Emergence of a R-Type Ca$^{2+}$ Channel (CaV 2.3) Contributes to Cerebral Artery Constriction After Subarachnoid Hemorrhage

Masanori Ishiguro, Theresa L. Wellman, Akira Honda, Sheila R. Russell, Bruce I. Tranmer, George C. Wellman

Abstract—Cerebral aneurysm rupture and subarachnoid hemorrhage (SAH) inflict disability and death on thousands of individuals each year. In addition to vasospasm in large diameter arteries, enhanced constriction of resistance arteries within the cerebral vasculature may contribute to decreased cerebral blood flow and the development of delayed neurological deficits after SAH. In this study, we provide novel evidence that SAH leads to enhanced Ca$^{2+}$ entry in myocytes of small diameter cerebral arteries through the emergence of R-type voltage-dependent Ca$^{2+}$ channels (VDCCs) encoded by the gene CaV 2.3. Using in vitro diameter measurements and patch clamp electrophysiology, we have found that L-type VDCC antagonists abolish cerebral artery constriction and block VDCC currents in cerebral artery myocytes from healthy animals. However, 5 days after the intracisternal injection of blood into rabbits to mimic SAH, cerebral artery constriction and VDCC currents were enhanced and partially resistant to L-type VDCC blockers. Further, SNX-482, a blocker of R-type Ca$^{2+}$ channels, reduced constriction and membrane currents in cerebral arteries from SAH animals, but was without effect on cerebral arteries of healthy animals. Consistent with our biophysical and functional data, cerebral arteries from healthy animals were found to express only L-type VDCCs (CaV 1.2), whereas after SAH, cerebral arteries were found to express both CaV 1.2 and CaV 2.3. We propose that R-type VDCCs may contribute to enhanced cerebral artery constriction after SAH and may represent a novel therapeutic target in the treatment of neurological deficits after SAH. (Circ Res. 2005;96:419-426.)

Key Words: calcium channels ▪ vascular smooth muscle ▪ cerebral arteries ▪ subarachnoid hemorrhage ▪ α1E

The consequences of subarachnoid hemorrhage (SAH) after cerebral aneurysm rupture are devastating, with mortality rates as high as 50% and the majority of survivors left with moderate to severe disability. Cerebral vasospasm, characterized as a delayed and sustained arterial constriction, is a major contributor to these high morbidity and mortality rates associated with SAH, and current therapies in the treatment of this phenomenon are less than ideal. Classically, cerebral vasospasm has been diagnosed in SAH patients by the use of angiography to detect cerebral artery narrowing. However, small diameter arteries, below the resolution limits of standard angiography, may also be affected by subarachnoid blood. We have recently documented that small diameter (100 to 200 μm diameter) cerebral arteries from a rabbit model of SAH are significantly more constricted at physiological intravascular pressures compared with similar arteries from healthy animals. This enhanced pressure-induced constriction of small diameter arteries may contribute to decreased cerebral blood flow after SAH.

In vitro, elevation of intravascular pressure within a physiological range (60 to 100 mm Hg) will constrict small diameter cerebral arteries in the absence of other vasoactive stimuli. In cerebral arteries from healthy animals, increased intravascular pressure leads to vascular smooth muscle membrane potential depolarization and increased global cytosolic free Ca$^{2+}$ concentration ([Ca$^{2+}$]) because of an increase in the open-state probability of L-type voltage–dependent Ca$^{2+}$ channels (VDCCs). L-type VDCC antagonists cause a maximal decrease in [Ca$^{2+}$], and abolish pressure- and agonist-induced constriction in small diameter arteries. Although L-type Ca$^{2+}$ channels are widely accepted to be the dominant type of voltage-dependent Ca$^{2+}$ channels expressed in arterial myocytes, studies have also reported the presence of T-type, P/Q-type, and nifedipine-resistant high voltage–activated Ca$^{2+}$ Channels. Expression of L-type Ca$^{2+}$ channels in vascular smooth muscle has been reported to change both during development and hypertensive. The goal of our study was to examine whether the properties of voltage-dependent Ca$^{2+}$ channels are altered in...
100 to 200 μm diameter cerebral arteries obtained from a rabbit SAH model. In this article, we provide evidence that enhanced cerebral artery constriction after SAH is mediated by an increase in the current density of voltage-dependent Ca2+ channels. Our data also suggest that the expression of an additional VDCC family member (R-type, CaV 2.3) may confer a partial resistance of cerebral arteries to L-type Ca2+ channel blockers after SAH. We propose that R-type VDCCs may contribute to enhanced cerebral artery constriction after SAH.

