Electromechanical Alterations in the Cerebrovasculature of Stroke-Prone Rats

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Background and Purpose—Cerebrovascular pressure-dependent constriction (PDC) is associated with smooth muscle (SM) depolarization and Ca$^{2+}$ influx through voltage-gated channels. We studied the alterations in electromechanical contraction in the middle cerebral arteries (MCAs) of stroke-prone Wistar-Kyoto spontaneously hypertensive rats (SHRsp) in relation to the stroke-related loss of PDC.

Methods—Constriction to pressure, elevated [K$^+$], and/or [Ca$^{2+}$], and SM membrane potentials (E_m) were measured in isolated pressurized MCAs of SHRsp and stroke-resistant SHR.

Results—MCAs of SHRsp exhibited an age-related decrease in PDC before hemorrhagic stroke and a loss of PDC after stroke. At 100 mm Hg, the MCAs of poststroke SHRsp maintained partial constriction that was not altered with pressure but was inhibited by nifedipine (1 μmol/L). The MCAs of poststroke SHRsp constricted to vasopressin (0.17 μmol/L) but not to elevated [K$^+$]. When pressure was reduced from 100 to 0 mm Hg, the MCAs from young prestroke SHRsp exhibited SM hyperpolarization (−38 to −46 mV), whereas those of poststroke SHRsp maintained a constant, depolarized E_m (−34 mV). Alterations in E_m with varying [K$^+$] suggested that there was a decrease in SM K$^+$ conductance in the MCAs of poststroke SHRsp.

Conclusions—The observation that the MCAs of poststroke SHRsp depolarize but do not constrict to elevated [K$^+$], suggests the presence of dysfunctional voltage-gated Ca$^{2+}$ channels. The inability to alter E_m with pressure or to constrict to depolarization could partially contribute to the loss of PDC in the MCAs of poststroke SHRsp. (Stroke. 2000;31:751-759.)

Key Words: calcium channels • membrane potentials • myogenic regulatory factors • nifedipine • rats

Pressure-dependent constriction (PDC) of smaller blood vessels is thought to play an important role in the autoregulation of blood flow.\(^1\) Elevations in blood pressure (BP) that might increase blood flow are counteracted by an increase in vascular resistance to flow, which helps maintain blood flow constant. In preliminary studies, we have observed that PDC is attenuated in the middle cerebral arteries (MCAs) of the Wistar-Kyoto strain of stroke-prone spontaneously hypertensive rats (SHRsp) before stroke in the MCAs of SHRsp.\(^2\) We believe that the inability to decrease lumen diameter in response to elevated pressure during hypertension could promote cerebrovascular overperfusion. The increased downstream pressure and elevated endothelial shear resulting from the loss of PDC could compromise the blood-brain barrier and contribute to the initiation of cerebral hemorrhage.

The signal transduction mechanisms promoting PDC are not fully understood but probably involve both an intercellular influx of Ca$^{2+}$ and the activation of protein kinase C.\(^2\)–\(^8\) Electrophysiological studies of both cerebral and renal vessels have shown that PDC is associated with smooth muscle (SM) depolarization.\(^4\)–\(^7\)–\(^8\) In vitro and in vivo studies involving pressurized rat and cat MCAs or rat pial cerebral arteries, as well as dog renal interlobular and preglomerular arterioles, have indicated that PDC is dependent on the presence of external Ca$^{2+}$ and is inhibited by L-type Ca$^{2+}$ channel blockers.\(^2\)–\(^4\)–\(^8\) It is possible that constriction in response to elevated pressure could be partially produced by pressure-dependent depolarization and the subsequent opening of voltage-gated Ca$^{2+}$ channels.\(^9\)

The present study was undertaken to determine whether the electromechanical function of the MCAs was altered with stroke development in SHRsp in a manner that could contribute to the loss of PDC. In the study, the relative sensitivity and reactivity of PDC at 100 mm Hg to nifedipine and external Ca$^{2+}$ were measured in the MCA. In addition, PDC was temperature-inactivated, and the contractile reactivity of the MCA to depolarization (produced by altering external [K$^+$]) was determined. Electrophysiological studies were performed to determine whether the SM resting membrane potential (E_m) and pressure-dependent depolarization were altered in relation to stroke development. Through the experiments we have performed, we will attempt to demonstrate...
that stroke development in SHRsp is associated with a defect in the ability of the MCA to alter $E_m$ in relation to pressure and to constrict in response to depolarization. We believe that the inability to constrict in response to depolarization in the MCA of poststroke SHRsp may be partially promoted by the inability of SM depolarization to initiate Ca$^{2+}$ entry into the SM through L-type Ca$^{2+}$ channels.

