Oxyhemoglobin-Induced Suppression of Voltage-Dependent K⁺ Channels in Cerebral Arteries by Enhanced Tyrosine Kinase Activity

Masanori Ishiguro, Anthony D. Morielli, Katarina Zvarova, Bruce I. Tranmer, Paul L. Penar, George C. Wellman

Abstract—Cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH) has devastating consequences. Oxyhemoglobin (oxyhb) has been implicated in SAH-induced cerebral vasospasm as it causes cerebral artery constriction and increases tyrosine kinase activity. Voltage-dependent, Ca²⁺-selective and K⁺-selective ion channels play an important role in the regulation of cerebral artery diameter and represent potential targets of oxyhb. Here we provide novel evidence that oxyhb selectively decreases 4-aminopyridine sensitive, voltage-dependent K⁺ channel (Kᵥ) currents by ≈30% in myocytes isolated from rabbit cerebral arteries but did not directly alter the activity of voltage-dependent Ca²⁺ channels or large conductance Ca²⁺-activated (BK) channels. A combination of tyrosine kinase inhibitors (tyrphostin AG1478, tyrphostin A23, tyrphostin A25, genistein) abolished both oxyhb-induced suppression of Kᵥ channel currents and oxyhb-induced constriction of isolated cerebral arteries. The Kᵥ channel blocker 4-aminopyridine also inhibited oxyhb-induced cerebral artery constriction. The observed oxyhb-induced decrease in Kᵥ channel activity could represent either channel block, or a decrease in Kᵥ channel density on the plasma membrane. To explore whether oxyhb altered trafficking of Kᵥ channels to the plasma membrane, we used an antibody generated against an extracellular epitope of Kᵥ1.5 channels. In the presence of oxyhb, staining of Kᵥ1.5 on the plasma membrane surface was markedly reduced. Furthermore, oxyhb caused a loss of spatial distinction between staining with Kᵥ1.5 and the general anti-phosphotyrosine antibody PY-102. We propose that oxyhb-induced suppression of Kᵥ currents occurs via a mechanism involving enhanced tyrosine kinase activity and channel endocytosis. This novel mechanism may contribute to oxyhb-induced cerebral artery constriction following SAH. (Circ. Res. 2006;99:1252-1260.)

Key Words: voltage-dependent potassium channels □ vascular smooth muscle □ cerebral arteries □ subarachnoid hemorrhage □ oxyhemoglobin

Voltage-dependent K⁺ (Kᵥ) channels represent a large family of potassium-selective ion channels characterized by voltage-dependent activation and block by 4-aminopyridine (4-AP). Among their diverse functions, Kᵥ channels play an important role in the regulation of cerebral artery diameter both in vitro and in vivo. In isolated cerebral artery myocytes, steady-state 4-AP-sensitive membrane Kᵥ currents exist at physiological membrane potentials. Inhibition of Kᵥ channels leads to membrane depolarization, increased free intracellular Ca²⁺ concentration ([Ca²⁺]), and vasoconstriction attributable to an increase in the open-state probability of voltage-dependent calcium channels (VDCCs). Decreased Kᵥ channel activity may contribute to a number of vascular pathologies, including systemic and pulmonary hypertension, diabetes, and subarachnoid hemorrhage (SAH)-induced cerebral vasospasm. The reduction of Kᵥ channel activity associated with these pathological conditions may reflect decreased channel expression and/or suppression of channel activity.

Activators of protein kinase C (PKC) can decrease Kᵥ channel activity in vascular smooth muscle, and enhanced tyrosine kinase activity has been demonstrated to suppress the activity of Kᵥ1 family members in other cell types. The exact mechanisms linking enhanced kinase activity to Kᵥ channel suppression in cerebral arteries is unclear. However, a recent report has demonstrated that, in a model system, muscarinic receptor activation leads to Kᵥ1.2 channel suppression by enhanced tyrosine kinase activity and channel internalization via endocytosis.

