Fundamental increase in pressure-dependent constriction of brain parenchymal arterioles from subarachnoid hemorrhage model rats due to membrane depolarization

Matthew A. Nystoriak,1 Kevin P. O’Connor,1 Swapnil K. Sonkusare,1 Joseph E. Brayden,1 Mark T. Nelson1,3 and George C. Wellman1,2

1Department of Pharmacology and 2Division of Neurological Surgery, Department of Surgery, University of Vermont, College of Medicine, Burlington, Vermont; and 3Department of Cardiovascular Medicine, Faculty of Medical and Human Sciences, University of Manchester, Manchester, United Kingdom

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Nystoriak MA, O’Connor KP, Sonkusare SK, Brayden JE, Nelson MT, Wellman GC. Fundamental increase in pressure-dependent constriction of brain parenchymal arterioles from subarachnoid hemorrhage model rats due to membrane depolarization. Am J Physiol Heart Circ Physiol 300: H803–H812, 2011. First published December 10, 2010; doi:10.1152/ajpheart.00760.2010.—Intracerebral (parenchymal) arterioles are morphologically and physiologically unique compared with pial arteries and arterioles. The ability of subarachnoid hemorrhage (SAH) to induce vasospasm in large-diameter pial arteries has been extensively studied, although the contribution of this phenomenon to patient outcome is controversial. Currently, little is known regarding the impact of SAH on parenchymal arterioles, which are critical for regulation of local and global cerebral blood flow. Here, we sought to determine the role of voltage-dependent Ca2+ channels (VDCCs), and membrane potential measurements were used to assess the function of intact brain parenchymal arterioles isolated from unoperated (control), sham-operated, and SAH model rats. At low intravascular pressure (5 mmHg), membrane potential and [Ca2+]i were not different in arterioles from control, sham-operated, and SAH animals. However, raising intravascular pressure caused significantly greater membrane potential depolarization, elevation in [Ca2+]i, and constriction in SAH arterioles. This SAH-induced increase in [Ca2+]i, and tone occurred in the absence of the vascular endothelium and was abolished by the L-type voltage-dependent calcium channel (VDCC) inhibitor nimodipine. Arteriolar [Ca2+]i, and tone were not different between groups when smooth muscle membrane potential was adjusted to the same value. Protein and mRNA levels of the L-type VDCC Cav1.2 were similar in parenchymal arterioles isolated from control and SAH animals, suggesting that SAH did not cause VDCC upregulation. We conclude that enhanced parenchymal arteriolar tone after SAH is driven by smooth muscle membrane potential depolarization, leading to increased L-type VDCC-mediated Ca2+ influx.

Cerebral blood flow is regulated by the diameter of resistance arteries and arterioles both on the surface of the brain and within the brain parenchyma. Parenchymal arterioles, unlike pial arteries and arterioles, lack extrinsic innervation and are encased by astrocytic processes (“endfeet”) (17, 27). The close association of this microvasculature with astrocytic endfeet is essential for functional hyperemia, whereby focal increases in neuronal activity are coupled to vasodilation of nearby arterioles and increased blood flow (6, 14, 27, 45). In addition to their role in neurovascular coupling, parenchymal arterioles also contribute significantly to autoregulation of global cerebral blood flow and account for ~40% of total cerebral vascular resistance (12). Thus parenchymal arterioles are a unique vascular bed, central in the regulation of both local and global cerebral blood flow.

Pial arteries and arterioles constrict to physiological increases in intravascular pressure via a process involving vascular smooth muscle membrane potential depolarization, activation of voltage-dependent Ca2+ channels (VDCCs), and increased global intracellular Ca2+ concentration ([Ca2+]i) (1, 13, 19, 32, 44). Recent work has established that pressure-dependent constriction, or myogenic tone, is significantly greater in isolated parenchymal arterioles than pial arteries (3). The cellular basis for enhanced pressure-dependent myogenic tone in the parenchymal vasculature is unclear. Furthermore, the relationship between intravascular pressure, membrane potential, [Ca2+]i, and diameter of isolated parenchymal arterioles is not known.

