Oxyhemoglobin-Induced Expression of R-Type Ca$^{2+}$ Channels in Cerebral Arteries

Timothy E. Link, MD; Kentaro Murakami, PhD; Micah Beem-Miller, BS; Bruce I. Tranmer, MD; George C. Wellman, PhD

**Background and Purpose**—Cerebral vasospasm after subarachnoid hemorrhage (SAH) is a major contributor to mortality and morbidity after aneurysm rupture. Recently, R-type voltage-dependent Ca$^{2+}$ channel (VDCC) expression has been associated with increased cerebral artery constriction in a rabbit model of SAH. The goal of the present study was to examine whether the blood component oxyhemoglobin (oxyHb) can mimic the ability of SAH to cause R-type VDCC expression in the cerebral vasculature.

**Methods**—Rabbit cerebral arteries were organ cultured in serum-free media for up to 5 days in the presence or absence of purified oxyHb (10 $\mu$mol/L). Diameter changes in response to diltiazem, (L-type VDCC antagonist) and SNX-482 (R-type VDCC antagonist) were recorded at day 1, 3, or 5 in arteries constricted by elevated extracellular potassium. RT-PCR was performed on RNA extracted from arteries cultured for 5 days ($\pm$oxyHb) to assess VDCC expression.

**Results**—After 5 days, oxyHb-treated arteries were less sensitive and partially resistant to diltiazem compared to similar arteries organ cultured in the absence of oxyHb. Further, SNX-482 dilated arteries organ cultured for 5 days in the presence, but not in the absence, of oxyHb. RT-PCR revealed that oxyHb treated arteries expressed R-type VDCCs (CaV 2.3) in addition to L-type VDCCs (CaV 1.2), whereas untreated arteries expressed only CaV 1.2.

**Conclusions**—These results demonstrate that oxyhemoglobin exposure for 5 days induces the expression of CaV 2.3 in cerebral arteries. We propose that oxyhemoglobin contributes to enhanced cerebral artery constriction after SAH via the emergence of R-type VDCCs. (Stroke. 2008;39:2122-2128.)

**Key Words:** calcium channels  ■ cerebral arteries  ■ subarachnoid hemorrhage  ■ vasospasm  ■ vascular smooth muscle

Oxyhemoglobin (oxyHb) has been implicated in the development of cerebral vasospasm and the associated delayed neurological deficits frequently encountered after aneurysmal subarachnoid hemorrhage (SAH). For example, the peak in free oxyHb concentrations in the cerebral spinal fluid of SAH patients, attributable to lysis of red blood cells, correlates with the onset of cerebral vasospasm after aneurysm rupture. Furthermore, in vivo application of oxyHb into the subarachnoid space can mimic the effect of whole blood to induce cerebral vasospasm. Although evidence suggests oxyHb contributes to the development of SAH-induced vasospasm, the mechanisms involved in this phenomenon are less clear.

Acute application of oxyHb constricts cerebral arteries via a number of mechanisms including suppression of K$^+$ channel activity, enhanced calcium entry, and increased activity of protein kinase C and Rho kinase. Currently, it is uncertain whether altered gene expression may also contribute to the ability of oxyHb to decrease cerebral artery diameter. Our laboratory has recently shown expression of R-type VDCCs, encoded by the gene CaV 2.3, in small diameter cerebral arteries in a rabbit model of SAH. The emergence of R-type VDCCs in cerebral artery myocytes, combined with existing L-type VDCCs, would promote elevated intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) and ultimately increased cerebral artery constriction.

The objective of the present study was to examine whether purified oxyHb mimics SAH to induce CaV 2.3 expression in the cerebral vasculature. Here, we report the emergence of R-type VDCCs in cerebral arteries from healthy rabbits organ cultured in the presence of purified oxyHb for a period of 5 days. Further, our data suggest that L-type VDCCs in cerebral arteries become less sensitive to antagonists such as diltiazem after 5-day exposure to oxyHb. These findings are consistent with a role of oxyHb-induced R-type VDCC expression in enhanced constriction of small diameter cerebral arteries after SAH.