Oxyhemoglobin (oxyhb) causes cerebral artery constriction and is among the blood components contributing to the pathogenesis of cerebral vasospasm following SAH. A
number of cellular mechanisms have been reported to contribute to the vasoconstrictor actions of oxyhb and SAH, including increased PKC and tyrosine kinase activity.16–20 The objective of the present study was to examine the acute impact of oxyhb on K⁺ currents in freshly isolated cerebral artery myocytes. Here we demonstrate that oxyhb leads to K⁺ channel suppression via a mechanism involving tyrosine kinase, but not PKC activity. Furthermore, we provide novel evidence indicating that oxyhb decreases staining of Kv1.5 channels on the cell surface, consistent with increased channel endocytosis. This mechanism of K⁺ channel suppression may contribute to oxyhb-induced cerebral artery constriction.

Materials and Methods

Tissue Preparation

Posterior cerebral and cerebellar arteries were obtained from healthy New Zealand White rabbits (males, 3.0 to 3.5 kg, Charles River Laboratories, Saint Constant, Quebec, Canada) as described previously.21 In 1 experimental series, cerebral arteries were also obtained from an established rabbit model of SAH.21,22 5 days following intracisternal injection of 3 mL of whole blood. All procedures and 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. Human cerebral arteries, removed as a necessary part of a required procedure, were obtained from 1 consenting surgical patient. The University of Vermont has an approved assurance of compliance with the Department of Health and Human Services covering this activity (Assurance Identification no. FWA723; Institutional Review Board identification no. 0485).

Measurements of Arterial Diameter

Cerebral arterial segments were cannulated on glass pipettes mounted in a 5-mL myograph chamber (Living Systems Instruments, Burlington, Vt), as previously described.22,23 Arteries were discarded if an initial constriction representing less than a 50% decrease in diameter was observed when arteries were exposed to elevated extracellular K⁺ (60 mmol/L).

Measurements of K⁺ and VDCC Currents

Vascular smooth muscle cells were enzymatically isolated from cerebral arteries24 and the perforated-patch configuration of the Vascular smooth muscle cells were enzymatically isolated from cerebral arteries24 and the perforated-patch configuration of the Vascular smooth muscle cells were enzymatically isolated from cerebral arteries24 and the perforated-patch configuration of the Vascular smooth muscle cells were enzymatically isolated from cerebral arteries24 and the perforated-patch configuration of the Vascular smooth muscle cells were enzymatically isolated from cerebral arteries24 and the perforated-patch configuration.5 Oxyhb (10 mmol/L) markedly decreased outward K⁺ currents in cerebral arteries, isolated rabbit cerebral arteries by 36.2±7.2 m (n=6), representing an approximate 16% decrease in diameter. Purified oxyhb (10 μmol/L) caused an additional constriction in these arteries of 28.2±6.7 μm (n=6) (Figure 1a and 1b). The constriction caused by oxyhb reached a maximum within 5 minutes and was sustained for the duration of the oxyhb application.

VDCCs play an important role in the regulation of cerebral artery diameter and L-type calcium channel antagonists, such as diltiazem, are potent vasodilators of these arteries (Figure 1a and 1b). To examine whether oxyhb directly enhances VDCC activity, the whole-cell patch-clamp technique, with 10 mmol/L Ba²⁺ as the charge carrier, was used to measure VDCC currents in freshly isolated rabbit cerebral artery myocytes. From a holding potential of −80 mV, depolarizing voltage steps to +20 mV elicited inward membrane currents characteristic of VDCCs.31 Oxyhb (10 μmol/L or 100 μmol/L for 10 minutes) did not alter VDCC currents in cerebral artery myocytes isolated from healthy animals (Figure 1c and 1d). These data demonstrate that Ca²⁺ influx via VDCCs may be involved in oxyhb-induced cerebral artery constriction; however, oxyhb does not directly alter their open-state probability.