### Materials and Methods

#### Animals

Wistar-Kyoto stroke-resistant spontaneously hypertensive rats (srSHR) and poststroke SHRsp were sampled from colonies of animals maintained at our institution, and the experiments were performed with institutional approval in a manner consistent with the guidelines on animal care. SHRsp fed a Japanese-style diet containing 4% NaCl and 0.75% K$^+$ develop stroke at 12 weeks of age (the first behavioral signs of stroke observed are involuntary forepaw flexion, lethargy, some paralysis) and exhibit a 100% mortality associated with stroke by $\sim$17 weeks of age. SrSHR fed the same diet do not develop stroke. In the present study, SHRsp between the ages of 9 and 16 weeks that had not yet developed stroke and poststroke SHRsp 12 to 18 weeks of age were sampled and compared with each other and with SrSHR between 10 and 18 weeks of age. In our colony of SHRsp, a 12.5-week-old SHRsp that has not developed behavioral signs of stroke and (on sampling) shows no evidence of cerebral hemorrhage has an $\sim$50% chance of developing stroke in the near future. The probability of stroke development in the near future increases dramatically in prestroke SHRsp when they surpass 12.5 weeks of age. Hence, physiological/pharmacological changes occurring in SHRsp after 12.5 weeks of age best represent the changes that occur just before stroke development. Such alterations may be important in the development of stroke. Therefore, in some experiments, prestroke SHRsp were partitioned into animals older or younger than 12.5 weeks. The systolic blood pressure (BP) of the animals was measured with a tail-cuff compression method (PE 300, Programmed Electrophysgmonanometer, Narco Biosystems Inc.).

Animals exhibiting and lacking symptoms of stroke were anesthetized with sodium pentobarbital 60 mg/kg IP and exsanguinated. The brain (including the brain stem) was removed and placed in oxygenated ice-cooled (3°C) Krebs physiological salt solution (95% O$_2$, 5% CO$_2$, pH 7.4). The right or left MCA at a point at which it crosses the rhinalis fissure was removed and mounted in a pressure myograph. The rest of the brain was placed in fixative (4% formaldehyde, 1% glutaraldehyde, 84 mmol/L PO$_4$, pH 7.4) and histologically examined for lesions. In our colony of SHRsp, stroke development is associated with the occurrence of intracerebral hemorrhage, and the observation of subarachnoid hemorrhage is rare. All poststroke SHRsp and SrSHR used in the study lacked behavioral symptoms of stroke and had brains lacking cerebral hemorrhage. The presence of cerebral hemorrhage was identified in all poststroke SHRsp.

#### Pressure Myograph Studies

The experiments were performed at an end pressure of 100 mm Hg. There is a pressure drop of $>50\%$ between the BP measured in the femoral or carotid arteries and distal segments of the middle cerebral vasculature. Therefore, studies performed at a pressure of 100 mm Hg represent a realistic mean BP that the distal MCAs of SrSHR and SHRsp (systolic BP averages between 200 and 230 mm Hg) might experience in vivo.

The techniques and equipment used to measure PDC are described in detail elsewhere. The technique was developed to differentiate between the degree of constriction being maintained as a result of pressure activation (ie, PDC) versus pressure-independent tone (PIT) in a pressurized artery. Initially, the MCAs were excised from the brain of the SHRsp and mounted on the pipette submerged in oxygenated Krebs solution at 37°C in the pressure myograph. This aspect of the procedure usually takes 10 to 15 minutes. Subsequently, they were pressurized to 100 mm Hg for 45 minutes. Typically, under these conditions the MCAs of srSHR or SHRsp develop tone during the equilibration period. To distinguish between PIT and PDC, the MCAs were then subjected to a subsequent 6-minute equilibration period at 0 mm Hg followed by 4 minutes at 100 mm Hg. By definition, PDC should be abolished if the artery is maintained at a pressure of 0 mm Hg. To measure the degree of tone that maintains PDC at 100 mm Hg pressure, one cannot simply measure the differences in lumen diameter at 0 or some other low pressure versus that present at 100 mm Hg. At the low pressure, the decreased PDC (which would tend to dilate the artery) is counteracted by the presence of a smaller distending pressure that would tend to reduce the arterial lumen size. The latter problem was overcome by equilibrating the artery to 0 mm Hg for 6 minutes to deactivate PDC, then rapidly applying a 100-mm Hg pressure step. The lumen diameter present at 100 mm Hg shortly (1 second) after pressurization, before a significant degree of PDC developed, was measured. The degree of constriction that occurred between 1 second and 4 minutes after the application of the 100-mm Hg pressure step was recorded as a measure of PDC. Previous time-course analyses of the rate of constriction indicated that the predicted lumen diameter that would be present at time 0 differed by $<5\%$ of that measured 1 second after pressurization. Therefore, the latter lumen diameter represents the luminal approximation of the lumen diameter that would exist at 100 mm Hg pressure if the artery lacked the ability to constrict to pressure. PDC was typically complete within 3 minutes after pressurization to 100 mm Hg. The difference in lumen diameter present between 1 second after pressurization and 100 mm Hg pressure step that present at 100 mm Hg under conditions in which the artery was maximally dilated represented the degree of constriction being maintained by PIT. PIT can be defined as the endogenous constriction present in the artery that cannot be increased by elevating arterial pressure or decreased by lowering pressure.

#### Nifedipine and Ca$^{2+}$ and K$^+$ Dose-Response Curves

The ability of nifedipine to relax the MCA was tested at a pressure of 100 mm Hg under conditions in which the MCAs were bathed with Krebs solution containing a normal [Ca$^{2+}$], of 2.5 mmol/L in the presence of 4.6 or 100 mmol/L [K$^+$], (Krebs solution was kept isomotic by the removal of Na$^+$). The maximal ability of nifedipine to relax the MCAs was compared with that of 10$^{-3}$ mmol/L verapamil and Ca$^{2+}$-free Krebs solution containing 1 mmol/L EGTA.

