**SK$_{Ca}$ and IK$_{Ca}$ Channels, Myogenic Tone, and Vasodilator Responses in Middle Cerebral Arteries and Parenchymal Arterioles**

**Effect of Ischemia and Reperfusion**

Marilyn J. Cipolla, PhD; Jeremiah Smith, BS; Meghan M. Kohlmeyer, BS; Julie A. Godfrey, BS

**Background and Purpose**—The role of SK$_{Ca}$ and IK$_{Ca}$ channels in myogenic tone and endothelium-derived hyperpolarizing factor (EDHF) responsiveness was investigated under control conditions and after ischemia and reperfusion in parenchymal arterioles (PA) versus middle cerebral arteries (MCA).

**Methods**—MCA and PA were dissected from male Wistar rats that were ischemic for 1 hour with 24 hours of reperfusion ($n=110$) or sham controls ($n=12$). Basal tone and reactivity to apamin (300 nmol/L), TRAM-34 (1.0 µmol/L), and nitro-L-arginine (0.1 mmol/L) were compared in PA and MCA pressurized to 40 mm Hg and 75 mm Hg, respectively. SK$_{Ca}$ and IK$_{Ca}$ channel mRNA expression was measured using real-time PCR.

**Results**—PA developed greater basal tone than MCA (42±4% versus 19±3%; $P<0.01$). Addition of apamin and TRAM-34 increased tone of PA by 25±3% and 16±2%, respectively, whereas MCA had no response to either inhibitor. After ischemia and reperfusion, the response to nitric oxide synthase inhibition (NOS) was diminished in PA, whereas EDHF responsiveness was preserved. In addition, stimulated EDHF dilation was partially reversed by apamin and completely reversed by TRAM-34 in both control and ischemic PA. SK$_{Ca}$ and IK$_{Ca}$ channel mRNA expression was similar in PA and MCA and not altered by ischemia and reperfusion. However, IK$_{Ca}$ channel mRNA expression was 4- to 5-fold greater than SK$_{Ca}$ channels.

**Conclusions**—It appears that SK$_{Ca}$ and IK$_{Ca}$ channel activity diminishes basal tone of PA, but not MCA. The preservation of EDHF responsiveness of PA after ischemia and reperfusion suggests an important role for this vasodilator under conditions when NOS is inhibited. *(Stroke. 2009;40:1451-1457.)*

**Key Words:** ischemic stroke parenchymal arterioles potassium channels vascular tone

The vasoactive response of cerebral arteries and arterioles to changes in pressure, termed myogenic reactivity, is likely an important contributor to autoregulation of cerebral blood flow. In addition, myogenic tone is a major determinant of cerebrovascular resistance, providing a crucial protective mechanism by limiting transmission of damaging hydrostatic pressure to the microcirculation. In the middle cerebral artery (MCA) territory, both cerebral (pial) arteries and penetrating parenchymal arterioles (PA) contribute significantly to cerebrovascular resistance. However, PA are unique in that they are long and largely unbranched vessels that connect the surface vessels to the microcirculation, where they appear to function as a bottleneck to blood flow. These vessels possess greater basal tone than the MCA and seem to be more resistant to ischemia and reperfusion (IR) in that they do not lose myogenic tone and reactivity to pressure in the same manner as MCA.