Oxyhb Decreases K⁺ Currents in Rabbit and Human Cerebral Artery Myocytes

As cerebral artery constriction requires Ca²⁺ entry through VDCCs, we next examined whether oxyhb indirectly increases VDCC activity via membrane potential depolarization through inhibition of K⁺ channels. Using the perforated patch-clamp technique, outward membrane K⁺ currents were elicited by a series of 10-mV depolarizing steps (to +50 mV) from a holding potential of −80 mV (Figure 2). Oxyhb (30 μmol/L) markedly decreased outward K⁺ currents in cerebral arteries, isolated rabbit cerebral arteries by 36.2±7.2 m (n=6), representing an approximate 16% decrease in diameter. Purified oxyhb (10 μmol/L) caused an additional constriction in these arteries of 28.2±6.7 μm (n=6) (Figure 1a and 1b). The constriction caused by oxyhb reached a maximum within 5 minutes and was sustained for the duration of the oxyhb application.

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Immunofluorescent Detection of Surface K₁,1,5

Freshly dissociated cerebral artery myocytes were incubated at 37°C either in the presence or absence of oxyhb for 10 minutes then fixed with 4% formaldehyde. Cells were washed, then incubated with a rabbit polyclonal anti-K₁,1,5 antibody (1:200 dilution) generated against an epitope in the second extracellular loop of the α subunit of the channel (a gift from Dr James Trimmer, University of California, Davis), and labeled with Alexa 568/goat anti-rabbit (Alexa 568-GAR) (1:500). This method produces staining of the channel on or near the cell surface and does not appear to stain intracellular channels. For cells sustained with anti-phosphotyrosine antibody, surface K₁,1.5 primary antibody was applied as described above; subsequently, cells were permeabilized with ice-cold acetone for 4 minutes, washed, blocked with PBS+5% goat serum for 20 minutes at 37°C, stained with the general anti-phosphotyrosine antibody PY-102 (1:200), and subsequently labeled with Alexa 568-GAR (1:500) and Alexa 647/goat anti-mouse (Alexa 647-GAM) (1:500). F-Actin was visualized using Alexa 488-phalloidin (1:200). Six to 10 cells were averaged from each animal (n).

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’s t test.
freshly isolated rabbit cerebral artery myocytes (Figure 2a and 2c). At +40 mV, oxyhb decreased outward K⁺ channel current density by approximately 40%. Although oxyhb reduced Kᵥ currents, the activation time constants for these currents were similar in the absence (+40 mV, τ=56.4±6.8 ms; n=11) and presence of oxyhb (+40 mV, τ=54.2±5.0 ms; n=11).

Few studies have examined K⁺ channel function in the human cerebral vasculature. We therefore also examined the ability of oxyhb to influence Kᵥ currents in small-diameter human cerebral arteries obtained from a consenting surgical patient. At +40 mV, the Kᵥ channel current density was 16.5±6.9 pA/pF (n=3 cells from 1 individual), compared with the current density of 27.9±3.4 pA/pF observed in rabbit cerebral myocytes using similar recording conditions. Oxyhb reduced currents in human cerebral artery myocytes by approximately 40% (Figure 2b and 2d), a decrease similar to that observed in the rabbit.