Materials and Methods

SAH Model
New Zealand White rabbits (males, 3.0 to 3.5 kg; Charles River Laboratories, Saint Constant, Quebec, Canada) were initially anesthetized by isoflurane (5%) using an induction chamber, then intubated and maintained on isoflurane (2 to 3%) for the duration of the surgical procedure. As described previously,2 3 mL of unheparinized blood was injected into the cisternal magna. Buprenorphine (0.01 mg/kg) was given every 12 hours (for 36 hours, then as needed) as an analgesic. In the present study, cerebral arteries were obtained from control (no surgery) and SAH (5 days after surgery) animals. Rabbits were euthanized by exsanguination under deep pentobarbital anesthesia (IV; 60 mg/kg body weight). Posterior cerebral and cerebellar arteries (100- to 200-μm diameter), which are consistently located within the clot region of SAH animals, were dissected from the brain in cold (4°C), oxygenated (20% O2/5% CO2/75% N2) physiological saline solution (PSS) of the following composition (in mmol/L): 119 NaCl, 4.7 KCl, 24 NaHCO3, 1.2 KH2PO4, 1.6 CaCl2, 1.2 MgSO4, 0.023 EDTA, and 10 glucose. All protocols were conducted in accordance with the guidelines for the care and use of laboratory animals (NIH publication 85-23, 1985) and followed protocols approved by the Institutional Animal Use and Care Committee of the University of Vermont, USA.

Diameter Measurements in Isolated Arteries
Cerebral artery segments were cannulated on glass pipettes mounted in a 5 mL myograph chamber (Living Systems Instruments) and superfused with aerated PSS (20% O2/5% CO2/75% N2) at 37°C and pH 7.4, as previously described.5 Arterial diameter was measured with video edge detection equipment and recorded using data acquisition software (Dataq Instruments Inc).

Measurements of VDCC Currents
Vascular smooth muscle cells were enzymatically isolated from cerebral arteries as previously described.19 The conventional whole cell configuration of the patch clamp technique was used to measure whole-cell VDCC currents in cerebral artery myocytes. The external (bath) solution contained (in mmol/L): 125 NaCl, 10 KCl, 5 CaCl2, 10 HEPES, 1 MgCl2, and 10 glucose; pH adjusted to 7.4 with NaOH. Patch pipettes (3 to 5 MΩ) were filled with an internal solution that contained (in mmol/L) 130 CsCl, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N’N’,N’-tetraacetic acid, 10 HEPES, 1 MgCl2, 2 ATP, 0.5 GTP, 5 phosphocreatine, and 10 glucose; pH adjusted to 7.2 with CsOH. Ki values for diltiazem and nisoldipine were determined from Hill plot of concentration-response curves of currents using the following equation: y = Imax/(1 + (drug/Ki)n) + r, where Imax denotes the maximum effect, concentration of drug (diltiazem or nisoldipine), Hill coefficient, and drug resistant current, respectively. Experiments were performed at 24 to 26°C.

RNA Isolation and RT-PCR
Total RNA was extracted and quantified from cerebral arteries and first-strand cDNA was synthesized using Omniscript RT kit (Quagen). To perform RT-PCR, α CaV subunit–specific primers were generated from unique coding regions for CaV 2.3 and CaV 1.2. The following sets of primers were used: CaV 2.3 (GenBank Accession no. X67855) sense nucleotides 5692 to 5709 (5’-CCCGAGAAATCATCGCC-3’), and antisense nucleotides 6771 to 6750 (5’-GTCTTCAGTGCTACTCAG-3’); CaV 1.2 (GenBank Accession no. X55763) sense nucleotides 5380 to 5398 (5’-ATGGCAAAAACAATCGG-3’), antisense nucleotides 6211 to 6193 (5’-CTCCGAAAATCAAGACGC-3’). cDNA was amplified under the following RT-PCR conditions: 94°C 3 minutes for preincubation, 94°C 1 minute, 55 to 57°C 1 minute,
and 72°C for 1.5 minutes for 35 cycles followed by an incubation at 72°C for 10 minutes.