The cerebral endothelium controls vascular tone through production of vasoactive factors, most notably nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). The relative contribution of these vasodilators to vascular tone may depend on the size of the vessel. In particular, EDHF’s contribution to regulation of cerebral blood flow and vascular resistance may be greatest at the level of small PA. In addition, whereas prostacyclin has little influence in cerebral vessels, both cerebral arteries and arterioles have considerable basal NO production that mitigates tone under resting conditions. This is demonstrated by the fact that NO synthase (NOS) inhibition produces constriction and increases tone in both MCA and PA. Activation of ion channels has an important role in regulating endothelial membrane potential and calcium homeostasis, and hence vasodilator production, in response to stimulation. In particular, small and interme-
iate calcium-activated potassium channels (SK\textsubscript{Ca} and IK\textsubscript{Ca}) produce endothelial hyperpolarization when stimulated.\textsuperscript{14–16} In the absence of voltage-operated calcium channels, hyperpolarization of endothelial cells may increase the electrochemical driving force for calcium entry and appears to be an important initial step in endothelial-mediated smooth muscle hyperpolarization necessary for EDHF dilation.\textsuperscript{17,18} SK\textsubscript{Ca} and IK\textsubscript{Ca} channels are expressed on endothelium, not vascular smooth muscle,\textsuperscript{14,19} and their inhibition by specific antagonists blocks EDHF-mediated relaxation and hyperpolarization.\textsuperscript{20–22} In the rat MCA, IK\textsubscript{Ca} inhibition alone is sufficient to block EDHF dilation.\textsuperscript{20,21} However, when NOS is active, SK\textsubscript{Ca} channels also contribute to endothelial hyperpolarization.\textsuperscript{22} Interestingly, the level of basal tone in the MCA is unaffected by apamin and TRAM-34, specific inhibitors of SK\textsubscript{Ca} and IK\textsubscript{Ca} channels, respectively.\textsuperscript{21,22} This result suggests that unlike NO, EDHF does not affect basal tone under normal conditions. However, the functional importance of EDHF in PA, a vessel that has been shown to possess a greater EDHF component than MCA, is not known.\textsuperscript{19} The functional role for EDHF under basal conditions may represent an important yet unrecognized determinant of cerebrovascular resistance.

In the present study, we investigated whether SK\textsubscript{Ca} and IK\textsubscript{Ca} inhibition affects basal tone in PA and the role of these channels in EDHF-mediated dilation. In addition, we investigated the effect of I/R on these channels and the role of EDHF in PA because this is a condition in which NO-mediated responses are largely abolished and it has been hypothesized that EDHF may act as a backup vasodilator when NO production is compromised.\textsuperscript{5,9}

### Materials and Methods

#### Animal Model of Focal Ischemia

All procedures were approved by the Institutional Animal Care and Use Committee and complied with the National Institutes of Health guidelines for the use and care of animals. Temporary filament occlusion of the MCA was used to induce I/R in male Wistar rats (N = 35; 280 to 300 g; Harlan; Dublin, Va) as previously described.\textsuperscript{5,7} Ischemic animals were exposed to 1 hour of ischemia and 24 hours of reperfusion by suture removal. Sham control animals underwent anesthesia and a midline incision but were not exposed to I/R. All animals received buprenorphine (0.025 mg/kg subcutaneously) for analgesia.

#### Preparation of Cerebral Vessels and Pressurized Arteriograph

Animals were anesthetized with isoflurane (2% in oxygen), decapitated, and the brain quickly removed and placed in cold, oxygenated physiological saline solution (PSS). PAs were identified as branches of the MCA that penetrate at right angles into the brain parenchyma. Once identified, surrounding brain tissue was carefully cleared and the vessel removed, placed in the arteriograph chamber (Living Systems Instrumentation, Burlington, Vt), and mounted on glass cannulas. In some experiments, we compared the response of PA with that of MCA from the same animal. In these cases, both MCA and PA were carefully dissected and placed in arteriograph chambers. Isolated vessel experiments were conducted as previously described.\textsuperscript{5,7,11}