In other experiments, MCAs were pressurized to 100 mm Hg under conditions in which the arteries were bathed with normal (4.6 mmol/L) or high- (100 mmol/L) [K$^+$], Krebs solution. The arteries were initially suffused with Ca$^{2+}$-free Krebs solution containing 200 μmol/L EGTA for 2 minutes to remove external Ca$^{2+}$ bound to the artery and the perfusion chamber. Subsequently, the suffusate was replaced with Ca$^{2+}$-free Krebs solution lacking EGTA. Ca$^{2+}$ was then introduced in a dosewise fashion from 1-μmol/L to 9.0-mmol/L levels. The contractile reactivity (amplitude of constriction) in response to [Ca$^{2+}$], was measured.

The reactivity of the MCAs to varying levels of [K$^+$], ranging from 5 to 85 mmol/L was tested in the presence of a normal [Ca$^{2+}$], of 2.5 mmol/L. These experiments were performed at 23°C. At 37°C, pressurized MCAs from pretestroke SrSHR constrict to pressure, whereas those of poststroke SHRsp exhibit PIT. In view of this, we were concerned that the ability of the arteries to further constrict to elevations in [K$^+$], might be limited and differentially modified by the presence of differing levels of preexisting tone. To compare the MCAs under equivalent conditions, pressure-induced and pressure-independent constriction was inhibited in the MCAs of SrSHR and pretestroke SHRsp by lowering the bath temperature to 23°C. The notion of using this manipulation comes from an extrapolation of studies performed by Osol and Halpern, who observed that cooling (from 37°C to 35°C) pressurized posterior cerebral arteries sampled from SHRsp attenuates vasomotion and pressure-induced tone.


**E<sub>m</sub> Measurements**

Intercellular recording techniques were used to measure the SM resting E<sub>m</sub> (E<sub>m0</sub>) in MCAs sampled from prestroke and poststroke SHRsp at a variety of ages. Microelectrodes filled with 3 mol/L KCl having a tip resistance of between 40 and 60 MΩ were used to impale the SM of the MCAs. The SM E<sub>m</sub> was measured with a WPI 773 amplifier (World Precision Instruments). The electrophysiological signals were viewed with a Hameg M205 oscilloscope (Hameg Inc) and recorded on a Vetter 420 FM recorder (A.R. Vetter Co). We maintained the electrode in the cell to a point at which the E<sub>m</sub> remained stable and did not further change with time (often for many minutes), then withdrew the electrode. The E<sub>m0</sub> value was recorded only if the slightest withdrawal movement of the electrode produced an abrupt shift in the E<sub>m</sub> to baseline levels without an offset potential.

We are confident in our technique and in previous studies have used intercellular recording in combination with current injections to measure electrical conduction along the length of MCAs.<sup>14</sup>

In other experiments involving nifedipine, 10<sup>-6</sup> mmol/L nifedipine was introduced into the bath to prevent constriction, and the change in E<sub>m</sub> with varying concentrations of [K]<sup>+</sup> was assessed. The objective of these experiments was to determine whether (1) elevations in [K]<sup>+</sup> promoted SM depolarization, (2) the intercellular concentration of K<sup>+</sup> (in the SM) differed in prestroke versus poststroke SHRsp, and (3) there was evidence of a difference in K<sup>+</sup> conductance in the SM between the 2 groups. Both alterations in K<sup>+</sup> conductance in the SM and alterations in the intercellular concentrations of K<sup>+</sup> could alter the E<sub>m</sub> at rest and alter the rate of depolarization (and contraction) in response to graded elevations in [K]<sup>+</sup>. In plots of the E<sub>m</sub> change in response to altered [K]<sup>+</sup>, the [K]<sup>+</sup>, values that produce an E<sub>m</sub> of 0 mV will approximate the intercellular K<sup>+</sup> level, and changes in the slope of the plot of K<sup>+</sup> versus E<sub>m</sub> would suggest altered K<sup>+</sup> conductance. The most accurate assessment of the above parameters would be made under conditions in which alterations in [K]<sup>+</sup>, evoked minimal alterations in other ionic conductances that would exert secondary independent modifications in E<sub>m</sub>. In view of this, nifedipine was used to block the L-type voltage-gated channels. Such treatment prevented the (potential differential) influences of constriction and Ca<sup>2+</sup> entry on E<sub>m</sub> when [K]<sup>+</sup>, was elevated. In different arteries, the SM E<sub>m</sub> was also measured in the absence of nifedipine in pressurized MCAs bathed in normal Krebs solution at 0 mm Hg and after a 40-minute equilibration to 100 mm Hg.

**Statistical Analysis**

Comparisons involving 2 groups of data were assessed with Student’s t test. A 1-way ANOVA followed by Fisher’s post hoc test was used in comparisons involving >2 groups. Dose-response curves representing groups of animals were compared by a general linear model of ANOVA (GLM) to determine whether they differed significantly from each other. The curves were also assessed to determine whether a differential interactive effect between dose and response occurred between groups. A significant interactive effect with dose is usually associated with a situation in which curve crossover occurs or in which significant shifts in sensitivity are present. In addition, an 1-way ANOVA followed by Fisher’s post hoc test was used to assess differences between groups at each dose. Regression analysis and the calculation of a Pearson product of correlation (r value) was used to determine whether a significant relationship existed between 2 parameters. Results were considered significant at P<0.05 and were expressed as the mean±SEM.