**Oxyhb Decreases Kᵥ, but Not BK, Currents in Rabbit Cerebral Artery Myocytes**

In cerebral artery myocytes, K⁺ currents evoked by membrane depolarization reflect the combined activity of large-conductance Ca²⁺-activated (BK) channels and delayed rectifier (Kᵥ) channels. A hallmark of Kᵥ channels is their block by 4-aminopyridine (4-AP).1 4-AP (10 mmol/L) reduced outward K⁺ current densities at 60 mm Hg, with no further reduction by oxyhb (13.9±0.8 pA/pF in the presence of 4-AP and oxyhb). Additionally, we used iberiotoxin (IBTX) (100 mmol/L), a selective blocker of BK channels, to examine whether oxyhb could also alter voltage-dependent BK channel activity. Consistent with a contribution of BK channels to outward K⁺ currents, IBTX decreased whole-cell Kᵥ currents by approximately 40% at +40 mV. However, oxyhb still reduced outward K⁺ channel current density in the presence of IBTX (Figure 3e, 3d, and 3f). Furthermore, IBTX-sensitive BK current density at +40 mV was not significantly different in the absence (11.3±1.3 pA/pF) and presence (9.0±0.4 pA/pF) of oxyhb. These data demonstrate that oxyhb decreases the amplitude of Kᵥ, but not BK, currents evoked by membrane potential depolarization in cerebral artery myocytes isolated from healthy rabbits. Functional studies examining diameter changes in isolated pressurized cerebral arteries were in accord with the actions of 4-AP and IBTX on Kᵥ channel current densities (Figure 3e). Both IBTX and 4-AP constricted pressurized cerebral arteries, demonstrating the functional presence of BK and Kᵥ channels. Consistent with our electrophysiological data, inhibition of Kᵥ channels with 4-AP abolished oxyhb-induced cerebral artery constriction. However, oxyhb-induced constriction was still observed following inhibition of BK channels with IBTX. These data are consistent with oxyhb-induced Kᵥ channel inhibition leading to cerebral artery constriction. In the presence of 4-AP, increasing extracellular K⁺ to 60 mmol/L caused an additional constriction (76±2%) decrease in diameter; n=5), ensuring that 4-AP was not causing a maximal constriction in this tissue.

**Oxyhb-Induced Suppression of Kᵥ Currents: Involvement of Tyrosine Kinase Activity**

We next sought to explore potential cell signaling pathways linking oxyhb to decreased Kᵥ channel current density in freshly isolated cerebral artery myocytes. Activation of protein tyrosine kinases (PTKs) leads to suppression of Kᵥ channels. Several PTKs have been implicated in the regulation of vascular tone. For example, growth factors and platelet-derived growth factor (PDGF) have been shown to activate PTK-dependent signaling pathways leading to reduced Kᵥ channel activity and arterial constriction. To examine the potential involvement of PTKs in oxyhb-induced constriction, we used several PTK inhibitors. While PDGF activates numerous PTKs, specific inhibitors were not available. Nevertheless, both PDGF (100 ng/mL) and oxyhb induced a significant constriction in isolated rabbit cerebral arteries (n=5) (P<0.05). In this study, we used KT5720 (5 µmol/L), a selective inhibitor of the PTK fyn. KT5720 alone did not constrict isolated rabbit cerebral arteries (n=5). KT5720 (5 µmol/L) partially reversed oxyhb-induced constriction (P<0.05). These data suggest that oxyhb-induced constriction in rabbit cerebral arteries involves activation of PTKs, possibly including fyn, leading to reduced Kᵥ channel current density.
currents in nonvascular cultured cells.\textsuperscript{12–14} To examine the involvement of PTKs in oxyhb-induced suppression of Kv currents in cerebral artery myocytes, we used a combination of PTK inhibitors including tyrphostin AG1478 (2.5 μmol/L), tyrphostin A23 (2.5 μmol/L), tyrphostin A25 (2.5 μmol/L), and genistein (15 μmol/L). This combination of PTK inhibitors abolished oxyhb-induced Kv current suppression (Figure 4a and 4b). Furthermore, PTK inhibitors abolished oxyhb-induced constriction of isolated pressurized cerebral arteries, whereas K\textsuperscript{+}-induced constriction was unaltered (Figure 4c).