**Materials and Methods**

All protocols were conducted in accordance with the *Guidelines for the Care and Use of Laboratory Animals* (NIH) and approved by the...
Organ Culture of Cerebral Arteries

Once blood was flushed from the lumen, anterior and posterior cerebral arteries were transferred into serum free Dulbecco modified Eagle medium (DMEM/F12 supplemented with penicillin (50 U/mL) and streptomycin (50 µg/mL) and placed in an incubator at 37°C with 5% CO₂ and 97% humidity. Arteries were cultured for up to 5 days in the presence or absence of purified hemoglobin Aₐ (oxy form; 10 µmol/L, Hemosol Inc: oxyHb). Culture medium was changed twice daily to maintain oxyHb levels, as our spectrophotometric studies indicate that oxyHb concentrations decreased approximately 20% after 8 hours in organ culture (data not shown). A concentration of 10 µmol/L oxyHb was chosen to approximate extracellular levels of this compound reported near cerebral arteries after SAH. Preliminary studies were also performed using arteries organ cultured with a higher concentration (100 µmol/L) of oxyHb. However, we observed a dramatic decrease in the viability of arteries organ cultured with 100 µmol/L oxyHb for a period of 5 days (5 of 5 arteries from 3 different animals).

Diameter Measurements in Isolated Arteries

Isolated arteries were cannulated in a 5-mL myograph chamber (Living Systems Instruments) and perfused with aerated physiological saline solution (PSS) with the following composition (in mmol/L): 118.5 NaCl, 4.7 KCl, 24 NaHCO₃, 1.18 KH₂PO₄, 1.6 CaCl₂, 1.2 MgCl₂, 0.023 EDTA, and 11 glucose, pH 7.4. Arteries were held at a relatively low intravascular pressure (20 mm Hg) to minimize responses to the endothelium-dependent vasoconstrictors adenosine and nitric oxide and to minimize potential depolarization accompanying this positive shift in EK leads from 6 mmol/L to 60 mmol/L, shifting the K⁺ equilibrium potential (E钾) from approximately −85 mV to approximately −20 mV. In cerebral artery myocytes, the membrane potential depolarization accompanying this positive shift in E钾 leads to an increase in the open-state probability of VDCCs, an increase in a marked difference emerged between arteries maintained in the presence and absence of oxyHb. K⁺ induced constrictions from arteries organ cultured for 5 days in the presence of oxyHb were significantly less sensitive to diltiazem as evidenced by a more than 10-fold increase in the IC₅₀ value (16.9±6.9 µmol/L). Further, approximately 20% of the K⁺-induced constriction in these arteries was resistant to 1 µmol/L diltiazem, a concentration nearly 1000-fold higher than the IC₅₀ for freshly isolated arteries (Figure 1c).

We next examined whether organ culture of arteries for 5 days affected responses to the endothelium-dependent vasodilator acetylcholine (Ach) or the endothelium-independent nitrovasodilator sodium nitroprusside (SNP). As Ach and

RNA Isolation and RT-PCR

Total RNA was extracted from freshly isolated and organ cultured cerebral arteries and brain using RNeasy Micro kit (QIAGEN). cDNA was synthesized by Omniscript reverse transcriptase (QIAGEN) using 250 ng of total RNA. Reactions were also performed in the absence of reverse transcriptase as a negative control, using an equal amount of total RNA. A unique coding region of the R-type VDCC α1 subunit (α1E, Ca₃.2 GenBank Accession # X67855) was amplified using the following set of primers: sense nucleotides 6562 to 6580 (5’- GACAGCGACACACTAC-3’) and antisense nucleotides 6996 to 6978 (5’-GCTGAGGAGAGATTGC-3’). Semiquantitative RT-PCR analysis was performed by comparing band intensities of arteries organ cultured in the absence and presence of oxyHb when equivalent amounts of cDNA were used for each preparation as determined by GAPDH PCR amplification. To further verify expression of Ca₃.2, nested PCR was used with the following sets of primers: forward primer 5’-ACCCCTCCTGCTCTGTC-3’ (3586–3605) and reverse primer 5’-TGCTCTGTTGAGCTTTGTC-3’ (3773–3754) for the 1st round PCR; 5’-CGAGGGTGTTGGGAAAAGAG-3’ (3607–3625) and 5’-CGTGGTTGACCTTGTCGTG-3’ (3767–3748) for the 2nd round PCR. The amplified DNA was extracted from gel bands and sequenced to confirm PCR products.

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 for comparisons between 2 groups or analysis of variance followed by Student-Newman-Keuls test for pairwise multiple comparisons.