**Results**

The systolic BP measured via a tail cuff compression method was lower (P<0.05) in srSHR (197±3 mm Hg, n=41) than in prestroke SHRSP (208±4 mm Hg, n=36), which in turn exhibited lower (P<0.05) BPs than poststroke SHRSP (233±6 mm Hg, n=32).

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**Figure 1.** PDC and PIT in the MCAs of srSHR and prestroke and poststroke SHRSP. The decrease in lumen diameter 1 second to 4 minutes after the application of a 100-mm Hg pressure step was used to measure the ability of the arteries to elicit PDC (A). The difference in lumen diameter between the start of PDC (1 second after the application of 100 mm Hg) and maximal relaxation (verapamil) was used as a measure of PIT (a, b, and c in A). The amplitudes of constriction produced by PDC and PIT are plotted in B. The MCAs of poststroke SHRSPs were unresponsive to pressure and maintained (because of PIT) a partially constricted state that could be inhibited by verapamil. Between-group statistical analysis: 1-way ANOVA followed by Fisher’s post hoc test to determine differences between groups; n values: 10 srSHR, 12 prestroke SHRSP, 9 poststroke SHRSP.

**Pressure-Dependent and Pressure-Independent Tone in the MCAs of SHR**

Figure 1, A and B, outlines the characteristics of PDC in response to a 100-mm Hg pressure step in the MCAs of srSHR and prestroke and poststroke SHRSP. The amplitude of PDC measured between 1 second and 4 minutes after the application of the 100-mm Hg pressure step (Figure 1A) was larger in the MCAs of srSHR than prestroke SHRSP. Poststroke SHRSP maintained a nearly constant lumen of diameter between 1 second and 4 minutes after the application of a 100-mm Hg pressure step, indicating the absence of PDC. After a 4-minute equilibration period to 100 mm Hg, lumen diameters maintained by the MCAs of srSHR were smaller than those of poststroke SHRSP. The loss of PDC in the MCAs of poststroke SHRSP occurred in both the right and left MCAs and was not related to the severity or location of the hemorrhagic lesions.

The degree of PIT that maintained constriction (the difference in lumen diameter under maximal dilation versus diameter 1 second after pressurization to 100 mm Hg, ie, a, b, and c in Figure 1A, plotted separately in Figure 1B) was highest in the MCAs of poststroke SHRSP. Prestroke SHRSP had larger levels of this tone than MCAs from srSHR. The
reduced lumen diameters observed 1 second after pressurization to a pressure of 100 mm Hg were not due to a structural reduction in lumen diameter, because in the presence of verapamil at maximal dilation, no differences in lumen diameter were observed between the groups. The total amplitude of constriction present in the MCAs of each animal group that is maintained by PDC and PIT is shown in Figure 1B. Under the latter conditions, the vast majority of constriction present in the MCAs of srSHR is pressure-dependent, whereas all the constriction present in the MCAs of poststroke SHRsp is pressure-independent.

Further studies were carried out to determine the mechanisms maintaining PDC in the MCAs of srSHR and PIT in those of poststroke SHRsp. Both types of constriction could be abolished by the removal of \([\text{Ca}^{2+}]_o\) (discussed later). As shown in Figure 2A, at 100 mm Hg, PDC in the MCAs of srSHR and PIT in the MCAs of poststroke SHRsp could be inhibited by the L-type \(\text{Ca}^{2+}\) channel antagonist nifedipine. However, the dose-response curves representing MCAs from poststroke SHRsp were shifted to the left of the curves representing srSHR, indicating that the sensitivity to nifedipine with respect to promoting the relaxation of PIT was higher than that promoting the relaxation of PDC.

The ability of nifedipine to block the L-type \(\text{Ca}^{2+}\) channel is affected by the SM \(E_{\text{m}}\) which, as will be shown, differs in relation to stroke development. Therefore, nifedipine relaxation-response curves were also constructed under conditions in which the SM of the arteries was depolarized with 100 mmol/L \([\text{K}^+]_o\). The latter concentration of \([\text{K}^+]_o\) depolarizes the SM to levels at which the L-type \(\text{Ca}^{2+}\) channel is in a state of maximal opening probability and minimizes the potential effects that differing \(E_{\text{m}}\) may have on the ability of nifedipine to block the \(\text{Ca}^{2+}\) channels. Under the above-described conditions (Figure 2B), the dose-response curves of the MCAs of SHRsp with stroke still remained shifted to the left of those representing srSHR, indicating that an increased relaxation sensitivity to nifedipine still existed in the MCAs of poststroke SHRsp.