Dysfunction of the cerebral microcirculation, including parenchymal arterioles, has been implicated in a number of brain pathologies, including vascular dementia, Alzheimer disease, and the development of delayed neurological deficits associated with cerebral aneurysm rupture and subarachnoid hemorrhage (SAH) (26, 65). A number of studies have suggested that SAH causes enhanced constriction of the microcirculation to limit parenchymal blood flow (34, 51, 58, 59, 65). However, the small size of parenchymal arterioles (maximum luminal diameter: 30–60 μm) poses a significant technical challenge to direct examination of their function.

The objective of the present study was to elucidate the relationship between intravascular pressure, [Ca2+]i, membrane potential, and diameter of isolated parenchymal arterioles from unoperated, sham-operated, and SAH model rats. We report that after SAH, augmented pressure-dependent depolarization of parenchymal arteriolar smooth muscle causes elevation [Ca2+]i, and enhanced vasoconstriction. Furthermore, this enhanced constriction of parenchymal arterioles after SAH occurs independently of endothelial dysfunction and is abolished by smooth muscle hyperpolarization or direct inhibition of L-type VDCCs.

METHODS

Animals. Sprague-Dawley rats (males, 300–350 g, 10–15 wk; Charles River Laboratories, Saint Constant, QC, Canada) were used in this study. All experiments were conducted in accordance with the
Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 1996) and followed protocols approved by the Institutional Animal Use and Care Committee of the University of Vermont.

Rat SAH model. Animals were initially anesthetized with isoflurane (5%) with an induction chamber and then maintained on isoflurane (2–2.5%) anesthesia with the aid of a nose cone. A small (0.5–1.5 cm), longitudinal, midline suboccipital incision was centered over the foramen magnum, and the neck muscles were dissected until dura was visualized. Autologous unheparinized blood (0.5 ml) drawn from the tail artery was injected into the cisterna magna (day 0) with a 25-gauge butterfly needle (37). The animal was then positioned on an incline board at a 45° angle with the head down in neutral position for 30 min. Twenty-four hours later, a second injection of blood was delivered by repeating the above procedure. Sham-operated animals were treated in a similar manner with the exception that injection of 0.5 ml of saline was substituted for injection of 0.5 ml of unheparinized blood. Buprenorphine (0.01 mg/kg) was given every 12 h (for 36 h, then as needed) as an analgesic. On day 4, animals were euthanized under deep pentobarbital anesthesia by decapitation. Parenchymal arterioles (30–60 μm in diameter) were dissected from the middle cerebral artery territory by a method previously described by Dacey and Duling (7). Briefly, the arachnoid mater around the middle cerebral artery was gently removed from the brain such that parenchymal arterioles (2–4 mm in length) were removed from the brain while attached to the middle cerebral artery. Arterioles were dissected in cold (4°C) 3-(N-morpholino)propanesulfonic acid (MOPS)-buffered saline solution of the following composition (in mM): 145 NaCl, 5 KCl, 1 MgSO4, 2.5 CaCl2, 1 KH2PO4, 0.02 EDTA, 3 MOPS, 2 pyruvate, 5 glucose, 1% bovine serum albumin, pH 7.4. Arteriole segments used for this study were located downstream of theVirchow–Robin space at depths >1 mm from the surface of the cortex.

Measurement of intracellular Ca2+ concentration and diameter. Freshly isolated parenchymal arterioles were cannulated on glass micropipettes mounted in a 5-ml myograph chamber (University of Vermont Instrumentation and Model Facility) as described previously (7, 29). Vascular endothelium was removed from some arterioles by passing an air bubble through the lumen after cannulation of one end (62). After cannulation, arterioles were loaded with the ratiometric Ca2+-sensitive dye fura-2 [acetoxyethyl ester (AM) membrane-permeant form] by incubating in MOPS-buffered saline solution containing fura-2 AM (5 μM) (Invitrogen, Carlsbad, CA) with plufronic acid (0.1%) (Invitrogen) at room temperature (~22°C) for 45 min. Next, the myograph chamber was mounted on a Nikon TE2000-S inverted fluorescence microscope. To allow for equilibration and deesterification of fura-2 AM, arterioles were pressurized to 5 mmHg and continuously superfused (37°C, 30 min) with artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 125 NaCl, 3 KCl, 18 NaHCO3, 1.25 NaH2PO4, 1 MgCl2, 2 CaCl2, 5 glucose aerated with 5% CO2, 20% O2, 75% N2. Bath pH was closely monitored and maintained at 7.30–7.35 (9).