Results

Five-Day Oxyhemoglobin Exposure Reduces the Efficacy of the L-Type VDCC Blocker Diltiazem to Dilate Cerebral Arteries

Our initial goal was to examine the impact of 1- to 5-day oxyHb exposure on VDCC function in small diameter cerebral arteries. Increasing extracellular K⁺ to 60 mmol/L caused freshly isolated cerebral arteries to constrict by 113±12 µm, representing a 59% decrease in diameter (n=5). In the presence of 60 mmol/L K⁺, the L-type VDCC blocker diltiazem evoked a concentration-dependent dilation of freshly isolated cerebral arteries, causing a maximum dilation of 98.8±0.4% at a concentration of 1 µmol/L (Figure 1a). To explore the influence of oxyHb on VDCC function, cerebral arteries were placed in serum-free organ culture media in the presence or absence of purified oxyHb (10 µmol/L) for a period of 1 to 5 days. After organ culture, arteries were removed from the media, placed in PSS (the absence of oxyHb), and cannulated for in vitro diameter measurements. After a 30-minute equilibration period, in vitro arterial diameters in PSS were not significantly different between organ cultured and freshly isolated arteries (Table). As with freshly isolated arteries, diltiazem (1 µmol/L) fully diluted arteries organ cultured for 24 or 72 hours (±oxyHb) that were constricted with 60 mmol/L extracellular K⁺ (Figure 1a and 1b). The IC₅₀ values for diltiazem-induced dilation were not significantly different (approximately 1 µmol/L, Table) in freshly isolated arteries and arteries organ cultured for 24 or 72 hours (±oxyHb). However, after 5 days of organ culture, a marked difference emerged between arteries maintained in the presence and absence of oxyHb. K⁺-induced constrictions from arteries organ cultured for 5 days in the presence of oxyHb were significantly less sensitive to diltiazem as evidenced by a more than 10-fold increase in the IC₅₀ value (16.9±6.9 µmol/L). Further, approximately 20% of the K⁺-induced constriction in these arteries was resistant to 1 µmol/L diltiazem, a concentration nearly 1000-fold higher than the IC₅₀ for freshly isolated arteries (Figure 1c).

We next examined whether organ culture of arteries for 5 days affected responses to the endothelium-dependent vasodilator acetylcholine (Ach) or the endothelium-independent nitrovasodilator sodium nitroprusside (SNP). As Ach and...
SNP may act in part through K⁺ channel activation, pathways were constricted with a combination of histamine (30 μmol/L) and serotonin (30 μmol/L) before addition of increasing concentrations of acetylcholine (Ach; a) or sodium nitroprusside (SNP; b). Day 0 represents freshly isolated cerebral arteries. **P<0.01, Day 0 vs Day 5 (±oxyHb). In many cases, where no statistical differences were observed, the power of the performed test was below the desired power of 0.800. The lack of statistical difference should be interpreted cautiously.  

**Table. Diltiazem-Induced Dilation of Fresh and Organ-Cultured Cerebral Arteries**

<table>
<thead>
<tr>
<th></th>
<th>Day 0 (n=5)</th>
<th>-oxyHb (n=5)</th>
<th>+oxyHb (n=6)</th>
<th>Day 3 (n=7)</th>
<th>-oxyHb (n=7)</th>
<th>+oxyHb (n=5)</th>
<th>Day 5 (n=4)</th>
<th>-oxyHb (n=4)</th>
<th>+oxyHb (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial diameter at 20 mm Hg (μm)§</td>
<td>192.6±24.0</td>
<td>256.6±25.7</td>
<td>259.5±20.1</td>
<td>215.1±17.5</td>
<td>257.6±20.6</td>
<td>212.3±26.4</td>
<td>184.0±17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K⁺-induced constriction (% decrease in diameter)§</td>
<td>59.0±1.4</td>
<td>57.9±3.9</td>
<td>58.9±3.0</td>
<td>59.0±1.0</td>
<td>61.2±2.9</td>
<td>57.7±3.1</td>
<td>62.4±1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diltiazem IC₅₀ (μmol/L)</td>
<td>0.93±0.06</td>
<td>0.76±0.07</td>
<td>0.95±0.14</td>
<td>0.78±0.12</td>
<td>1.07±0.06</td>
<td>1.1±0.10</td>
<td>16.9±6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diltiazem max dilation (% passive diameter)</td>
<td>98.8±0.4</td>
<td>99.5±0.5</td>
<td>99.0±0.2</td>
<td>99.3±0.3</td>
<td>98.6±0.4</td>
<td>98.3±0.6</td>
<td>80.7±1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05. Analysis of variance followed by Student-Newman-Keuls test for pairwise multiple comparisons.  
§The power of the performed test was below the desired power of 0.800. The lack of statistical difference should be interpreted cautiously.
culture or oxyHb treatment. These data suggest that the decreased efficacy of diltiazem to dilate arteries organ cultured in the presence of oxyHb reflects a selective and fundamental change in VDCCs of cerebral artery myocytes.