#### PCR Analysis of SK\textsubscript{Ca} and IK\textsubscript{Ca} Channels in MCA and PA

Expression of SK\textsubscript{Ca} and IK\textsubscript{Ca} mRNA was determined using standard techniques for real-time quantitative PCR and was performed by the DNA Facility at the University of Vermont. Briefly, MCA and PA from the ipsilateral and contralateral sides of the brain were dissected separately from ischemic (n = 4) and sham (n = 4) animals and rapidly frozen in RNAlater. The vessels were then removed and placed in an RNase-free microcentrifuge tube, homogenized in the presence of RLT buffer and total RNA isolated using an RNasy Micro Kit (Qiagen, Valencia, Calif). cDNA was made from total RNA using SuperScript III Kit (Invitrogen, Carlsbad, Calif). The cDNA reaction was according to standard protocol using random hexamers and 4.8 ng of total RNA. Real-time PCR was set up as follows: 10 μL Universal PCR Master Mix (Applied Biosystems, Foster City, Calif), 1 μL Assay on Demand (Applied Biosystems), 8 μL water, and 1 μL cDNA. Target genes SK3 and SK4 were used to assess mRNA expression of SK\textsubscript{Ca2.3} and SK\textsubscript{Ca3.1} channels, which are known to be expressed in endothelium and contribute to the EDHF response.\textsuperscript{14,15,17,18} All target genes (SK3 for SK\textsubscript{Ca2.3}, SK4 for IK\textsubscript{Ca}, and B2m for an endogenous control) were assessed using Assays on Demand from Applied Biosystems that were validated for efficiency and did not amplify genomic DNA. All samples were run in technical duplicates using a 7900HT Sequence Detection System (Applied Biosystems). The PCR was cycled for 2 minutes at 50°C, 10 minutes at 95°C, then 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Only Ct duplicate values of 35 were used (if replicates were >1.0 apart, they were reran). Ct values were averaged and used for comparison using the ΔΔCt method.\textsuperscript{23} Three comparisons of mRNA expression were made between MCA and PA and between ischemic and sham animals: (1) SK\textsubscript{Ca} and IK\textsubscript{Ca} expression of PA relative to MCA in sham and ischemic animals to determine if channel expression was different between these 2 segments of the vasculature; (2) SK\textsubscript{Ca} and IK\textsubscript{Ca} expression in PA after I/R relative to sham to determine if I/R affected channel expression; and (3) IK\textsubscript{Ca} channel expression relative to SK\textsubscript{Ca} channel expression in PA to determine if one channel was expressed to a greater extent than the other.

### SK\textsubscript{Ca} and IK\textsubscript{Ca} Channel Inhibition and Myogenic Tone

MCA and PA were dissected from animals that were either sham control (n = 6) or after I/R (n = 6), mounted in the arteriograph chamber, and equilibrated for 1 hour at either 40 mm Hg for PA or 75 mm Hg for MCA. These pressures were used because they approximate what these vessel experience in vivo.\textsuperscript{3} After the equilibration period in which spontaneous tone developed, apamin (300 nM), TRAM-43 (1.0 μmol/L), and nitro-L-arginine (L-NNA, 0.1 mmol/L) were sequentially added to the bath and lumen diameters and wall thicknesses measured. These concentrations of apamin and TRAM-43 have been shown to be selective for SK\textsubscript{Ca} and IK\textsubscript{Ca} channels, respectively.\textsuperscript{18} Because apamin and TRAM-43 can cause depolarization of perivascular sensory nerves that are present in MCA, but not PA,\textsuperscript{13} capsaicin (1.0 μmol/L) was added to the bath before addition of the inhibitors for the MCA only. At the conclusion of the experiment, papavenerine (0.1 mmol/L) in zero calcium PSS was added to obtain fully relaxed diameters.

### Effect of SK\textsubscript{Ca} and IK\textsubscript{Ca} Channel Inhibition on EDHF

Because the role of SK\textsubscript{Ca} and IK\textsubscript{Ca} in EDHF production has been previously studied in MCA,\textsuperscript{20–22} we investigated the role of SK\textsubscript{Ca} and IK\textsubscript{Ca} channels in EDHF responsiveness of PA only. PA were dissected from sham control (n = 6) or after I/R (n = 6) and mounted in the arteriograph chamber. Arterioles were equilibrated for 1 hour at 40 mm Hg during which time spontaneous tone developed. L-NNA (0.1 mmol/L) and indomethacin (10 μmol/L) were added to the bath to inhibit NOS and cyclooxygenase (COX), respectively. These concentrations have been shown to maximally inhibit NOS/COX in these vessels in the rat.\textsuperscript{10} In the presence of the inhibitors, A23817, a calcium ionophore and endothelium-dependent agonist,
IKCa channels alone could reverse the EDHF dilation. Finally, TRAM-34 was added before apamin to determine if inhibition of papaverine (0.1 mmol/L) in zero calcium PSS was added to obtain apamin, TRAM-34, or L-NNA and Sigma (St Louis, Mo).