Previous work by others has demonstrated that PKC can reduce Kv channel activity in vascular smooth muscle\textsuperscript{10,11} and enhanced activity of several PKC isoforms, including PKCa, -δ, and -ε, can occur during oxyhb exposure or following SAH.\textsuperscript{16,20} However, we found that chelerythrine (1 μmol/L), an inhibitor of PKC isoforms α, β\textsubscript{i}, β\textsubscript{2}, γ, and δ and GF109203X (1 μmol/L), an inhibitor of PKC isoforms ε, η, and θ, did not impact the ability of oxyhb to suppress Kv\textsuperscript{+} currents (Figure 4d). However, both chelerythrine and GF109203X were effective in blunting K\textsubscript{+} current suppression caused by the PKC activator 1,2-dioctanoyl-sn-glycerol (DOG) (1 μmol/L) (M. Koide and G.C.W., unpublished observations, 2006). These data suggest activity of PTKs, but not PKC, is involved in the inhibition of K\textsubscript{+} channels by oxyhb in cerebral myocytes.

**Oxyhb-Induced Decreased Surface K\textsubscript{v}1.5 Channel Staining Involves Tyrosine Kinase Activity**

Oxyhb-induced PTK activity could suppress K\textsubscript{v} currents through a reduction in channel open probability or through a decrease in channel number on the plasma membrane. The kinetic properties of the 4-AP and oxyhb-sensitive K\textsuperscript{+} currents seemed similar, suggesting that channel number may be reduced. K\textsubscript{v}1.5 is expressed in cerebral arteries\textsuperscript{25,26} and has been shown to undergo PTK-dependent phosphorylation in other cell types.\textsuperscript{27} In the absence of oxyhb, application of an antibody directed against an extracellular epitope of K\textsubscript{v}1.5 to formaldehyde-fixed myocytes revealed staining for surface K\textsubscript{v}1.5 within large but defined regions on the cell surface (Figure 5). Costaining of control cells with an anti-phosphotyrosine antibody (PY102) revealed phosphotyrosine...
rich vesicular compartments adjacent to the plasma membrane (see Figure 5, z-projection; and the online movie supplement, available at http://circres.ahajournals.org). Oxyhb elicited a redistribution of Kv1.5 into smaller, sharply defined foci (Figure 5). Reconstruction of a z-axis micrograph series suggests fusion of the phosphotyrosine-enriched vesicles with the surface Kv1.5 density (see Figure 5, z-projection; and online movie supplement). The above observations are consistent with oxyhb causing enhanced tyrosine kinase activity and tyrosine phosphorylation of Kv1.5, or a closely associated protein. In an attempt to strengthen these findings, efforts were made using Western blot to detect oxyhb-induced tyrosine phosphorylation of Kv1.5. However, using a variety of available antibodies generated against Kv1.5, or anti-phosphotyrosine residues, we were unable to immunoprecipitate sufficient quantities of the desired substrate to allow western blot detection of the phosphorylated protein. A number of factors, including inefficient detection of the specific rabbit epitope with available antibodies, comparatively low levels of Kv1.5 expression combined with a relatively small amount of total phosphorylated protein obtained from these 100 to 200 μm diameter cerebral arteries, or loss of phosphorylation by an associated phosphatase, may have contributed to our lack of success with this experimental series.

Using immunofluorescence, we did observe that oxyhb caused a significant reduction in the total amount of Kv1.5 detectable on the cell surface (Figure 6a), suggesting that oxyhb may elicit trafficking of Kv1.5 from the cell surface. Oxyhb did not change total F-actin staining, but did slightly reduce the average pixel area occupied by each cell, consistent with oxyhb-induced cell contraction. The oxyhb-induced decrease in surface staining of Kv1.5 was abolished by a combination of PTK inhibitors (Figure 6b). Collectively, these findings are consistent with the hypothesis that Kv1.5 suppression involves tyrosine phosphorylation-dependent trafficking of the channel from the cell surface.