The R-Type VDCC Blocker SNX-482 Dilates Cerebral Arteries After 5-Day Exposure to Oxyhemoglobin

The above data demonstrate that K⁺-induced constriction of cerebral arteries exposed to oxyHb for 5 days is partially resistant to the L-type VDCC blocker diltiazem. We have previously reported a similar phenomenon attributable to the emergence of R-type VDCCs in cerebral arteries obtained from a rabbit model of subarachnoid hemorrhage. We next examined whether diltiazem-resistant constrictions observed after 5-day oxyHb treatment could be reversed by SNX-482, a blocker of R-type VDCCs. In arteries treated with oxyHb for a period of 5 days, diltiazem (1 mmol/L) dilated K⁺-constricted arteries by 83.2±3.1%, and subsequent addition of SNX-482 (200 nmol/L) caused a further dilation of these arteries to their maximum diameter. Thus, the combination of diltiazem and SNX-482 caused a complete reversal of K⁺-induced constriction in oxyHb-treated arteries, similar to the effects of diltiazem alone in untreated organ cultured arteries (Figure 3a). In the absence of diltiazem, SNX-482 significantly dilated arteries treated with oxyHb for 5 days (33.5±4.7 μm, n=4), but did not significantly alter the diameter of arteries organ cultured for a similar period in the absence of oxyHb (Figure 3b). As predicted, SNX-482 did not dilate arteries exposed to oxyHb for shorter periods of time (1 or 3 days, n=3; data not shown). These results indicate the functional presence of R-type VDCCs in small diameter cerebral arteries treated with oxyHb for 5 days whereas arteries organ cultured for a similar period in the absence of oxyHb have a single population of diltiazem-sensitive L-type VDCCs.

Oxyhemoglobin Increases Expression of R-Type VDCCs in Cerebral Arteries

To further examine the ability of oxyHb to increase R-type VDCCs in cerebral arteries, RT-PCR was used to determine mRNA levels of Caᵥ 2.3, the gene encoding the pore-forming α1 subunit of R-type VDCCs. In the absence of oxyHb, Caᵥ 2.3 mRNA was not detected in arteries cultured for 5 days (n=5). However, after 5-day treatment with oxyHb, Caᵥ 2.3 mRNA was observed in 3 of 5 preparations, although the intensities of these bands were relatively faint (Figure 4a). RT-PCR studies were repeated on arteries obtained from an additional 6 animals. In this second RT-PCR series, semi-quantitative analysis revealed a significant increase in Caᵥ 2.3 mRNA levels in samples organ cultured in the presence compared to the absence of oxyHb (Figure 4b). To confirm the selective expression of Caᵥ 2.3 in arteries cultured in the presence of oxyHb, we performed nested PCR. Nested PCR is a highly sensitive method to detect low levels of gene expression by using 2 rounds of PCR. Here, the first round PCR product, as amplified using primers targeting a region that is unique to Caᵥ 2.3, was used as a template for the second round of PCR. Using nested PCR, expression of R-type Ca²⁺ channels after 5-day treatment with oxyHb was clearly seen in 5 of 5 preparations examined (Figure 4b). In arteries organ cultured in the absence of oxyHb, a PCR band corresponding to Caᵥ 2.3 was observed in 1 of 5 samples. DNA analysis confirmed that the sequence of these bands matched the Caᵥ 2.3 sequence in published database (GenBank Accession number X 67855). These results demonstrate that organ culture of arteries with oxyHb induces R-type VDCC gene expression.