Zero calcium PSS was made without adding calcium. Apamin, TRAM-34, and papaverine were purchased from Sigma (St Louis, Mo) and kept frozen until used. Capsaicin, L-NNA, indomethacin, and TRAM-34 were purchased from Tocris Biosciences (Ellisville, Mo) and the bath and diameter recorded. In some experiments (n = 3), TRAM-34 was added before apamin to determine if inhibition of IKCa channels alone could reverse the EDHF dilation. Finally, papaverine (0.1 mmol/L) in zero calcium PSS was added to obtain fully relaxed diameters.

### Drugs and Solutions
All experiments were conducted using Krebs PSS (a bicarbonate-based buffer aerated with 5% CO2, 10% O2, and 85% N2 to maintain pH); the ionic composition was (mmol/L): NaCl 119.0, NaCHO3 24.0, KCl 4.7, KH2PO4 1.18, MgSO4 1.6, EDTA 0.026, and glucose 5.5. PSS was made each week and stored without pH); the ionic composition was (mmol/L): NaCl 119.0, NaCHO3 24.0, KCl 4.7, KH2PO4 1.18, MgSO4 1.6, EDTA 0.026, and glucose 5.5. PSS was made each week and stored without glucose at 4°C; glucose was added to the PSS before each experiment. Zero calcium PSS was made without adding calcium. Apamin and TRAM-34 were purchased from Tocris Biosciences (Ellisville, Mo) and kept frozen until used. Capsaicin, L-NNA, indomethacin, calcium ionophore A23187, and papaverine were purchased from Sigma (St Louis, Mo).

### Data Calculations and Statistical Analysis
Vascular tone was calculated as a percent decrease in diameter from the fully relaxed diameter in papaverine by the equation: \((1 - \frac{\phi_{\text{tone}}}{\phi_{\text{baseline}}})\) x 100%; where \(\phi_{\text{tone}}\) is the diameter in papaverine. Percent constriction was calculated as a percent change in diameter from baseline by the equation: \((1 - \frac{\phi_{\text{drug}}}{\phi_{\text{baseline}}})\) x 100%; where \(\phi_{\text{drug}}\) is the diameter of vessel in drug and \(\phi_{\text{baseline}}\) is the diameter of vessel in baseline.

RQ values were determined by first calculating the \(\Delta Ct\) (difference between target gene Ct and endogenous control gene Ct) and the \(\Delta \Delta Ct\) (difference between \(\Delta Ct\)s to be compared). RQ value was then calculated as \(2^{-\Delta \Delta Ct}\). All data are presented as mean ± SEM. One-way analysis of variance was used to determine differences in tone and percent constriction with a post hoc Student-Newman-Keuls test for multiple comparisons, where appropriate.

### Results
**Effect of SKCa and IKCa Channel Inhibition on Myogenic Tone**
In the first experiments, we investigated the role of SKCa and IKCa in modulating basal tone in PA and MCA from the same animals. The percent tone of MCA and PA from sham control and ischemic animals is shown in Figure 1. Under control conditions, PA developed considerably greater tone compared with MCA (42 ± 4% versus 19 ± 3%; \(P < 0.01\)). Cumulative addition of apamin and TRAM-34 constricted the PA, suggesting that EDHF was affecting basal tone in these vessels (Figure 1A). Unlike the PA, and similar to previous studies,20–22 apamin and TRAM-34 had no effect on MCA diameter (Figure 1B). However, NOS inhibition with L-NNA caused constriction in both PA and MCA (Figure 1).

Similar to previous studies,5–7 I/R did not significantly affect myogenic tone in PA (42 ± 4% versus 33 ± 2%; \(P > 0.05\), but caused a significant loss of tone in MCA (19 ± 3% versus 8 ± 2%; \(P < 0.05\)). Addition of apamin and TRAM-34 to ischemic PA caused constriction but had no effect on MCA after I/R. Importantly, PA had little to no response to NOS inhibition with L-NNA after I/R (Figure 1).