Figure 3 demonstrates an age-related decrease in the proportion of tone that was maintained by PDC constriction in the MCAs of prestroke SHRsp. Regression analysis also indicated an age-related increase in the lumen diameter being maintained by the MCAs of prestroke SHRsp (n=13) after equilibration to 100 mm Hg (lumen diameter \([\text{mm}]\) at 100 mm Hg = 8.09 \(\times\) age + 22.4; \(r=0.556, P<0.05\)) and a direct relationship between the loss of PDC and increased lumen diameter size in the arteries (lumen diameter \([\text{mm}]\) at 100 mm Hg = 0.753 \(\times\) the amplitude of PDC in \(\mu\text{m} + 160\); \(r=0.761, P<0.01\)). The data indicate that the loss of PDC precedes stroke in SHRsp and that in prestroke SHRsp, there is an age-related decline in the ability of the MCA to constrict to pressure between 10 and 16 weeks of age.
higher levels of \([\text{Ca}^{2+}]_o\) in the MCAs of srSHR, followed by those of prestroke SHRsp. At varying \([\text{Ca}^{2+}]_o\), the sensitivity to \([\text{Ca}^{2+}]_o\) was greatest in MCAs of srSHR, whereas those of poststroke SHRsp were unresponsive to \([\text{Ca}^{2+}]_o\). Whole-curve analysis (GLM): srSHR vs prestroke SHRsp >12.5 weeks, \(P<0.001\); srSHR vs poststroke SHRsp, \(P>0.001\); prestroke SHRsp <12.5 vs >12.5 weeks, \(P<0.001\); prestroke SHRsp <12.5 or >12.5 weeks vs poststroke SHRsp, \(P<0.001\); between-group statistical analysis at each dose: 1-way ANOVA followed by Fisher’s post hoc test to determine differences between groups; n values: 8 srSHR, 7 SHRsp <12.5 weeks, 5 SHRsp >12.5 weeks, 8 SHRsp with stroke.

Figure 4. The constriction of pressurized MCAs from SHR to varying levels of external \([\text{Ca}^{2+}]_o\) in Krebs physiological saline containing 4.6 mmol/L (A) or 100 mmol/L (B) \([K^+]_o\). Altering \([K^+]_o\) from 4.6 mmol/L (A) to 100 mmol/L (B) \([K^+]_o\) abolished the reactivity differences to varying \([Ca^{2+}]_o\), observed between the MCAs of prestroke SHRsp and srSHR but did not affect the contractile reactivity of MCAs sampled from poststroke SHRsp. Whole-curve analysis (GLM): A, srSHR vs SHRsp, \(P<0.001\); srSHR vs poststroke SHRsp, \(P<0.001\); differential interaction with dose: srSHR vs poststroke SHRsp, \(P<0.001\); prestroke vs poststroke SHRsp, \(P<0.001\); prestroke vs poststroke SHRsp, \(P<0.001\); differential interaction with dose: srSHR vs poststroke SHRsp, \(P<0.001\); prestroke vs poststroke SHRsp, \(P<0.001\); between-group statistical analysis at each dose: 1-way ANOVA followed by Fisher’s post hoc test to determine differences between groups; n values: 7 srSHR, 11 prestroke SHRsp, 6 poststroke SHRsp; B, 6 srSHR, 8 prestroke SHRsp, 6 poststroke SHRsp.

Figure 5. Alterations in MCA lumen diameter in response to variations in external \([K^+]_o\). Experiments were performed at a temperature of 23°C to inactivate PDC. The MCAs of older prestroke SHRsp exhibited an attenuated ability to constrict to \([K^+]_o\), whereas those of poststroke SHRsp were unresponsive to \([K^+]_o\). The sensitivity to \([Ca^{2+}]_o\) was greatest in MCAs from prestroke SHRsp versus those of prestroke or poststroke SHRsp, the MCAs was not significantly different between prestroke SHRsp and srSHR at any external \([Ca^{2+}]_o\), level.

A key observation made was that elevation of \([K^+]_o\), from 4.6 to 100 mmol/L, a manipulation that should depolarize the SM of the MCAs, produced no change in the \([Ca^{2+}]_o\), dose-response curves in MCAs sampled from poststroke SHRsp with PTT. The conclusion reached was that \([Ca^{2+}]_o\) entry through L-type \([Ca^{2+}]_o\) channels in the SM of MCAs from srSHR and prestroke SHRsp was sensitive to manipulations that should modify the \(E_{ \text{K} } \) of the cells. The latter L-type \([Ca^{2+}]_o\) channel behaved in a voltage-gated manner, in that depolarization via 100 mmol/L \([K^+]_o\), increased the contractile reactivity to \([Ca^{2+}]_o\). Conversely, the same manipulation did not alter the configuration or the contractile reactivity in response to varying \([Ca^{2+}]_o\), observed in the MCAs of poststroke SHRsp. The pressure-independent constriction maintaining tone in the latter arteries appeared to be produced by an L-type \([Ca^{2+}]_o\) channel that could not be further activated by depolarization. To test further for this possibility, the ability of the MCAs to constrict to varying levels of \([K^+]_o\), was determined.

As demonstrated in Figure 1, at 100 mm Hg, the levels of tone in MCAs differed between srSHR, prestroke SHRsp, and poststroke SHRsp. To overcome this, the temperature was decreased to 23°C. Such manipulation inhibited PDC constriction. As shown in Figure 5, the ability to constrict to \([K^+]_o\), was greatest in MCAs from prestroke SHRsp <12.5 weeks of age. Prestroke SHRsp >12.5 weeks of age demonstrated an attenuated ability to constrict maximally to \([K^+]_o\), and MCAs sampled from SHRsp with stroke demonstrated a total inability to constrict to elevated \([K^+]_o\). The differences in response to \([K^+]_o\), could not be accounted for by general differences in the ability of the MCAs to constrict, because the latter MCAs constricted equally to vasopressin (% con-
striction of lumen in 4.6 mmol/L [K\textsuperscript{+}]\textsubscript{o} to 0.17 μmol/L vasopressin at 23°C, srSHR 58.3±2.5%; young prestroke SHRsp 49.4±3.1%; poststroke SHRsp 52.6±12.6%; no significant \([P>0.05]\) difference between the groups). In other experiments, the ability of the MCAs of srSHR and poststroke SHRsp to constrict to 100 mmol/L [K\textsuperscript{+}]\textsubscript{o} was determined at 37°C. MCAs from poststroke SHRsp still demonstrated an inability to constrict to an elevation in [K\textsuperscript{+}]\textsubscript{o} (mean lumen diameter change <1%, \(n=7\) SHRsp), whereas those of srSHR reduced their lumen size by 43.4±4.2% (\(n=4\) srSHR). Hence, the latter phenomenon was not a feature observed only at 23°C.