Discussion

Here we provide evidence that oxyhemoglobin can induce R-type VDCC expression in small diameter cerebral arteries. We report that arteries organ cultured for a period of 5 days in the presence of oxyHb exhibit constrictions that were partially resistant to the L-type VDCC antagonist, diltiazem. This diltiazem-resistant constriction was abolished by SNX-482, a blocker of R-type VDCCs. In contrast, diltiazem completely dilated freshly isolated arteries or arteries organ cultured in the absence of oxyHb, and SNX-482 was without effect. Consistent with functional observations, mRNA en-
by guest on April 12, 2017 http://stroke.ahajournals.org/ Downloaded from

OxyHb). b, Semiquantitative RT-PCR analysis was performed using primers specific for the R-type VDCC (CaV 2.3). The expected 435-bp product (arrow) was detected using 250 ng of total RNA extracted from cerebral arteries organ cultured for 5 days in the presence (−oxyHb), but not absence, of oxyhemoglobin (−oxyHb). b, Semiquantitative RT-PCR analysis was performed by comparing band intensities using equivalent amounts of cDNA (n = 6). c, Nested RT-PCR was performed using primers encoding a sequence unique to CaV 2.3.

Figure 4. CaV 2.3 expression in cerebral arteries after 5-day organ culture in the presence of oxyHb. a, RT-PCR was performed using primers specific for the R-type VDCC (CaV 2.3). The expected 435-bp product (arrow) was detected using 250 ng of total RNA extracted from cerebral arteries organ cultured for 5 days in the presence (+oxyHb), but not absence, of oxyhemoglobin (−oxyHb). b, Semiquantitative RT-PCR analysis was performed by comparing band intensities using equivalent amounts of cDNA (n = 6). c, Nested RT-PCR was performed using primers encoding a sequence unique to CaV 2.3.

Coding R-type VDCCs (CaV 2.3) was detected in arteries organ cultured for 5 days in the presence but not in the absence of oxyHb. These observations suggest that cerebral arteries typically contain L-type VDCCs, but prolonged exposure (5 days) to oxyHb can induce R-type VDCC expression that may contribute to cerebral artery constriction. Further, we report that L-type VDCCs become less sensitive to diltiazem after 5-day exposure to oxyHb.

The present work is consistent with our recent observations of the emergence of R-type VDCCs in small diameter cerebral arteries of a rabbit SAH model 5 days after the intracisternal injection of whole blood. In cerebral arteries from SAH rabbits, R-type VDCC expression was associated with enhanced VDCC membrane currents and increased constriction at physiological intravascular pressures. The present study suggests oxyHb, a major blood component, is the causative agent leading to the emergence of R-type VDCCs in cerebral artery myocytes after SAH. OxyHb-induced expression of R-type VDCCs would promote increased Ca2+ entry and enhanced constriction of cerebral arteries. We estimate, based on our present and past studies, that R-type VDCC expression caused by SAH or oxyHb is responsible for approximately 20% of the constriction in response to elevated extracellular K+ or increased intravascular pressure. Considering flow through a cylinder is a function of the radius to the fourth power (Poiseuille Law), we believe R-type VDCC expression could have a substantial impact on cerebral blood flow after SAH. It is interesting to note that dilations to SNX-482 were slightly larger in the absence of diltiazem compared to in the presence of diltiazem in arteries organ cultured for 5 days in the presence of oxyHb (Figure 3). Considering that SNX-482 had little, if any, effect in freshly isolated arteries or arteries organ cultured in the absence of oxyHb, it is unlikely that 200 μmol/L SNX-482 is blocking L-type VDCCs. However, it is possible that the high concentration of diltiazem used in the present study (1mmol/L) may be influencing R-type VDCC activity or that there may be some unknown interaction between R-type and L-type VDCCs.

