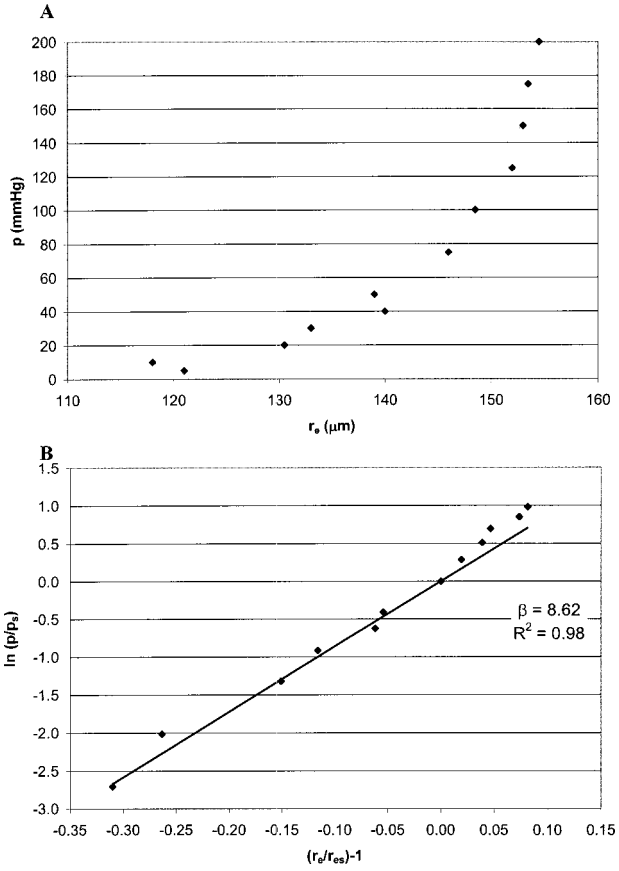

Fig. 3 Representative pressure-diameter relation (A) and logarithmic transformation with linear regression (B) to show how β was determined for each vessel where p is the transmural pressure, p_s is a reference pressure, r_e is the external radius, and r_{es} is the external radius measured at the reference pressure. The reference pressure chosen within the physiologic pressure range was 75 mmHg.

stress-strain curve seen in arteries: highly distensible at small strains, with increasing stiffness at higher strains. The active vessels, on the other hand, behaved much differently. A decrease in strain due to smooth muscle activation between 50 and 75 mmHg generated an almost parabolic stress-strain curve within the myogenic pressure range. Within this range, the circumferential stress and strain were lower in the active vessels than in the passive vessels for a given transmural pressure. Radial stress and strain are both negative due to the fact that radial stress reduces to $-p_i$ at the internal radius and in the radial strain calculation (Eq. 3) $1/\lambda_r > 1$.

The average β value for all passive MCAs was 9.11 ± 1.07 (mean $R^2 > 0.9$). 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 $R^2 = 0.52$). A representative pressure-diameter response and the regression used to find β for the vessels are shown in Fig. 3.

$E_{inc\text{-}p}$ increased significantly with pressure in the passive vessels ($p < 0.05$), and remained relatively constant in the active vessels. Passive $E_{inc\text{-}p}$ values (10^6 dynes/cm^2) within the myogenic pressure range were: 5.6 ± 0.5 (at 75 mmHg), 9.6 ± 2.1 (at 100 mmHg), and 14.7 ± 2.4 (at 125 mmHg). Comparable active $E_{inc\text{-}p}$ values (10^6 dynes/cm^2) were: 5.5 ± 2.4 (at 75 mmHg), 3.1 ± 0.4 (at 100 mmHg), and 6.2 ± 1.0 (at 125 mmHg). $E_{inc\text{-}p}$ was plotted versus both transmural pressure and circumferential strain in Fig. 4. When plotted versus pressure, it is evident that the incremental modulus for the passive vessels at higher pressures is larger than the modulus for active vessels. When plotted versus strain, on the other hand, $E_{inc\text{-}p}$ is higher in the active vessels for a given strain.

The activation modulus, $E_{inc\text{-}a}$, increased significantly with increasing pressure ($p < 0.001$). Values (10^6 dynes/cm^2) at each pressure were: 7.3 ± 1.2 (50 mmHg), 15.1 ± 2.3 (75 mmHg), 26.9 ± 6.1 (100 mmHg), and 49.4 ± 12.6 (125 mmHg) (Fig. 5).