**Alterations in the SM E\textsubscript{m} in MCAs of Prestroke and Poststroke SHRsp**

This aspect of the study was carried out to gain more information on why the MCAs of poststroke SHRsp lacked the ability to constrict to elevations in [K\textsuperscript{+}]\textsubscript{o}. Initially, we attempted to determine the degree of SM depolarization that occurred in response to variations to [K\textsuperscript{+}]\textsubscript{o}. In addition, the E\textsubscript{m} of the SM cells of the MCAs of poststroke SHRsp was assessed to determine whether it was of a magnitude that could cause the voltage-gated Ca\textsuperscript{2+} channels to be in a state of maximum opening probability. If such was the case, then elevations in [K\textsuperscript{+}]\textsubscript{o}, might not be expected to further increase the degree of constriction. Finally, we assessed the ability of the MCAs to elicit pressure-dependent SM depolarization in response to a 100-mm Hg pressure step.

MCAs were mounted in a pressure myograph and equilibrated to a pressure of 100 mm Hg for 40 minutes, after which time the SM cell E\textsubscript{m} was measured. Subsequently, the pressure was dropped to 0 mm Hg, and E\textsubscript{m} measurements were made. Finally, the ability of the MCAs to elicit PDC in response to a 100-mm Hg pressure step was measured. The E\textsubscript{m} values obtained at both pressures are outlined in the Table. When the pressure was reduced from 100 to 0 mm Hg, the SM E\textsubscript{m} hyperpolarized in MCAs sampled from prestroke SHR and remained constant in a depolarized state in MCAs sampled from poststroke SHRsp. MCAs sampled from prestroke SHRsp and srSHR but not poststroke SHRsp were capable of eliciting PDC.

Nonpressurized MCAs from both groups were treated with 3 μmol/L nifedipine to prevent constriction, and the SM E\textsubscript{m} was measured at varying [K\textsuperscript{+}]\textsubscript{o} levels (Figure 6). In the presence of nifedipine at 4.6 mmol/L (normal) [K\textsuperscript{+}]\textsubscript{o} levels, MCAs sampled from poststroke SHRsp still exhibited a more depolarized SM E\textsubscript{m} than those of prestroke SHRsp. The presence of a less steep decline in E\textsubscript{m} with respect to increased [K\textsuperscript{+}]\textsubscript{o}, was observed in MCAs sampled from poststroke versus prestroke SHRsp. The predicted [K\textsuperscript{+}]\textsubscript{o} level that produced an E\textsubscript{m} value of 0 mV, which should correspond to the SM [K\textsuperscript{+}]\textsubscript{o}, was similar in the 2 groups. The nature of the changes in the slope of the plot of E\textsubscript{m} versus [K\textsuperscript{+}]\textsubscript{o}, suggested that the more depolarized E\textsubscript{m} observed in the MCAs of poststroke SHRsp was not due to the presence of a smaller SM intercellular concentration of K\textsuperscript{+} but rather was consistent with a relative decrease in K\textsuperscript{+} to Na\textsuperscript{+} conductance in the SM cells.

**Discussion**

At 100 mm Hg pressure, poststroke SHRsp maintained constriction via a constant level of PIT. Although PIT developed while the MCAs of poststroke SHRsp were equilibrated to a pressure of 100 mm Hg, we think that development of this tone over the 45-minute equilibration period at 37°C represents a reestablishment of the ability of the arteries to develop tone after being excised from 3°C bath water. Once the constriction developed at 100 mm Hg, it was no longer influenced by modifications in pressure. On the basis of these observations, we believe that it is valid to call this tone pressure-independent. However, the exact role that the equilibration pressure plays in the initial induction of this tone during the equilibration process is not fully understood. In our view, it is important to distinguish PIT from PDC, because it is likely that PDC is involved in facilitating the cerebral

### Table 1: SM E\textsubscript{m} of MCA From Prestroke and Poststroke SHRsp

<table>
<thead>
<tr>
<th>Group</th>
<th>E\textsubscript{m} at 0 mm Hg, mV</th>
<th>E\textsubscript{m} at 100 mm Hg, mV</th>
<th>Change in E\textsubscript{m} between 0 vs 100 mm Hg, mV</th>
<th>Constriction (μm) to 100-mm Hg pressure step (1 s to 4 min)</th>
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<tr>
<td>Prestroke SHRsp</td>
<td>-46.4±1.5</td>
<td>-38.3±1.2</td>
<td>+8.1±1.8*</td>
<td>42.4±3.4</td>
</tr>
<tr>
<td>Poststroke SHRsp</td>
<td>-34.1±4.6</td>
<td>-34.7±1.8</td>
<td>-0.6±4.3†</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05‡</td>
<td>&lt;0.05§</td>
<td>&lt;0.05§</td>
<td></td>
</tr>
</tbody>
</table>

\*P<0.01 from 0, by paired t test; †NS from 0, by paired t test; ‡P<0.05; by unpaired t test.

