Altered Function of Inward Rectifier Potassium Channels in Cerebrovascular Smooth Muscle After Ischemia/Reperfusion

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Background and Purpose—Several recent studies have demonstrated that inward rectifier potassium channels (Kirs) are located on vascular smooth muscle of cerebral arteries in the rat. Activation of the Kirs dilates the arteries by relaxing the vascular smooth muscle. We tested the following hypothesis in the present study: function of inward rectifier potassium channels is altered after ischemia/reperfusion (I/R).

Methods—Temporary (2-hour) focal ischemia was induced in male Long-Evans rats (3% isoflurane anesthesia) by the intraluminal filament model. After 24 hours of reperfusion, ipsilateral and contralateral middle cerebral arteries (MCAs) were harvested and mounted on micropipettes, pressurized to 85 mm Hg, and luminally perfused.

Results—Resting diameters for contralateral (control) and ipsilateral (I/R) MCAs were not significantly different (215±4 μm and 211±5 μm [n=6 and n=7], respectively). Activation of the Ks by abluminal administration of 15 mmol/L KCl to the control MCAs dilated the MCA by 34±4% (n=8). Activation of the Ks in I/R MCAs produced a dilation of only 11±3% (n=8; P<0.001 compared with control). BaCl2 (75 μmol/L), a concentration-selective inhibitor of the Kirs, significantly attenuated the dilation produced by 15 mmol/L KCl in control MCAs but not in the I/R MCAs. Endothelial-mediated dilations elicited by the luminal administration of uridine triphosphate (10 μmol/L) produced similar dilations in both groups (32±5% for sham [n=4] and 33±2% for I/R [n=4]), indicating that dilator function in general was not altered in I/R vessels.

Conclusions—We conclude that Ks function is altered after I/R. The Ks altered function is likely to exacerbate the brain injury occurring after I/R. (Stroke. 1998;29:1469-1474.)

Key Words: cerebral arteries ■ ischemia ■ potassium channels ■ reperfusion ■ rats

In recent years, it has become apparent that potassium channels, located on VSM, are important determinants of resting and activated (pharmacological or functional activation) blood flow in brain and peripheral tissues.1–3 When opened, the potassium channels decrease the vascular resistance by dilating arteries and arterioles, thus increasing blood flow. The cascade between activation of the channels and increased flow involves an initial increase in the conductance of K+ across the VSM membrane. Driven by electrochemical forces and a concentration gradient, the increased conductance produces a net movement of K+ from the cytoplasm to the extracellular space.4 The loss of the positively charged K+ ions hyperpolarizes the VSM, which in turn closes voltage-gated Ca2+ channels. The result of the closing of these channels is relaxation of the VSM due to a decrease in the concentration of cytoplasmic Ca2+.5 Thus, opening or activation of potassium channels results in an increased blood flow.

To date, four types of potassium channels have been identified in cerebrovascular smooth muscle; they are the ATP-sensitive K+ channel (KATP), calcium-activated K+ channel (KCa), voltage-dependent or delayed rectifier K+ channel (Kr), and the inward rectifier K+ channel (Kir).3 In general, opening of any one of the four types of potassium channels results in the outward movement of K+. The differences in the channel types involve (1) the factor(s) that gate or activate the channels and (2) the conductance of the individual channel types. In contrast to the KATP and KCa, very little is known about the Ks and Kir in the cerebrovascular circulation.

The Ks are characterized as voltage-gated channels with the open state probability decreasing with depolarization, activation by modest increases in extracellular K+ (7 to 20 mmol/L), and rectification being modulated by intracellular polyamines.5–10 Recently it has been demonstrated that Ks are located on VSM of the posterior cerebral artery and its branches, MCA and its branches, and penetrating arterioles (References 5 through 8, Reference 11, and S.P.M. et al, unpublished data, 1996). Since extracellular K+ is increased during functional activation of neurons and increases in extracellular K+ activate the Ks, the Ks could link increased neuronal activity and metabolism to flow in the brain.12,13

Although the functional activity of the KATP and KCa has been studied during pathological conditions (hypertension,
diabetes, I/R, traumatic brain injury, and subarachnoid hemorrhage. The function of the Kir s in cerebral arteries has been studied only after chronic hypertension. Thus, the purpose of the present study was to answer the question: Is the activity of the Kir s altered after I/R in the rat?