Figure 2 shows the percent constriction of PA and MCA from sham controls and ischemic animals in response to cumulative addition of apamin, TRAM-34, and L-NNA. Comparing percent constriction allows for comparison of the reactivity of the vessels with each inhibitor. Figure 2A shows that in PA from control animals, apamin caused significantly greater constriction than TRAM-34, an effect that was reversed with I/R. Importantly, constriction to L-NNA was robust in control arterioles but almost completely abolished after I/R. Figure 2B shows that percent constriction to apamin and TRAM-34 was minimal in MCA from both control and ischemic animals. Unlike SKCa and IKCa inhibition, NOS inhibition caused considerable constriction in MCA that was not diminished after I/R.

**Effect of SKCa and IKCa Channels on EDHF in Parenchymal Arterioles**
Although the role of SKCa and IKCa channels in EDHF production has been established in MCA, the role of these channels in EDHF-mediated responses in PA is unknown. Figure 3 shows the response to A23187 in the presence of

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*Figure 1. Percent tone of PA (A) and MCA (B) at 40 mm Hg and 75 mm Hg, respectively. Vascular tone is shown before treatment and after cumulative addition of apamin (300 nM), TRAM-34 (1.0 μmol/L), and L-NNA (0.1 mmol/L) in sham control (gray bars) and after ischemia and reperfusion (black bars). *\(P < 0.01\) versus tone; ‡\(P < 0.05\) versus sham; ‡‡\(P < 0.01\) versus sham.*
NOS/COX inhibition from sham control and ischemic animals. Notice that the diameter of ischemic PA is greater than control vessels due to their lack of responsiveness to L-NNA. Addition of A23187 caused considerable dilation of both types of arterioles with NOS/COX inhibition that was maximal at 1.0 μmol/L and similar to fully relaxed diameters, suggesting a considerable role for EDHF in these arterioles. There was little difference in the EDHF component of the response with I/R except that ischemic arterioles started with less tone but still dilated fully.

It has been shown that EDHF-mediated dilation can be reversed by IK$_{Ca}$ inhibition alone with little to no contribution of SK$_{Ca}$ channels in MCA. However, it is not known what role these channels play in EDHF-mediated responses in PA. Figure 4A shows a diameter tracing of a PA in the presence of NOS/COX inhibition that dilated fully to A23187. Addition of apamin caused considerable vasomotion and partially reversed the EDHF-based dilation. However, addition of TRAM-34 completely reversed this dilation and restored diameter to starting levels. When TRAM-34 was given before apamin (n = 3), it alone fully reversed the EDHF dilation (Figure 4B). These data suggest that similar to the MCA, IK$_{Ca}$ inhibition alone is sufficient to reverse EDHF dilation in PA.

Figure 4C shows the percent tone in PA from control and ischemic animals under these conditions. Similar to the first set of experiments, PA developed less tone after I/R but was not significantly different than controls. PA from control animals responded to L-NNA with increased tone but had little reactivity to NOS inhibition after I/R. However, arterioles from both groups dilated fully to 1.0 μmol/L A23187, again suggesting a considerable role for EDHF in these vessels. Interestingly, apamin caused constriction in both types of vessels, but only partially reversed the EDHF-based response, whereas TRAM-34 completely reversed the dilation. It is worth noting that although the percent tone in TRAM-34 was less in PA after I/R compared with sham controls, these vessels started out less constricted because of their lack of response to L-NNA. However, TRAM-34 reversed the dilation to the starting diameter of those vessels as well.

**Expression Levels of SK$_{Ca}$ and IK$_{Ca}$ Channels in MCA and PA After I/R**

To determine if the differential response to apamin and TRAM-34 between MCA and PA was due to differences in mRNA expression of SK$_{Ca}$ and IK$_{Ca}$, we used quantitative PCR to compare channel mRNA expression. Figure 5A shows SK$_{Ca}$ and IK$_{Ca}$ expression of PA and MCA from sham and ischemic animals. Notice that despite the lack of response of MCA to apamin and TRAM-34, both vessels expressed SK$_{Ca}$ and IK$_{Ca}$. However, there was no significant difference in expression levels between PA and MCA, suggesting that differences in expression cannot account for the observed functional changes.