After the equilibration period, studies were performed in which intravascular pressure was increased to 10, 20, 40, and 60 mmHg or monitored and maintained at 7.30–7.35 (9).

Luminal diameter of arterioles was simultaneously recorded with [Ca2+]i, with a charge-coupled device (CCD) camera and the edge-detection function of IonOptix software. Constriction is presented as a decrease in arterial diameter relative to the maximum diameter at a given pressure obtained at the end of each experiment using Ca2+-free aCSF containing nimodipine (300 nM) (29, 66).

Measurement of membrane potential. Arterioles were cannulated as described above and pressurized to either 5 or 40 mmHg. Smooth muscle membrane potential was measured by insertion of a sharp glass microelectrode (~100-MΩ resistance) filled with 0.5 M KCl into the vessel wall. The criteria for successful impalement were 1) an abrupt negative potential deflection upon entry, 2) a stable membrane potential for ≥30 s, and 3) an abrupt positive potential deflection upon removal (32, 53). Measurements were made with an electrometer (World Precision Instruments) and recorded via computer with Axotape and Dataq software.

Quantitative real-time polymerase chain reaction. Total RNA was extracted with RNA STAT-60 total RNA/mRNA isolation reagent (Tel-test, Friendswood, TX) and reverse transcribed to cDNA with the SuperScript First-Strand synthesis system (Invitrogen) (49). Polymerase chain reaction was performed with primers detecting CaV1.2 (GenBank accession no. AA974797; forward: 5′-CAAGAGCTCTGAGCTGAGA-3′; reverse: 5′-CCGACAGCAGTGAATGAGA-3′) and 18S (GenBank accession no. M11188; forward: 5′-AGTCGC-GTGTCCTACCAT-3′; reverse: 5′-GCTGCTGCTTCCCTGTTG-3′). Amplification was performed with SYBR Green JumpStart Taq ReadyMix (Sigma, St. Louis, MO) and a real-time PCR system (Applied Biosystems, Carlsbad, CA). Quantification was performed by comparison of threshold cycles (CT) relative to a standard curve constructed from serially diluted plasmids containing target sequences. Expression levels were normalized to 18S rRNA, which was not statistically different between arterioles isolated from SAH animals and controls. [CTcalcium/CACVG1.2]i vs. CTcalcium/C18S was determined from a separate set of arterioles (see METHODS). No differences were found among control and subarachnoid hemorrhage (SAH) arterioles. Pooled values shown were used for analysis of intracellular Ca2+ concentration measurements.

<table>
<thead>
<tr>
<th>n</th>
<th>Rmin</th>
<th>Rmax</th>
<th>β</th>
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<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>0.371 ± 0.008</td>
<td>9.969 ± 0.565</td>
</tr>
<tr>
<td>SAH</td>
<td>4</td>
<td>0.378 ± 0.005</td>
<td>10.064 ± 0.756</td>
</tr>
<tr>
<td>Pooled</td>
<td>0.374 ± 0.004</td>
<td>10.017 ± 0.483</td>
<td>7.898 ± 0.451</td>
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</table>