Oxyhb Does Not Suppress Kv Channel Activity in Cerebral Artery Myocytes Following SAH
Oxyhb is among the blood components contributing to SAH-induced cerebral vasospasm, and previous work by others suggests altered Kv function following SAH. We next examined the ability of oxyhb to suppress Kv channel
activity and constrict small-diameter cerebral arteries following experimental SAH. If oxyhb-induced K\textsuperscript{+} currents were similar in the absence and presence of oxyh, the K\textsuperscript{+} currents from a cell exposed to tyrosine kinase inhibitors were similar in the absence (a) and presence (b) of oxyhb (30 μmol/L). The tyrosine kinase inhibitors included the following: tyrphostin AG1478 (2.5 μmol/L), tyrphostin A23 (2.5 μmol/L), tyrphostin A25 (2.5 μmol/L), and genistein (15 μmol/L). c, Summary of diameter measurements from 4 arteries exposed to tyrosine kinase inhibitors, then subsequently to oxyhb. Oxyhb-induced constrictions were abolished in arteries treated with tyrosine kinase inhibitors; however, arteries still exhibited robust constrictions to elevated extracellular K\textsuperscript{+}. d, Suppression K\textsuperscript{+} current density at 40 mV by oxyhb in the presence of chelerythrine (1 μmol/L; n = 4), GF109203X (1 μmol/L; n = 5), or the combination of tyrosine kinase inhibitors described above. **P<0.01 vs cells treated with tyrosine kinase inhibitors in the absence of oxyhb.

SAH animals (Figure 7a and 7b). Based on the inability of oxyhb to suppress K\textsuperscript{+} currents following SAH, we hypothesized that oxyhb-induced constriction would be impaired in cerebral arteries from SAH animals. Consistent with this hypothesis, oxyhb did not constrict cerebral arteries from SAH animals in vitro (Figure 7c and 7d). As we have reported previously,21 in the absence of exogenous oxyhb, arteries from SAH animals exhibit enhanced pressure-induced constrictions that are abolished by the combination of an L-type VDCC blocker, diltiazem, and an R-type VDCC blocker, SNX-482. These findings suggest that oxyhb-induced K\textsuperscript{+} channel suppression may contribute to the complex series of events leading to enhanced cerebral artery constriction following SAH.
depolarization, an increase in the open-state probability of VDCCs, enhanced Ca\textsuperscript{2+} influx and ultimately, vasoconstriction.

Recent studies have revealed that in vascular myocytes, functional K\textsubscript{v} channels likely represent heterotetramers of 2 K\textsubscript{v} family members, K\textsubscript{v,1.2} and K\textsubscript{v,1.5}.\textsuperscript{26-30} In arterial myocytes, mRNA levels of K\textsubscript{v,1.5} are the most abundant of known K\textsubscript{v} family members.\textsuperscript{25,31} Using an antibody generated against an extracellular epitope of K\textsubscript{v,1.5}, we observed that oxyhb caused a marked decrease in K\textsubscript{v,1.5} staining on the surface of nonpermeabilized cells. A recent study by Choi et al\textsuperscript{32} found that disruption of the endocytotic machinery leads to increased K\textsubscript{v,1.5} currents and a corresponding increase in surface expression of this channel, whereas Nesti et al\textsuperscript{14} have found tyrosine kinase activity promoted suppression of K\textsubscript{v,1.2} current via increased channel endocytosis. Thus, although our present study does not directly demonstrate oxyhb enhances K\textsubscript{v,1.5} channel endocytosis, our observations are consistent with recent reports demonstrating that endocytotic activity can regulate K\textsubscript{v} currents on the plasma membrane.

In the present study, we also provide 2 lines of evidence that enhanced tyrosine kinase activity is involved in oxyhb-induced suppression of K\textsubscript{v} currents. Firstly, pharmacological inhibition of tyrosine kinases blocked the ability of oxyhb to suppress K\textsubscript{v} current density (Figure 4) and abolished both oxyhb-induced decreased surface K\textsubscript{v,1.5} staining (Figure 6) and cerebral artery constriction (Figure 4). Secondly, staining with a general anti-phosphotyrosine antibody revealed that oxyhb caused colocalization of phosphotyrosine and K\textsubscript{v,1.5} signals (Figure 5). Although the identity of the specific tyrosine kinase linked to oxyhb-induced suppression of K\textsubscript{v,1.5} remains to be determined, other investigators have demonstrated that Src kinase can bind to and phosphorylate K\textsubscript{v,1.5}.\textsuperscript{27} It is also possible that oxyhb could increase the activity of receptor-mediated tyrosine kinases such as the EGF receptor, which has been implicated in the suppression of K\textsubscript{v,1.2} membrane currents.\textsuperscript{33} Although it is likely that K\textsubscript{v,1.5} is a direct target for tyrosine phosphorylation, it is conceivable that phosphorylation of other K\textsubscript{v} subunits within the channel or a closely associated protein may also play a role in the loss of surface channel.