To determine if the effect of I/R on the response to apamin and TRAM-34 in PA related to channel mRNA expression, we compared the mRNA expression of SK$_{Ca}$ and IK$_{Ca}$ after I/R with sham controls (Figure 5B). There was no difference in channel expression with I/R, again suggesting that the
differential response to apamin and TRAM-34 in PA was not related to differences in mRNA expression.

Lastly, because of the different roles of SKCa and IKCa channels in mediating EDHF in MCA and PA, we compared the mRNA expression of IKCa relative to SKCa. Figure 5C shows that there was a 4- to 5-fold increase in IKCa mRNA expression compared with SKCa in PA that was not altered by I/R.

**Discussion**

In the present study, we show for the first time that SKCa and IKCa channels are involved in modulating basal tone in PA, but not MCA. This is demonstrated by the finding that apamin and TRAM-34, selective SKCa and IKCa inhibitors, both caused a significant increase in tone in PA, whereas the MCA was unaffected. The lack of response in MCA to these inhibitors confirms previous findings. The increase in basal tone in PA with channel inhibition importantly suggests that similar to NO, EDHF is affecting basal tone in the PA. A second major finding was that although I/R significantly diminished NO responsiveness, the reactivity to apamin and TRAM-34 was preserved. The decreased constriction to L-NNA in PA confirms our previous finding and suggests that basal NO production is decreased after I/R. This is not likely due to diminished responsiveness of vascular smooth muscle to NO (or the guanylate cyclase–cyclic GMP pathway) because sensitivity to the NO donor sodium nitroprusside was increased after I/R. It has been hypothesized that EDHF acts as a backup vasodilator under conditions when NO production is compromised. The finding that NO responsiveness was significantly diminished after I/R but the role for EDHF was preserved seems consistent with this hypothesis. However, it appears that the EDHF-mediated response may be resistant to I/R.

Similar to previous studies, I/R caused a significant loss of myogenic tone in MCA, whereas tone in PA was more preserved. Clearly, diminished tone in MCA after I/R was not due to SKCa and IKCa channels because inhibition of these channels did not restore tone. This result was somewhat expected, however, because even under nonischemic conditions, MCA did not respond to apamin and TRAM-34. The explanation for the lack of responsiveness of the MCA to apamin and TRAM-34 under basal conditions is not clear but does not appear to be related to lack of channel mRNA expression because quantitative PCR in isolated MCA revealed that both channels were expressed at similar levels as...
PA. It is therefore possible that another component of the EDHF response is differentially affected in MCA and PA. The basic mechanism of EDHF-mediated response is that increased intracellular calcium in endothelium activates SK$_{Ca}$ and IK$_{Ca}$ channels causing K$^+$ efflux and membrane hyperpolarization. Hyperpolarization of endothelium is then transferred to vascular smooth muscle through synthesis or generation of signals capable of diffusing through membranes or myoendothelial gap junctions. In vascular smooth muscle, EDHF activates K$^+$-channels, causing hyperpolarization and relaxation. There are several steps in this pathway that may be different between MCA and PA. First, basal endothelial cell calcium may be higher in PA compared with MCA such that SK$_{Ca}$ and IK$_{Ca}$ channels are more active basally in PA. Second, it may be that the generation of the signal responsible for transferring hyperpolarization from endothelium to vascular smooth muscle is different under basal conditions and/or in response to agonists. Third, there may be anatomic differences between MCA and PA such that there is a difference in how the signals are transferred from endothelium to vascular smooth muscle. Due to its smaller size and fewer smooth muscle cell layers, the endothelium of PA may have a greater influence on smooth muscle. It is therefore possible that this structural arrangement allows for transfer of hyperpolarization that is not possible in MCA under basal conditions.