Immunofluorescence
Whole-mount arteries were fixed with 10% formalin at room temperature for 1.5 hours then permeabilized and blocked with 0.1% Triton-X100/2% bovine serum albumin in PBS (pH 7.4). Arteries were stained using rabbit anti-CaV 2.3 (primary antibody obtained from Alomone labs) and Cy5 anti-rabbit as a secondary antibody. Images were obtained using a Bio-Rad laser confocal microscope (excitation 650 nm and emission 670 for Cy5). The cyanine nuclear dye, YoYo-1 (excitation 490 nm and emission 510 nm), was used to identify cell nuclei.

Statistical Analysis
Data are presented as mean±SEM. Statistical significance was considered at the level of *P<0.05 (*) or **P<0.01 (**) using Student t test.

Results
Cerebral Artery Constriction Becomes Partially Resistant to L-Type VDCC Blockers After SAH
To examine the effects of subarachnoid blood on small diameter cerebral arteries, we have used an established model of SAH, where blood (3 mL) is injected into the cisterna magna of anesthetized rabbits. Five days after this surgery, we isolated and cannulated 100- to 200-μm diameter cerebral arteries to obtain in vitro diameter measurements using video microscopy. Whereas arteries from both control and SAH animals constrict to increase intravascular pressure, we have observed significant differences in the characteristics of these pressure-induced responses between groups. Notably, pressure-induced constriction is approximately twice as great in arteries from SAH animals (Figure 1). We have also observed a fundamental difference in how these small-diameter arteries from SAH animals respond to L-type VDCC antagonists. In arteries from control animals, pressure-induced constriction at 100 mm Hg was abolished by diltiazem (50 μmol/L), an L-type VDCC antagonist (Figure 1A). However, in marked contrast to arteries from healthy animals, pressure-induced constriction of cerebral arteries from SAH animals was partially resistant to a combination of diltiazem (50 μmol/L) and nisoldipine (1 μmol/L). In arteries from SAH animals, the constriction resistant to L-type VDCC blockers, representing approximately 20% of the total constriction, was reversed by removal of extracellular Ca2+ (Figure 1B and 1C). These data suggest an additional Ca2+ entry pathway resistant to blockers of L-type VDCCs emerges in cerebral arteries after SAH and contributes to enhanced pressure-induced constriction.

Enhanced VDCC Currents With Distinct Biophysical Properties After SAH
To further explore the contribution of VDCCs to enhanced cerebral artery constriction after SAH, conventional whole-cell patch clamp electrophysiology was performed on freshly isolated cerebral artery myocytes. No gross morphological distinctions were apparent between myocytes from control and SAH animals. Cell capacitance, an index of cell membrane surface area, was similar between the two groups (control: 12.6±0.3 pF, n=252; SAH: 12.9±0.2 pF, n=315). Using 10 mmol/L Ba2+ as a charge carrier, we observed that depolarizing voltage steps from a holding potential of −80 mV elicited greater inward membrane currents in myocytes isolated from SAH animals (Figure 2A and 2B). Membrane currents from both control and SAH myocytes exhibited a “V-shaped” current-voltage relationship, with peak current at +20 mV (Figure 2C). Current density at +20 mV was markedly enhanced (≈50%) in myocytes from SAH (−12.8±1.5 pA/pF) versus control (−8.3±1.3 pA/pF) animals. The voltage for half-maximal activation (V0.5,act) obtained from Boltzmann fit of tail currents was similar between myocytes isolated from control animals (0.8±0.7 mV) and SAH animals (−2.3±0.6 mV), consistent with the activation of high voltage-activated channels, but not low voltage-activated channels, in both cell types (Figure 2D).

We next explored whether enhanced VDCC currents in cerebral artery myocytes from SAH animals had inactivation properties distinct from the L-type VDCCs in myocytes of control animals. We found that the voltage for half-maximal steady-state inactivation (V0.5,inact) was shifted by about −15 mV in SAH myocytes (Figure 3A). In addition, membrane currents from SAH myocytes exhibited a more rapid inactivation. Membrane current decay from myocytes of both
control and SAH animals were best fit to a double exponential decay. Time constants for fast inactivation ($\tau_1$) were decreased by 40% to 50% at voltages between +10 mV and +30 mV in SAH compared with control myocytes (Figure 3B). Time constants for slow inactivation ($\tau_2$) were not significantly different between groups (Figure 3C). Further, $\tau_1$ makes a greater contribution to current decay in cerebral artery myocytes isolated from SAH compared with control animals (Figure 3D). These data demonstrate a fundamental change in the properties of VDCC currents, suggesting the possibility that a second, more rapidly inactivating, high voltage-activated Ca\(^{2+}\) current is determined at $-80$ mV. Voltage for half-maximal ($V_{1/2}\text{m}ax$) inactivation for control (1.4±1.6 mV, n=6) and SAH (-13.8±1.2 mV, n=10) cells were determined from Boltzmann fit of data. B, Summary of fast inactivation time constants from control (n=10) and SAH (n=10) myocytes. Fast inactivation time constants ($\tau_1$) were obtained by double exponential fit of current decay ($I=I_0+e^{-t/\tau_1}+A_2e^{-t/\tau_2}$) during a 800-ms voltage step from $-80$ to $+20$ mV. C, Summary of slow inactivation time constants ($\tau_2$) from control (n=10) and SAH (n=10) myocytes. D, Contribution of fast time constant to current decay ($A_1/A_1+ A_2 + C$) during a 800-ms voltage step from $-80$ to $+20$ mV. *$P<0.05$, **$P<0.01$.