**Figure 6.** The alterations in SM E\textsubscript{m} with varying [K\textsuperscript{+}]\textsubscript{o} in MCAs sampled from SHR. The experiment was performed in the presence of 3 μmol/L nifedipine to prevent constriction. Between-group analysis at each dose: unpaired t test; \(n=5\) prestroke (<12.5 weeks) and 6 poststroke SHRsp.
autoregulation of blood flow. The presence of PIT also emphasizes that the observation of tone in a pressurized artery that is reversed by vasodilators such as L-type Ca$^{2+}$ antagonists cannot always be interpreted as indicating a nature of tone that is subsequently dynamically altered with pressure or is truly pressure-dependent.

The MCAs of poststroke SHRsp could not elicit PDC and did not achieve the reductions in lumen diameter that could be achieved by the MCAs of prestroke SHRsp or sHR. The decline in the ability of MCAs to elicit PDC preceded stroke development in SHRsp and occurred between 10 and 16 weeks of age in prestroke SHRsp. We believe that the development of the latter defect could play an important role in the initiation of cerebral hemorrhage in SHRsp. Under hypertensive conditions, a decline in the ability to elicit PDC could result in the overperfusion of the distal vasculature fed by the MCAs and promote the elevation of downstream intravascular pressures. Such changes would be conducive to the formation of cerebrovascular hemorrhage. The balance of the study attempted to investigate the potential vascular defects that could contribute to the loss of PDC in the MCAs of SHRsp.

Studies involving pressurized rat, cat, and rabbit MCAs or smaller dog renal arteries have indicated that PDC is inhibited by L-type Ca$^{2+}$ channel antagonists. In these arteries, PDC was associated with SM membrane depolarization, perhaps produced by the direct activation of an SM arachidonic acid product, such as 20-hydroxyeicosatetraenoic acid, or a process involving the activation of select CI$^{-}$ channels. In cat, rat, and rabbit MCAs, including MCAs of sHR, the magnitude of depolarization observed in response to elevated pressure was sufficient to open voltage-gated L-type Ca$^{2+}$ channels. In rabbit MCAs also modified the amplitude of PDC. Therefore, it was suggested that PDC could be produced by an influx of Ca$^{2+}$ through voltage-gated L-type Ca$^{2+}$ channels in response to pressure-induced depolarization.

Experimental evidence presented in this paper suggests that some of the above-described mechanisms are altered in relation to stroke development in SHRsp. At 100 mm Hg pressure, the SM E$_{m}$ of MCAs was comparable between prestroke and poststroke SHRsp. However, when pressure was lowered to 0 mm Hg, the SM E$_{m}$ of MCAs from poststroke SHRsp did not alter to more hyperpolarized levels but rather was maintained at levels similar to those observed at 100 mm Hg pressures (Table). If the signal transduction mechanisms promoting vasomotor responses to pressure involved the opening or closing of SM voltage-gated L-type Ca$^{2+}$ channels in response to depolarization or hyperpolarization, respectively, the maintenance of a constant SM E$_{m}$ in the SM of MCAs at 0 and 100 mm Hg pressures could have prevented the arteries from altering tone in response to pressure. However, we believe that functional changes in the L-type Ca$^{2+}$ channel must also exist in the SM of the MCAs of poststroke SHRsp to account for their unresponsiveness to elevated [K$^{+}$]. Studies of isolated SM from rat cerebral arteries have indicated that the maximal opening probability of the voltage-gated L-type Ca$^{2+}$ channel occurs at E$_{m}$ levels less negative than −20 mV. At 100 or 0 mm Hg pressures, the SM E$_{m}$ of MCAs from poststroke SHRsp was near −35 mV. Depolarization with 100 mmol/L [K$^{+}$]$_{o}$ should have produced an E$_{m}$ of −15 mV (Figure 6) and further constriction. However, elevations in [K$^{+}$]$_{o}$ only enhanced constriction in MCAs sampled from prestroke SHRsp and sHR (Figure 4). The inability of the MCAs of poststroke SHRsp to constriuct to elevation in [K$^{+}$]$_{o}$ could occur if the maximum opening probability of the Ca$^{2+}$ channel was achieved at E$_{m}$ levels already present in the SM of the MCAs of SHRsp or if there was a defect in the voltage sensor in the channel causing the channel not to respond to further depolarizing changes in SM E$_{m}$. Further confirmation of the above hypothesis will require the use of patch-clamp studies of isolated muscle cells to determine the voltage-dependence of L-type Ca$^{2+}$ channel-mediated Ca$^{2+}$ currents.