The present work also suggests that oxyhb-induced suppression of K\textsubscript{v} channels may contribute to the vascular pathology of cerebral vasospasm following aneurysm rupture and SAH. We found K\textsubscript{v} currents were decreased in myocytes obtained from a rabbit SAH model and that the effects of SAH and acute exposure of oxyhb were not additive. Our data are consistent with previous reports suggesting K\textsubscript{v} channel activity is decreased following SAH.\textsuperscript{3,34} and provides the first evidence implicating oxyhb in this phenomenon. Alternatively, it is possible that the suppression of K\textsuperscript{\textsuperscript{+}} currents following SAH represent a decrease in K\textsuperscript{\textsuperscript{+}} channel expression, or altered expression of a signaling protein involved in the action of oxyhb on K\textsubscript{v} channels. Additional studies are required to firmly establish the mechanism of decreased K\textsuperscript{\textsuperscript{+}} channel activity following SAH.

In summary, this work demonstrates that oxyhb-induced suppression of K\textsubscript{v} currents in cerebral artery myocytes occurs via tyrosine kinase-dependent reduction of K\textsubscript{v,1.5} channels on the plasma membrane. This novel mechanism contributing to
cerebral artery constriction may represent a more widespread mechanism whereby endogenous vasoactive substances modulate cerebral artery function. Furthermore, oxyhb-induced suppression of K+ currents may contribute to decreased cerebral blood flow and the accompanying neurological deficits associated with SAH.

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Disclosures

None.

References


Figure 7. Oxyhb does not suppress K+ channel activity in cerebral artery myocytes following SAH. a. Voltage-dependent K+ currents from a cerebral myocyte isolated from a SAH rabbit 5 days after intracisternal injection of whole blood. Currents were obtained in the absence (left) and presence (right) of purified oxyhb. b. Summary of current/voltage relationship of K+ currents obtained from SAH myocytes in the presence and absence of oxyhb (n=6). c, Diameter recording from a cerebral artery isolated from a SAH rabbit pressurized to 60 mm Hg. Oxyhb (10 μmol/L) did not constrict this artery. The L-type Ca2+ channel blocker diltiazem (50 μmol/L) caused no further dilation. d. Summary of oxyhb-induced constriction of cerebral artery from healthy (control, n=6) and SAH (n=4) rabbits. Constrictions are presented as a percentage decrease in arterial diameter in arteries pressurized to 60 mm Hg. **P<0.05.


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Online Movie Files

In order to accurately depict the three dimensional separation between phosphotyrosine positive vesicles within the cytoplasm and Kv1.5 at the cell surface, a Z-axis image series was collected using the DeltaVision deconvolution microscope system and processed with the Volocity software package (Improvision, Lexington MA) to produce a rotatable 3-D projection.

Supplemental Movie A. In cells not treated with oxyhemoglobin, a distinct separation between the phosphotyrosine positive vesicles (red) and Kv1.5 (green) at the cell surface can be discerned upon rotation of the image. Phalloidin stained F-actin (blue) indicates the cell’s overall dimensions.

Supplemental Movie B. After treatment with oxyhemoglobin individual phosphotyrosine positive vesicles distinct from the Kv1.5 signal are not apparent. Instead, most of the vesicles have moved into close proximity with the plasma membrane in an area tightly overlapping that occupied by Kv1.5 remaining at the cell surface.