Figure 3. Inactivation of VDCC currents in cerebral artery myocytes isolated from control and SAH animals. A, Steady state inactivation of VDCC currents from control and SAH myocytes were obtained using a double-step voltage protocol (see inset) where every 10 seconds cells are stepped from $-80$ mV to a membrane potential between $-70$ and $+20$ mV for 1 second; before membrane current is determined at $+20$ mV. Voltage for half-maximal ($V_{1/2}\text{m}ax$) inactivation for control (1.4±1.6 mV, n=6) and SAH (-13.8±1.2 mV, n=10) cells were determined from Boltzmann fit of data. B, Summary of fast inactivation time constants from control (n=10) and SAH (n=10) myocytes. Fast inactivation time constants ($\tau_1$) were obtained by double exponential fit of current decay ($I=I_0+e^{-t/\tau_1}+A_2e^{-t/\tau_2}$) during a 800-ms voltage step from $-80$ to $+20$ mV. C, Summary of slow inactivation time constants ($\tau_2$) from control (n=10) and SAH (n=10) myocytes. D, Contribution of fast time constant to current decay ($A_1/A_1+ A_2 + C$) during a 800-ms voltage step from $-80$ to $+20$ mV. *$P<0.05$, **$P<0.01$.

SNX-482–Sensitive VDCC Currents Emerge in Cerebral Artery Myocytes After SAH

We next examined whether pharmacological agents that specifically block various VDCC subtypes differentially impact currents from control and SAH myocytes. Consistent with our functional data (Figure 1), the L-type VDCC blocker diltiazem was less effective inhibitor of membrane currents in cerebral artery myocytes isolated from SAH animals. Although the highest concentration of diltiazem (1000 μmol/L) used in this study completely blocked currents from control myocytes, approximately 20% of the current in SAH myocytes was resistant to this L-type VDCC blocker (Figure 4A and 4B). The current voltage relationship of the diltiazem-resistant current was consistent with high voltage-activated VDCCs and similar to membrane currents from control and SAH animals in the absence of diltiazem (Figure 4C). The diltiazem-resistant component of VDCC currents present in SAH myocytes exhibited rapid inactivation kinetics ($\tau=10.5±1.0$ ms at $+20$ mV, n=10). Excluding the diltiazem-resistant component, VDCC currents from SAH myocytes were also less sensitive to diltiazem ($K_i=99.5±10.4$ μmol/L) compared with control ($K_i=41.4±5.7$ μmol/L) myocytes. In a similar manner (ie, excluding the resistant component), SAH myocytes exhibited decreased sensitivity to the dihydropyridine L-type Ca\(^{2+}\) channel antagonist, nifedipine (SAH: $K_i=125.8±6.4$ nmol/L versus control: $K_i=55.6±3.9$ nmol/L). VDCC currents resistant to high concentrations of nifedipine (10 μmol/L) in SAH myocytes were abolished by cadmium (200 μmol/L), a nonselective Ca\(^{2+}\) channel blocker (n=4, data not shown). The selective blocker of N-type Ca\(^{2+}\) channels, $\alpha_1$-Conotoxin GVIA$^{21}$ (1 μmol/L) and P/Q type Ca\(^{2+}\) channel blocker, $\alpha_2$-Agatoxin IVA$^{22}$ (100 nmol/L) did not alter peak inward currents at $+20$ mV in SAH myocytes (Figure 4D). Whereas blockers of N- and P/Q-type channels were without effect, the R-type Ca\(^{2+}\) channel blocker, SNX-482 (200 nmol/L),$^{23}$ reduced VDCC currents by approximately 30% in myocytes from SAH, but had no effect on VDCC currents in myocytes from control animals (Figure 5A and 5B). Consistent with this electrophysiological data, SNX-482 dilated pressurized cerebral arteries from SAH animals by approximately 30%, but had no significant effect on pressurized arteries from controls (Figure 5C and 5D). These data suggest that the enhanced VDCC currents observed in cerebral artery myocytes after SAH may represent the expression of R-type Ca\(^{2+}\) channels.