The finding that both apamin and TRAM-34 caused constriction in PA suggests that both SK$_{Ca}$ and IK$_{Ca}$ channels are involved in mediating this response. Under nonischemic conditions, contraction to apamin was greater than that of TRAM-34, a result that was reversed after I/R. It is unclear what caused the differential response, or its significance, but it does not appear to be related to differences in channel mRNA expression based on our PCR results. Because PCR was done on isolated PA that contained both endothelium and vascular smooth muscle, we cannot rule out the possibility that these channels are expressed on vascular smooth muscle. In a study by McNeish et al., immunohistochemical staining of MCA found positive staining of IK$_{Ca}$ in vascular smooth muscle, although a functional role for these channels in smooth muscle has yet to be demonstrated. In regard to the present study, we cannot rule out the possibility that these channels are expressed in vascular smooth muscle of PA and/or are altered by I/R that might contribute to the differential response.

Agonist stimulation of endothelial cell calcium in the presence of NOS/COX inhibition is commonly used to investigate EDHF responses. Previous studies in mesenteric vessels found that both SK$_{Ca}$ and IK$_{Ca}$ inhibi-

![Figure 5](http://stroke.ahajournals.org/)

**Figure 5.** Real-time qualitative PCR data for SK3 gene (SK$_{Ca}$, channel) and SK4 gene (IK$_{Ca}$, channel) expression. A, Change in channel expression of PAs relative to MCAs isolated from sham control and ischemic brains (ipsilateral side only). B, Change in channel expression of PAs from sham controls compared with after I/R. C, Comparison of IK$_{Ca}$ channel expression relative to SK$_{Ca}$ channel in PA from sham controls and after I/R.
tion are necessary to block endothelial hyperpolarization and hence EDHF, making activation of these channels critical to agonist-induced EDHF production.9,15–17 Cerebral arteries are thought to be different than peripheral vessels in that IK_{Ca} channel inhibition alone is sufficient to block endothelial hyperpolarization and EDHF-mediated relaxation.20–22 In one study, endothelial and vascular smooth muscle membrane potential was measured in response to endothelial stimulation, and it was found that SK_{Ca} channels can contribute to endothelium-dependent hyperpolarization in MCA, but only when the arteries could produce NO (eg, not in the presence of NOS inhibition).22 In the present study, under conditions of NOS/COX inhibition, A23187 caused dilation in PA that was similar in magnitude to that elicited by papaverine and zero calcium PSS, suggesting a major influence of EDHF on tone in both control and ischemic vessels. Addition of the specific SK_{Ca} inhibitor apamin partially reversed the dilation in both vessel types. This is in contrast to previous studies in the MCA in which apamin had no effect on EDHF-mediated dilation.20–22 However, similar to previous studies in the MCA, IK_{Ca} channel inhibition with TRAM-34 completely reversed the EDHF response in both types of PA. These data demonstrate another fundamental difference between MCA and PA in that there appears to be some involvement of SK_{Ca} channels in mediating agonist-induced EDHF responsiveness in PA, albeit to a lesser extent than IK_{Ca} channels. The finding that SK_{Ca} inhibition with apamin only partially reversed, whereas IK_{Ca} inhibition with TRAM-34 completely reversed EDHF-mediated vasodilation may be related to differences in channel expression in PA. Quantitative PCR revealed that IK_{Ca} expression was 4- to 5-fold higher than SK_{Ca} in these vessels.

Summary

There appear to be fundamental differences in mechanisms of endothelium-dependent relaxation between MCA and penetrating PA, including the influence of SK_{Ca} and IK_{Ca} on basal tone and the involvement of both channel types in agonist-induced EDHF-mediated dilations in PA, but not in MCA. During I/R, basal tone and reactivity to pressure are relatively intact in PA, whereas it is significantly diminished in MCA as demonstrated by this and other studies.5–7 There is also a selective loss of NO responsive-ness in PA after I/R, yet the influence of EDHF, both under basal and stimulated conditions, appears to be preserved. The functional importance of this is not entirely clear but may serve to preserve cerebral blood flow under conditions in which NOS is impaired by disease.

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Disclosures

None.

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