Values are means ± SE for n arterioles. Ratios of emission signals under Ca2+-free and Ca2+-saturated conditions (Rmin and Rmax, respectively) and ratio of F390 intensities at Rmin and Rmax (β) were determined from a separate set of arterioles (see METHODS). No differences were found among control and subarachnoid hemorrhage (SAH) arterioles. Pooled values shown were used for analysis of intracellular Ca2+ concentration measurements.
membrane at 80 V for 2 h (4°C). The membranes were washed in Tris-buffered saline with 0.1% Tween 20 (TBS-T) and blocked with 10% nonfat dried milk in TBS-T (1 h, room temperature). Membranes were then incubated with the specific polyclonal antibodies against Cav1.2 (1:200, overnight, 4°C; Alomone, Jerusalem, Israel) or monoclonal antibody against GAPDH (1:100,000; 30 min, room temperature; Sigma) prepared in 5% nonfat dried milk in TBS-T. Membranes were then incubated (1 h, room temperature) with horseradish peroxidase-labeled donkey anti-rabbit IgG (Cav1.2, 1:5,000; GAPDH, 1:10,000, GE Healthcare, Waukesha, WI) in TBS-T containing 5% nonfat dried milk. Bands were identified by chemiluminescence and exposed to X-ray films. Densitometry for immunoreactive bands was performed with National Institutes of Health software (ImageJ), and density was expressed as percentage of GAPDH density for each lane. The quantity of GAPDH protein, normalized to total protein, was similar between control and SAH groups.

**Statistical analysis.** Values are presented as means ± SE. One-way analysis of variance followed by Tukey multiple comparison test was used in the comparison of multiple groups. Student’s t-test was used in the comparison of two groups. Statistical significance was considered at the level of $P < 0.05$ or $P < 0.01$.

**RESULTS**

Parenchymal arterioles isolated from SAH model animals exhibit elevated cytosolic Ca$^{2+}$ and enhanced tone at physiological intravascular pressures. To assess the role of cytosolic Ca$^{2+}$ in pressure-induced parenchymal arteriolar constriction, simultaneous measurements of [Ca$^{2+}$], and diameter were obtained from freshly isolated parenchymal arterioles loaded with the ratiometric Ca$^{2+}$ indicator fura-2. In response to stepwise increases in intravascular pressure, arterioles from unoperated (control), sham-operated, and SAH animals exhibited graded increases in cytosolic Ca$^{2+}$ ([Ca$^{2+}$]) and active constriction. At low intravascular pressure (5–10 mmHg), similar levels of [Ca$^{2+}$] and tone were observed in vessels isolated from all groups. However, as intravascular pressure was increased to physiological levels (i.e., 20–60 mmHg) (12, 21), arterioles from SAH animals exhibited markedly enhanced levels of [Ca$^{2+}$], and constriction compared with arterioles from control and sham-operated animals (Fig. 1, A–E). For example, at 40 mmHg, [Ca$^{2+}$] was ~33% higher in arterioles

![Graph and Data](http://ajpheart.physiology.org/Downloaded_from/10.220.33.4/November_10_2017/Enhanced_Parenchymal_Arteriolar_Constriction_After_SAH)
from SAH animals (349 ± 11 nM, n = 5) compared with arterioles isolated from control animals (261 ± 8 nM, n = 6).

Table 2. Active and passive diameters of parenchymal arterioles isolated from control, sham-operated, and SAH animals

<table>
<thead>
<tr>
<th>Intravascular Pressure</th>
<th>Control</th>
<th>Sham Operated</th>
<th>SAH</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Active</td>
<td>Passive</td>
<td>Active</td>
</tr>
<tr>
<td>5 mmHg</td>
<td>39 ± 2</td>
<td>41 ± 2</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>10 mmHg</td>
<td>39 ± 2</td>
<td>43 ± 2</td>
<td>44 ± 5</td>
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<tr>
<td>20 mmHg</td>
<td>38 ± 2</td>
<td>45 ± 3</td>
<td>44 ± 6</td>
</tr>
<tr>
<td>40 mmHg</td>
<td>36 ± 3</td>
<td>49 ± 3</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>60 mmHg</td>
<td>33 ± 3</td>
<td>50 ± 3</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>*+Nimodipine</td>
<td>49 ± 4</td>
<td>57 ± 8</td>
<td>57 ± 8</td>
</tr>
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Values (in μm) are means ± SE. *P < 0.05 vs. control active and sham operated active.
intact parenchymal arterioles were impaled from the adventitial surface with intracellular microelectrodes. At low intravascular pressure (5 mmHg), membrane potential was similar in arterioles isolated from control (n = 4) and SAH (n = 4) animals. Membrane potential was measured at 30 mmHg using a micromanipulator to control plasma membrane potential hyperpolarization. Membrane potential hyperpolarization in arterioles from both groups. However, parenchymal arteriolar myocytes from SAH animals were significantly more depolarized at 40 mmHg (−58 ± 2 mV, n = 4) compared to arterioles from control animals (−35 ± 1 mV, n = 5) (Fig. 5, B and C). These data suggest that greater pressure-dependent membrane potential depolarization underlies enhanced parenchymal arteriolar tone after SAH.