Discussion

Active and passive mechanical properties were measured for normal, isolated rat MCAs. Our results indicate that vascular smooth muscle activation caused by changes in transmural pressure (i.e., myogenic reactivity) had a significant influence on the mechanical behavior of these arteries. The shift to the left of the stress-strain curves for the active vessels (Fig. 2) indicates a stiffening of the vessels due to SMC activation. Because the active vessels experience smaller strains than the passive vessels for the same range of stress, they are effectively stiffer. In addition, the shape of the active stress-strain curve is very different than the passive response due to the active constriction of vessels at the lower limit of the myogenic range. Between 50 and 125 mmHg the vessels actively constrict such that they do not dilate with increasing pressure. If smooth muscle tone is lost and a vessel is overly distensible, it will not be able to effectively control the blood flow by expanding and contracting the lumen diameter. This

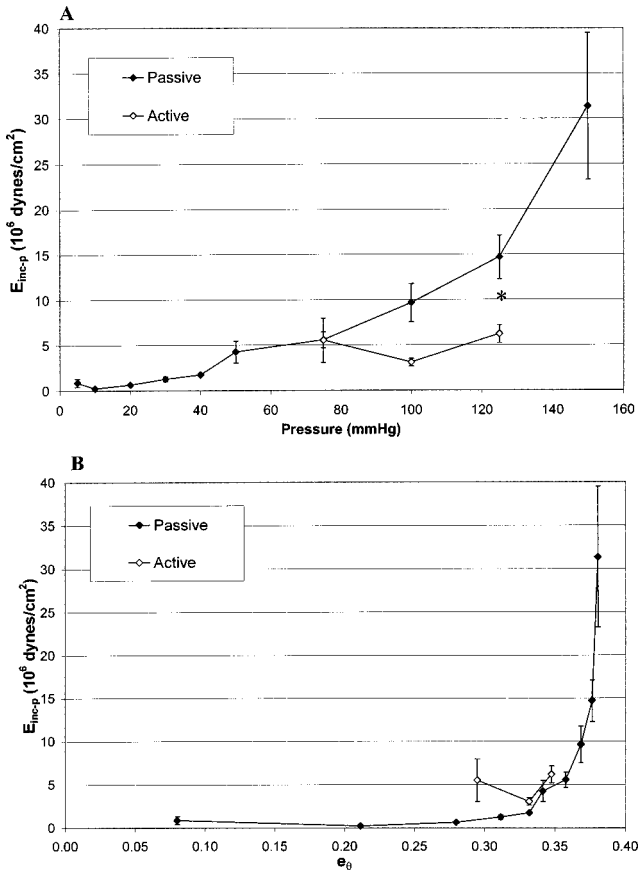

Fig. 4 Incremental elastic modulus, E_{inc-p}, for passive (♦) and active (◇) vessels versus transmural pressure (A) and circumferential Almansi strain (B). Values are mean±SE. *p<0.05.

demonstrates how the mechanical properties (e.g., stiffness) of the cerebral vessels can contribute to the autoregulation of cerebral blood flow.

The stiffness parameter, β, has been used as a simple and reliable parameter for describing the pressure-diameter relationship in arteries [1]. Hayashi et al. found β values for rabbit thoracic and femoral arteries ranging from 3.3 to 20, respectively [19]. Thus, our average β value (9.01) for rat MCAs is comparable to those found previously. This parameter is useful because it describes the intrinsic material properties dimensionlessly [20], so comparisons can easily be made between different vessel types and states (healthy, aged, diseased or damaged). The drawback to this parameter is that it is only useful for passive wall mechanics or vessels with exponential pressure-diameter relationships. In our experiments, active vessels did not display an exponential pressure-diameter relationship; so β was not a useful parameter for arteries with active SMCs.

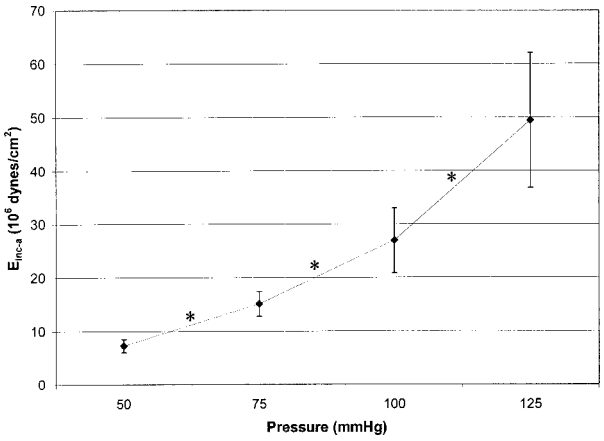

Fig. 5 Activation modulus, E_{inc-a}, calculated at transmural pressures ranging from 50 to 125 mmHg. The slope of the monotonic increase is significantly greater than zero between all pressures (*p<0.001). Values are mean±SE.

Incremental elastic moduli, by constrast, quantify nonexponential and nonlinear stress-strain relationships in an arterial wall. They have been measured for many different artery types over a wide range of pressure. Values for rabbit carotid, femoral and thoracic arteries at 100 mmHg have been reported to be 35, 23, and 13×10^6 dynes/cm^2, respectively [19]. Human anterior cerebral and internal carotid arteries at 100 mmHg have been reported to have incremental moduli of 57 and 30 $\times10^6$ dynes/cm^2, respectively [21]. Our values for passive rat middle cerebral arteries are slightly lower, but still within a comparable range. This wide range of moduli is attributable to the fact that different arteries with various functions and structures are being compared, and, in addition, different experimental methods and modulus formulations were used.