Studies have indicated that PDC in rat posterior cerebral arteries is inhibited not only by L-type Ca$^{2+}$ antagonists but also by protein kinase C inhibitors. We have also observed that the inhibition of protein kinase C with staurosporine (40 nmol/L), chelerythrine (12 μmol/L), or bisindolylmaleimide (5 μmol/L) inhibits PDC in the MCAs of poststroke SHRsp. In addition, the MCAs of poststroke SHRsp exhibited an attenuated ability to constric in response to protein kinase C activation by phorbol esters in the presence of nifedipine. SM Ca$^{2+}$ influx and protein kinase C activation may act in a cooperative manner to promote PDC. In rabbit facial veins, an influx of Ca$^{2+}$ has been suggested to promote protein kinase C activation, leading to increased constriction in response to stretch. Other studies have hypothesized that during pressure- or tension-related constriction, an influx of Ca$^{2+}$, possibly through voltage-gated channels, and protein kinase C activation (through a separate signal transduction pathway possibly involving phospholipase C activation and diacylglycerol formation) may combine to promote constriction. These studies implied that neither the influx of Ca$^{2+}$ nor the activation of protein kinase C (promoted by an elevation in pressure) can independently promote constriction in response to elevated pressure and that the cooperative action of both mechanisms is necessary to contract the blood vessels. Hence, in the latter model, either protein kinase C or Ca$^{2+}$ channel inhibitors would independently inhibit PDC, a finding that is consistent with our observations. In the context of the present study, elevations in pressure may induce SM depolarization, which could open voltage-gated L-type Ca$^{2+}$ channels. The subsequent entry of Ca$^{2+}$ into the SM may act as a trigger to activate PDC in the arteries via an activation of protein kinase C or facilitate constriction in conjunction with protein kinase C activation through an alternative activation pathway. Other sequences of activation are also possible. For example, in cultured vascular SM cell lines (A7r5) clamped at a constant E$_{m}$, activation of protein kinase C by phorbol esters resulted in the initiation of an L-type Ca$^{2+}$ current that can be blocked by dihydropyridine antagonists. It is possible that elevations in pressure, perhaps via activation of phospholipase C and production of diacylglycerol, might activate protein kinase C, which in turn could be involved in promoting PDC by opening L-type Ca$^{2+}$ channels directly, in a voltage-independent manner. Regardless of the sequence of events,
if PDC in the MCAs of SHR involved pressure-induced depolarization and Ca\(^{2+}\) entry through voltage-gated L-type Ca\(^{2+}\) channels, the maintenance of a constant SM E\(_{m}\) at varying pressure and an inability to constrict in response to depolarization would act to inhibit PDC. Likewise, even if Ca\(^{2+}\) entry into the SM through L-type Ca\(^{2+}\) channels was initiated via other signal transduction mechanisms that did not involve SM depolarization (such as protein kinase C activation), a dysfunction in the Ca\(^{2+}\) channel, such as that observed in the present study, could also affect the ability of the latter signals to operate the channel and initiate PDC.

**Acknowledgments**

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**References**

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**Editorial Comment**

The findings in this article are important because they describe the development of functional defects in the electromechanical properties of cerebrovascular smooth muscle from stroke-prone, genetically hypertensive rats (SHRsp). By decreasing basal arterial tone and compromising blood flow autoregulation, these alterations not only precede but may also contribute to the development of hemorrhagic stroke.

A pharmacological approach (modulation of extracellular calcium and potassium concentrations; use of a calcium channel blocker) in combination with electrophysiology (microelectrode impalement of smooth muscle) was applied to isolated, pressurized segments of the middle cerebral artery from 9- to 18-week-old SHRsp and stroke-resistant rats (SHRsr). The results show that arterial smooth muscle from SHRsp animals that have recently undergone a stroke is unable to hyperpolarize when pressure is decreased (Table 1) and that vessels lose their ability to change diameter in response to pressure (Figures 1A and 1B). SHRsp arteries were also more found to be more sensitive to nifedipine (Figure 2A) and less sensitive to extracellular calcium (Figure 4B), even under depolarized conditions (Figures 2B and 4B), and did not constrict to potassium depolarization (Figures 5 and 6). This loss of reactivity could not be attributed to a defect in the contractile apparatus, because responsiveness to
vasopressin, a constrictor agonist, was preserved. Together, these observations suggest that cerebral arteries from post-stroke SHRsp possess dysfunctional voltage gated calcium channels, and that the consequence of this defect is to uncouple the mechanosensory elements within the vascular wall from the regulation of smooth muscle membrane potential, cytosolic calcium, and arterial diameter.

Some of these functional alterations precede the onset of stroke; hence, severe and progressive hypertension, and not stroke per se, appears to be the most likely culprit. It deserves note that systolic pressures in SHRsp continued to increase from just over 200 mm Hg (statistically similar to SHRsp) to a significantly higher mean value of 233 mm Hg in the poststroke SHRsp group and that the severity of the defects increased accordingly over time.

I found the PIT versus PDC terminology somewhat confusing, since a diminished but significant degree of tone (approximately 30%; see Figure 1) does develop in poststroke SHRsp vessels during equilibration at 100 mm Hg, presumably in response to the stimulus of pressure. If it is pressure induced, can it really be called pressure independent? This is partly semantic and of secondary importance. The key point, however, is that the combination of reduced pial artery tone, and an inability to modulate that tone (via changes in membrane potential and calcium) in response to considerable (100 mm Hg) fluctuations in pressure, would most likely affect both cerebrovascular resistance and blood flow autoregulation, and might therefore be important in the etiology of hemorrhagic stroke. Both changes favor exposure of smaller, more fragile downstream vessels to higher transmural pressures, thereby increasing wall tension and potentially leading to rupture and intracerebral hemorrhage.

The challenge ahead is to understand just how high intravascular pressures alter cellular function and to determine whether this defect persists in the intact, living animal. Although a number of studies have reported effects of pressure or stretch on gene activation in a variety of cells, the link between chronic hypertension and vascular smooth muscle calcium channel expression and function has yet to be described in any detail.

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Electromechanical Alterations in the Cerebrovasculature of Stroke-Prone Rats
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