**DISCUSSION**

Here we report that cytosolic Ca\(^{2+}\) is elevated and pressure-dependent constriction is enhanced in parenchymal arterioles from experimental SAH animals. Our data demonstrate that elevations in intravascular pressure cause greater membrane potential depolarization after SAH, leading to increased activity of L-type VDCCs and enhanced constriction.

Previously, we observed that SKCa and IKCa channels are functional and their basal activity is unchanged after SAH. In addition, we observed that an opener of SKCa and IKCa channels, apamin and TRAM-34, respectively, on parenchymal arteriolar tone. At 40 mmHg, application of apamin (300 nM) and TRAM-34 (1 μM) caused a similar level of constriction in arteries isolated from control (11 ± 2%, n = 4) and SAH (10 ± 1%, n = 6) animals, suggesting that these channels are functional and their basal activity is unchanged after SAH. In addition, we observed that an opener of SKCa and IKCa channels, NS-309 (1 μM), hyperpolarized smooth muscle membrane potential to similar levels in endothelium-intact arteries from control (−64 ± 2 mV, n = 5) and SAH (−67 ± 2 mV, n = 4) animals. Consistent with membrane potential hyperpolarization, NS-309 significantly decreased [Ca\(^{2+}\)]\(_i\) in arteries from control (242 ± 7 nM to 141 ± 15 nM, n = 4) and SAH (330 ± 11 nM to 151 ± 14 nM, n = 4) animals and induced >90% vasodilation in both groups (Fig. 6, A and C). These results demonstrate that activation of endothelial SKCa and IKCa channels results in enhanced hyperpolarization and vasodilation of arterioles from control and SAH animals.