The form of the equation used to calculate our incremental elastic modulus, E_{inc-p}, was chosen because it was developed for an incompressible, orthotropic artery held at a fixed length, which was preferable to other moduli calculations which were developed using an isotonic (fixed force) assumption [1,9]. In this study, E_{inc-p} was calculated for arteries with both active and passive SMC states. E_{inc-p} values are different between active and passive vessels, but whether they are higher or lower for passive vessels depends upon whether they are compared at the same transmural pressure or the same circumferential strain. E_{inc-p} is higher in passive vessels when plotted versus pressure, but it is higher in active vessels when plotted versus strain. According to Dobrin, the explanation for this disparity is that different strains are associated with each pressure when the vascular muscle is active and when the muscle is relaxed [10]. The higher passive stiffness at a given pressure is due to the fact that the vessel wall is already strained at higher pressures and there is ultimately a smaller increment in strain for a given increment in pressure. Activation of the muscle clearly causes constriction to smaller overall strains, and this reduction in diameter decreases the elastic modulus of the vessel. This activity of the SMCs is important in maintaining and controlling the vessel diameter within the myogenic pressure range.

The activation modulus, E_{inc-a}, was developed to provide a direct indication of the contribution of vascular SMC activity to vessel wall stiffness, unlike the somewhat complex comparison between active and passive E_{inc-p} values. This measure increased with higher pressures as expected. Active SMCs in canine carotid arteries have been shown to exhibit a maximum elastic modulus of 4×10^6 dynes/cm^2 for the whole wall and an estimated 12.7 $\times10^6$ dynes/cm^2 for the smooth muscle component of the wall [10]. This active elastic modulus was calculated by subtracting the elastic modulus of the potassium cyanide-treated vessel from the modulus of the norepinephrine-treated vessel at equivalent strains. The correction for the proportion of the wall that is smooth muscle is obtained by dividing the whole wall modulus by (1—SMC fraction). If the same procedure is performed on our rat MCA, we find that the maximum active elastic modulus is 6.6 $\pm2.3\times10^6$ dynes/cm^2, with an estimated SMC component modulus of 26.4×10^6 dynes/cm^2. These values are similar to those reported previously [10], and the SMC component modulus is comparable to our activation moduli values at 100 mmHg.

Because axial force and strain were not measured in this study, 3-D constitutive modeling was not performed. Attempts to fit these data to pseudo-strain energy equations using a plane strain

assumption with a stretch ratio of 1 resulted in poorly fit parameters, and was an unreliable use of these data. Also, we have assumed that the MCA was radially (and axially) homogeneous, when in fact it has distinct layers. Thus, our results reflect the bulk elastic behavior of the tissue. Other limitations of the model include the assumptions of orthotropy, which fails if the SMCs have a substantially helical arrangement, incremental linearity, and the nonmechanistic nature of the equations. We furthermore did not analyze tissue viscoelastic behavior or residual stress, and did not explore other parameterized, continuous functions to fit the data.

With regard to the experiments, there is some variability in the literature regarding the use of cyclic preconditioning before active SMC measurements in isolated vessels. In the study of myogenic reactivity in MCAs, protocols more uniformly do not use preconditioning (see [13,14] and [22–26], for example). We chose not to use cyclic preconditioning before active measurements to be consistent with the majority of previous work on this vessel type. However, as Fridez et al. state for drug-induced vasoconstriction in rat carotid arteries, preconditioning should decrease the SMC contractile response [11]; if this is also true for myogenic reactivity, the data reported here may overestimate pressure-induced SMC activity. In the passive vessels, one inflation to the maximum pressure followed by one complete deflation/inflation was sufficient to achieve reproducible results. More cycles may not have been required because the vessels were already preconditioned by prior steps.

These experiments also did not consider the effects of luminal flow, which modulates the development and degree of myogenic tone. In particular, in the cerebrovascular circulation, a change in flowrate can cause either dilation or constriction, depending on the pressure and the level of induced myogenic tone [27]. We did not investigate the interactions between pressure and flow on SMC activity in these vessels. In addition, it has been reported that ex vivo measurements of vascular biomechanics may differ substantially from in vivo measurements [28]. Thus, extrapolation from these data to the complex situation *in vivo*, with time-varying blood pressure and flow, should be done with caution.

Conclusions

There are few reports on the mechanical properties of MCAs and fewer still regarding the mechanics of rat MCAs. Because these properties are important in the study of cerebrovascular conditions such as cerebral aneurysms, vasospasm and ischemia, a baseline understanding of normal MCA myogenic tone and reactivity is useful. We have shown that the pressure-induced activation of vascular smooth muscle contributes greatly to the effective modulus of the arteries. In addition, the amount of SMC activation increases significantly with increases in pressure. These findings could be important in future studies regarding cerebral autoregulation and clinical treatments for stroke and hypertension and their concurrent effects on vascular mechanics and vascular biology.

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