R-Type (Ca\(_\gamma\) 2.3) VDCC Expression in Cerebral Artery Myocytes After SAH

We next performed RT-PCR to examine whether mRNA of the pore-forming $\alpha_\gamma$ subunit of the R-type VDCC ($\alpha_{\gamma_{\text{II}}}$ encoded by the gene Ca\(_\gamma\) 2.3) is present in cerebral artery myocytes after SAH. Using $\alpha_{\gamma_{\text{II}}}$-specific primers generated from rabbit Ca\(_\gamma\) 2.3 (see Materials and Methods for sequence), we detected the expected 1.1-kb RT-PCR product of these primers in cerebral arteries obtained from SAH animals, but not control animals (n=4) (Figure 6A). DNA sequencing verified that the product generated matched the expected sequence of Ca\(_\gamma\) 2.3. No product was detected in reactions performed in the absence of reverse transcriptase consistent with the absence of genomic DNA contamination of the RNA samples. We did, however, detect the pore-forming $\alpha_\gamma$ subunit of the L-type VDCC ($\alpha_{\text{IC}}$ encoded by the gene Ca\(_\gamma\) 1.2) in both the...
control and SAH mRNA samples that were also used to examine CaV 2.3 expression (Figure 6B). RT-PCR did not detect CaV 2.3 mRNA in larger diameter basilar arteries obtained from control or SAH animals.

Using confocal microscopy and the nuclear dye, YoYo, to identify vascular smooth muscle cells, we detected much greater immunofluorescent labeling of CaV 2.3 in the smooth muscle of arteries from SAH, compared with control animals (Figure 7).
The enhanced fluorescence in arteries from SAH animals was abolished by either preadsorption of the primary (CaV 2.3) antibody with a specific blocking peptide or exclusion of the CaV 2.3 antibody from the staining protocol (labeling with secondary antibody only). These qualitative data provide the first evidence for R-type VDCC expression in small diameter cerebral arteries. Further, these data suggest that expression of pore-forming VDCC /H9251 subunits is altered in cerebral artery myocytes after subarachnoid hemorrhage with expression of only CaV 1.2 in control animals, but with expression of both CaV1.2 and CaV 2.3 after subarachnoid hemorrhage.

Discussion

In this study, we provide evidence indicating that R-type Ca2⁺ channel (CaV 2.3) expression contributes to enhanced cerebral artery constriction after SAH. The following observations are consistent with this hypothesis: (1) pressure-induced cerebral artery constriction is enhanced and partially resistant to L-type Ca2⁺ channel blockers after SAH; (2) VDCC currents in cerebral artery myocytes after SAH are enhanced, but partially resistant to L-type Ca2⁺ channel blockers; (3) the R-type Ca2⁺ channel antagonist, SNX-482, reduced VDCC currents in cerebral artery myocytes after SAH, but partially resistant to L-type Ca2⁺ channel blockers; (3) the R-type Ca2⁺ channel antagonist, SNX-482, reduced VDCC currents in cerebral artery myocytes after SAH, but not control animals; (4) SNX-482 dilated cerebral arteries from SAH, but not control animals; and (5) CaV 2.3 expression occurs in cerebral arteries after SAH.

Our in vitro data demonstrate enhanced constriction over a physiological range of intravascular pressures in 100 to 200 µm diameter cerebral arteries obtained from a rabbit SAH model. In vivo, enhanced constriction of these arteries could limit cerebral blood flow and potentially contribute to the delayed neurological deficits after cerebral aneurysm rupture. Our observations are consistent with previous reports suggesting the possible involvement of the resistance vasculature in the neurological complications that often follow cerebral aneurysm rupture and SAH. However, future studies are required to define the clinical significance of our present findings.