Numerous reports have provided evidence suggesting oxyHb plays a role in the development of SAH-induced vasospasm. An action commonly attributed to oxyHb is vasoconstriction occurring shortly on exposure of this agent to cerebral arteries in vitro. Several mechanisms have been proposed to contribute to this acute oxyHb-induced constriction including K+ channel suppression, inhibition of Ca2+ sparks, elevated intracellular Ca2+ levels, increased Ca2+ sensitivity of the contractile apparatus attributable to increased activity of Rho kinase or protein kinase C, decreased availability of nitric oxide, and increased synthesis of the vasoconstrictor 20-HETE. In the present study, arteries were organ cultured with oxyHb however, oxyHb was not included in the physiological saline solution used during functional assessment after the organ culture period. Thus, our present findings suggest that in addition to the acute effects detailed above, long-term (5 days) oxyHb can impact cerebral artery function via changes in VDCC expression. It should be noted that the observed changes in CaV 2.3 expression cannot be simply attributed to the organ culture conditions used in the present study. Firstly, a large body of previous work supports the approach of organ culturing intact artery segments in serum-free media. Secondly, in the present study, cerebral arteries organ cultured in the absence of oxyHb exhibit properties comparable to freshly isolated arteries (eg, Figures 1 and 2). Although organ culture conditions clearly do not exactly replicate in vivo conditions, we believe the approach used in the current study has allowed us to examine whether oxyHb can induce CaV 2.3 expression in contractile cerebral artery myocytes. Considering that multiple cell types are present in intact cerebral arteries, it is, however, possible that cells other than vascular smooth muscle may contribute to the observed increase in CaV 2.3 mRNA levels after 5-day oxyHb exposure. Although we cannot definitively rule out this possibility, functional data within this article support the notion that expression of CaV 2.3 occurs primarily in cerebral artery myocytes after oxyHb exposure.

At present, the signaling pathway linking oxyHb to CaV 2.3 expression in cerebral arteries is unclear. However, reactive oxygen species (ROS) produced during the oxidation of oxyHb can regulate gene expression and may play a role in
the pathogenesis of cerebral vasospasm.\textsuperscript{23} Further, Peiro et al\textsuperscript{30} have demonstrated that glycosylated human oxyHb leads to the activation of 2 transcription factors, nuclear factor-xB (NFxB) and activator protein 1 (AP-1), in cultured human aortic smooth muscle via redox-sensitive pathways. Interestingly, activated NFxB has also been detected in cerebral cortices of a mouse SAH model in wild-type but not superoxide dismutase–overexpressing mice.\textsuperscript{31} Additionally, oxyHb–induced elevations in [Ca\textsuperscript{2+}]) could lead to the activation of Ca\textsuperscript{2+}–dependent transcription factors such as cyclic-AMP–dependent response element binding protein (CREB)\textsuperscript{32,33} and nuclear factor of activated T-cells (NF\textsuperscript{a}c).\textsuperscript{34} Future studies are needed to determine the transcriptional events associated with oxyHb–induced R-type VDCC expression.

In addition to enhanced expression of R-type VDCCs, 5-day exposure to oxyHb caused a decrease in the sensitivity of cerebral arteries to the L-type VDCC antagonist diltiazem. The IC\textsubscript{50} value of diltiazem to reverse K\textsuperscript{+}–induced constriction was 10-fold higher in arteries organ cultured for 5 days in the presence, compared to the absence, of oxyHb (Figure 1). This action of oxyHb is similar to the decreased functional response to L-type VDCC blockers reported by others after SAH\textsuperscript{15,16} or oxyHb incubation.\textsuperscript{37} We have also demonstrated that VDCC membrane currents obtained from cerebral artery myocytes isolated from 5-day SAH rabbits were less sensitive to diltiazem and nisoldipine.\textsuperscript{10} These findings may help to explain why L-type VDCC antagonists are potent dilators of arteries from healthy individuals yet exhibit only modest efficacy in the improvement of clinical outcome in SAH patients.

In summary, here we have demonstrated that oxyHb can induce R-type VDCC gene expression and decrease the sensitivity of cerebral arteries to the L-type VDCC antagonist diltiazem. We propose that oxyHb–induced changes in VDCCs are likely to play an important role in diameter regulation in cerebral arteries after SAH.

Acknowledgments

The authors thank Hemosol Inc for their gracious gift of the purified oxyhemoglobin used in this study. The authors also thank Drs Masanori Ishiguro and Masayo Koide for their helpful comments on this study.

Sources of Funding

This work was supported by the Totman Medical Research Trust Fund, the Peter Martin Brain Aneurysm Endowment, the American Heart Association (SDG # 003029N), and the NIH (NCRR, P20 RR16435 and NHLBI, R01 HL078983).

Disclosures

None.

References


Oxyhemoglobin-Induced Expression of R-Type Ca\textsuperscript{2+} Channels in Cerebral Arteries
Timothy E. Link, Kentaro Murakami, Micah Beem-Miller, Bruce I. Tranmer and George C. Wellman

*Stroke*. 2008;39:2122-2128; originally published online April 24, 2008;
doi: 10.1161/STROKEAHA.107.508754
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/39/7/2122

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/