To further examine whether decreased basal endothelial function contributes to elevated [Ca\(^{2+}\)]\(_i\) and enhanced constriction of parenchymal arterioles from SAH animals, [Ca\(^{2+}\)]\(_i\) and diameter were measured in endothelium-denuded vessels. The endothelium was removed from parenchymal arterioles by passing an air bubble through the vessel lumen during the cannulation procedure. Although endothelium-denuded arterioles displayed increased [Ca\(^{2+}\)]\(_i\) and constriction compared with corresponding endothelium-intact vessels in both groups, these parameters remained significantly higher in parenchymal arterioles from SAH animals (Fig. 6, B and D). Application of NS-309 was used to confirm absence of endothelial function in denuded vessels. Consistent with expression of SKCa and IKCa channels in endothelial cells but not smooth muscle, NS-309 had no effect on [Ca\(^{2+}\)]\(_i\), or diameter in denuded arterioles from either control or SAH animals (Fig. 6, B and D). These results suggest that SAH-induced elevated [Ca\(^{2+}\)]\(_i\) and enhanced arteriolar tone result from a direct modulation of smooth muscle membrane potential rather than decreased endothelial vasodilatory influence. 
This work also provides novel information regarding the physiological function of arterioles within the brain parenchyma in the absence of SAH. Our data, consistent with other recent studies (3, 4), demonstrate that parenchymal arterioles develop significantly greater pressure-induced myogenic tone at lower intravascular pressures than pial surface arteries, such as middle cerebral arteries (32). At a given level of intravascular pressure, parenchymal arteriolar tone at the lower physiological pressures experienced in the cerebral microcirculation is unaltered. Furthermore, the contribution of endothelial dysfunction to cerebral artery constriction is controversial, and others have reported that endothelium-dependent vasodilation is impaired in large-diameter cerebral arteries in experimental models of SAH (5, 13). Similar findings have been reported in basilar arteries of SAH patients (23). However, the contribution of endothelial dysfunction to cerebral artery constriction is controversial, and others have reported that endothelium-dependent relaxation is unchanged after SAH (8, 39). The relative contribution of endothelium-dependent vasodilators to vascular tone may depend on vessel size, and the effects of SAH on endothelial function may differ between large- and small-diameter vessels. Whereas basal NO production seems to control tone in both cerebral arteries and arterioles, the contribution of endothelium-derived hyperpolarizing factor (EDHF) to blood flow regulation may be greater at the level of the microcirculation (64). Previous studies suggest that EDHF-mediated relaxation requires the activity of endothelial SKCa and IKCa channels (10, 67), and these channels are involved in modulating basal tone in parenchymal arterioles but not in middle cerebral arteries (4). Here we show that constrictions following SKCa and IKCa inhibition are similar between control and SAH arterioles. Thus, while EDHF activation following endothelial stimulation (i.e., ATP) may be impaired in parenchymal arterioles after experimental SAH (32), our data suggest that the basal activity of these channels in the cerebral microcirculation is unaltered. Furthermore, activation of endothelial SKCa and IKCa channels with NS-309 caused a reduction of \([Ca^{2+}]_i\) to basal levels and profound dilation of parenchymal arterioles from both control and SAH animals. These results are consistent with activation of endothelial SKCa and IKCa channels by NS-309 hyperpolarizing electrically coupled smooth muscle cells toward \(E_K\). In addition, endothelium removal did not ablate SAH-induced pressure-induced increases in \([Ca^{2+}]_i\) and myogenic tone.
fore, reduced endothelial vasodilator influence cannot account for enhanced vasoconstriction of parenchymal arterioles after SAH. These results suggest that a direct alteration of smooth muscle membrane potential regulation contributes to dysfunction of intracerebral arterioles from SAH animals.

Our results indicate that elevated parenchymal arteriolar [Ca$^{2+}$], and tone after SAH depend on intravascular pressure. Accordingly, the effects of SAH on parenchymal arteriolar constriction observed in the present study may be exacerbated by elevated blood pressures commonly observed in SAH (15, 40). At this time, it is unclear how SAH causes enhanced pressure-dependent depolarization in parenchymal arterioles. Activation of mechanosensitive nonselective cation channels belonging to the transient receptor potential family (i.e., TRPC6, TRPM4) is reported to contribute to pressure-dependent membrane potential depolarization of cerebral artery myo-

Fig. 4. Elevated arteriolar Ca$^{2+}$ and enhanced constriction after SAH depend on membrane potential depolarization. A: summary membrane potential data obtained from isolated pressurized (5 mmHg) parenchymal arterioles bathed in 60 mM [K$^+$]. Membrane potential closely matched the theoretical equilibrium potential ($E_K$) of −22 mV in arterioles from both control ($n = 4$) and SAH ($n = 4$) animals. B and C: summary data showing [Ca$^{2+}$], (B) and constriction (C; % decrease in diameter) in control ($n = 7$) and SAH ($n = 5$) arterioles exposed to aCSF containing either 3 mM or 60 mM [K$^+$]. Nimodipine (300 nM) reduced [Ca$^{2+}$], to basal levels and dilated arterioles. NS $P > 0.05$ vs. control.