We feel it likely that an increase in VDCC current density contributes to enhanced vasoconstriction after SAH. We have observed a substantial (~50%) increase in VDCC current density in cerebral artery myocytes isolated from SAH animals. Increased VDCC currents are consistent with the pivotal role of this Ca2⁺ entry pathway in dictating the contractile state of arterial smooth muscle. Our findings suggest that SAH not only increases VDCC current density, but leads to the expression of an additional VDCC family member, encoded by the gene CaV 2.3, that is distinct from L-type VDCCs (CaV 1.2) found in cerebral artery myocytes of healthy animals. In cerebral artery myocytes from control animals, nifedipine and diltiazem abolished VDCC currents, consistent with a predominant role of L-type VDCCs (CaV 1 family members) in the cerebral vasculature in healthy animals. In marked contrast, VDCC currents obtained from cerebral artery myocytes of SAH animals were...
partially resistant to L-type Ca\(^{2+}\) channel blockers. Selective blockers of P/Q type VDCCs (Ca\(_V\) 2.1) and N-type VDCCs (Ca\(_V\) 2.2) had no effect on VDCC currents from SAH myocytes. However, SNX-482, a peptide found in the venom of the African tarantula Hysteroctates gigas that blocks R-type VDCCs (Ca\(_V\) 2.3), reduced currents by \(\approx 50\%\) in myocytes from SAH, but not control animals. There has been considerable variability with respect to the reported voltage for half-maximal activation (\(V_{0.5}\text{act}\)) of the R-type or Ca\(_V\) 2.3 calcium channel. Soong et al.\(^{29}\) in their initial study of the cloned rbE-II (alpha1E) calcium channel, reported membrane currents that peaked around \(-10\) mV with a \(V_{0.5}\text{act}\) of approximately \(-25\) mV and described this channel as low voltage–activated. A number of subsequent studies\(^{30–32}\) have described R-type VDCCs to be high voltage–activated with a \(V_{0.5}\text{act}\) similar to that of L-type VDCCs. These latter observations are consistent with our present findings that inward currents from both control and SAH myocytes exhibited current–voltage relationships and voltage–dependent activation kinetics characteristic of high voltage–activated VDCCs. We have also observed that inactivation time constants were shorter for VDCC currents from SAH myocytes. Native R-type and expressed Ca\(_V\) 2.3 Ca\(^{2+}\) channels are known for their more rapid rate of inactivation compared with L-type Ca\(_V\) 2.1 channels. Indeed, the fast inactivation time constant (\(\tau_I\)) of the diltiazem-resistant current present in myocytes from SAH animals (\(\tau_I=10.5\pm 1.0\) ms at \(+20\) mV) is comparable to values of \(\tau_I\) previously reported for R-type Ca\(_V\) 2.3 channels.\(^{33,34}\) We also found a \(-15\) mV shift in the steady-state voltage–dependent inactivation of VDCC currents after SAH, consistent with previous studies that have found steady-state inactivation of R-type VDCCs occurs at more hyperpolarized membrane potentials than L-type VDCCs.\(^{35}\) Supporting a role for the emergence of R-type Ca\(_V\) 2.3 channels in cerebral artery myocytes after SAH, we have also detected Ca\(_V\) 2.3 mRNA and protein in cerebral arteries after SAH and have found that SNX-482 dilates cerebral arteries from SAH, but not healthy animals. Our electrophysiological and functional studies suggest that after SAH, this novel Ca\(^{2+}\) entry pathway (R-type VDCCs) contributes approximately 20% to 30% of the total Ca\(^{2+}\) influx and resulting contraction in myocytes of small diameter cerebral arteries.

It should be noted that despite the emergence of R-type Ca\(^{2+}\) channels in small diameter cerebral arteries, calcium influx via these myocytes after SAH. In this study, we report that in small diameter cerebral arteries, excluding the potential contribution of R-type Ca\(_V\) 2.3 channels, L-type VDCC currents after SAH are less sensitive to L-type Ca\(_V\) 2.3 channel blockers. The mechanism underlying this decrease in the sensitivity of L-type VDCCs to pharmacological agents is currently uncertain. We cannot exclude the potential contribution of altered expression of other pore-forming \(\alpha\) VDCC subunits (including Ca\(_V\) 1.2 splice variants) or possible alterations in auxiliary (\(\alpha_\delta, \beta, \gamma\)) subunits that may impact \(\alpha\) trafficking to the cell membrane, drug binding to the channel pore, or modulate channel activation/inactivation kinetics.\(^{36–38}\)

In summary, the present study suggests the emergence of R-type VDCCs in small diameter cerebral arteries after SAH may contribute to enhanced constriction and decreased cerebral blood flow after SAH. We propose that R-type Ca\(^{2+}\) channels may represent a novel target in the search for more effective agents to prevent neurological deficits associated with SAH.

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