Fig. 5. Myocytes of pressurized parenchymal arterioles are depolarized after SAH. A and B: representative smooth muscle membrane potential measurements in arterioles isolated from control and SAH animals at 5 mmHg (A) and 40 mmHg (B) intravascular pressure. Scale bars represent 10 s. C: summary of membrane potential measurements obtained at 5 and 40 mmHg. At 40 mmHg, myocytes of arterioles isolated from SAH animals were significantly more depolarized (−28 ± 1 mV, $n = 4$) compared with myocytes of arterioles isolated from control animals (−35 ± 1 mV, $n = 5$). **$P < 0.01$ vs. control. NS $P > 0.05$ vs. control.
cytes (11, 63). Thus increased expression or activity of these channels after SAH may lead to membrane depolarization and enhanced VDCC activity. On the other hand, SAH-induced depolarization may also result from suppressed hyperpolarizing influence. For example, significant voltage-dependent K\(^+\) (K\(_V\)) channel-mediated current is observed at physiological membrane potentials (54), and activation of these channels represents an important feedback mechanism in response to pressure-induced depolarization. Consistent with this hypothesis, inhibition of K\(_V\) channels with 4-aminopyridine causes membrane potential depolarization and vasoconstriction (33, 56). Numerous studies have suggested that substances within blood may lead to membrane potential depolarization of cerebral artery myocytes via suppression of K\(^+\) channel activity (13, 20, 55). For example, using rabbit pial arteries, our laboratory has shown (28, 35) that acute exposure of vascular smooth muscle to the blood component oxyhemoglobin can cause internalization of K\(_V\)1.5 via a mechanism involving protein tyrosine kinase activation. Others have additionally demonstrated that mRNA and protein levels of K\(_V\)2.2 channels were decreased in basilar arteries from a canine SAH model (31). Further studies are required to determine whether K\(_V\) channel suppression or additional mechanisms contribute to enhanced pressure-dependent depolarization of parenchymal arteriolar myocytes in SAH animals.

Our present findings indicate a dominant role for L-type VDCC-mediated Ca\(^{2+}\) influx in enhanced parenchymal arteriolar constriction following SAH. Previous reports have demonstrated upregulated expression of R-type (CaV2.3) and T-type (CaV3.1 and CaV3.3) channels in pial cerebral arteries after experimental SAH (30, 47, 61). In contrast to these findings, we observed no effect on arteriolar [Ca\(^{2+}\)] or diameter of the R-type VDCC inhibitor SNX-482 or the T-type VDCC inhibitor mibefradil (after L-type VDCC inhibition) in the present study. There are several potential explanations that could account for the observed differences between our present study and those suggesting functional involvement of R-type and T-type VDCCs after SAH, including type of blood vessel studied (pial artery vs. parenchymal arteriole), cellular environment, and proximity to the induced blood clot. It is also of interest that two previous studies have reported no difference in spontaneous tone development in parenchymal arterioles and brain stem-penetrating arterioles after experimental SAH (52, 60). The basis for the observed differences between those studies and our present work is not readily apparent. However, in support of our present findings, histological studies have found evidence of increased constriction of intracerebral arterioles from SAH model animals (22, 37, 50).

Although rat models of SAH have been used widely to study the impact of subarachnoid blood on the brain and cerebral...
vasculature (37, 46), caution should be used in extrapolating data obtained from rodents to SAH-induced pathologies observed in humans after cerebral aneurysm rupture. For example, pial artery vasospasm in SAH model rats is less severe and of shorter time course than reported in canine and primate SAH models or in many cases of SAH-induced vasospasm in humans (41, 42). It should also be noted that in vivo cerebral blood flow measurements were not performed in the present study and represent an important future direction to establish whether SAH-induced decreased cortical blood flow parallels the enhanced parenchymal arteriolar constriction that we observed in vitro.

To summarize, we provide evidence that myocytes of parenchymal arterioles obtained from SAH model rats exhibit enhanced membrane potential depolarization at physiological intravascular pressures leading to increased L-type VDCC activity, elevated \([Ca^{2+}]_i\), and enhanced constriction. Furthermore, we demonstrate that inhibitors of L-type VDCCs (i.e., nimodipine) as well as agents that cause membrane potential hyperpolarization (i.e., NS-309) are effective in reducing \([Ca^{2+}]_i\), and dilating parenchymal vessels from SAH animals. We propose that therapeutic strategies targeting enhanced vasocostriction of the cerebral microcirculation may be beneficial in reducing neurological deficits associated with SAH.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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