Materials and Methods

The experimental protocol was approved by the Animal Protocol Review Committee at Baylor College of Medicine. Before surgery all rats were allowed free access to food and water.

Thirteen male Long-Evans rats (weight: 280 to 320 g) were subjected to reversible MCA occlusion. Anesthesia was induced and maintained with isoflurane (3% delivered through a face mask. The right MCA was occluded with the use of a nylon monofilament, as previously described. Briefly, a monofilament line, approximately 240 \( \mu \)m in diameter, was inserted into the right external carotid artery and advanced into the circle of Willis and beyond the ostium of the MCA. The diameter of the monofilament was sufficiently large to occlude blood flow into the MCA. After 2 hours the occluder was removed, thereby restoring the blood flow. Heparin (50 U) was administered before occluder insertion and after 1 hour of occlusion to reduce blood clot formation. Rectal temperature was maintained at 37 ± 0.5°C during the entire procedure with the use of a temperature controller coupled to a heat lamp. After gaining consciousness, each rat was returned to the animal holding facilities until the following day.

After 24 hours of reperfusion, rats were anesthetized with isoflurane and decapitated. The brain of each rat was removed from the cranium and placed in cold PSS solution (4°C). Both MCAs were rinsed and decapitated. Two monofilament lines, approximately 240 \( \mu \)m in diameter, were inserted into each end of the artery, 11–12 mm from the os. The diameter of the monofilament was increased to occlude blood flow into the MCA. The initial diameters of control (307 ± 6 \( \mu \)m) and I/R vessels (301 ± 5 \( \mu \)m) were measured using a video camera and a Videoplan image analysis software package (Optimas Corp) on a Hewlett Packard Pentium computer. The frequency of acquisition was 1.1 Hz. In cases in which vessel diameter was variable (Figure 2, bottom panel), the average minimum diameter was used. Control and I/R vessels were studied in three experimental protocols. In the first protocol (n = 5 each), KCl (15 mmol/L) was administered abluminally to the vessels and then washed out. After KCl washout, UTP (10 \( \mu \)mol/L) was delivered luminally (n = 4 each) to assess endothelium-dependent dilations. UTP is a potent endothelium-dependent dilator in the rat MCA.

In the second protocol (n = 3 each), KCl (15 mmol/L) was given initially, then in the presence of BaCl\(_2\), and finally after washout of BaCl\(_2\). BaCl\(_2\) was added to the extraluminal bath to give a concentration of 75 \( \mu \)mol/L, a concentration that selectively inhibits Kir s. Figure 6 is derived from data exclusively from protocol 1, while Figures 1 and 3 combine data from protocols 1 and 2. Figure 5 consists of data exclusively from protocol 2. In the third protocol, KCl was delivered in 5-mmol/L increments (5 to 20 mmol/L; n = 5 each). Data from protocol 3 are shown in Figure 4.

After removal of the MCAs, each brain was placed in a rat brain matrix (Braintree Scientific, Inc) and sectioned coronally in 2-mm sections. The sections were incubated in 2% TTC solution for 30 minutes and then placed in a formalin solution for at least 24 hours. Viable tissue stained deep red, while the lesion area due to the occlusion remained white. Lesion volumes were evaluated by image analysis (MCID, Imaging Research). Confirmation of finding by TTC was a prerequisite for all I/R vessels.

The PSS consisted of the following (mmol/L): NaCl 119, NaHCO\(_3\) 24, KCl 4.7, KH\(_2\)PO\(_4\) 1.18, MgSO\(_4\) 1.17, CaCl\(_2\) 1.6, and EDTA 0.026. All chemicals and reagents were purchased from Sigma Chemical Co. A stock solution of UTP was prepared in distilled water, divided into aliquots, and frozen.

All data are presented as mean ± SEM, with n representing the number of observations per group. Percent diameter change was calculated by the following formula: 

\[
\% \text{Change} = \left( \frac{D_i - D_f}{D_i} \right) \times 100
\]

where \( D_i \) is the initial diameter and \( D_f \) is the final diameter. Statistical comparisons between groups were made with the Student-Newman-Keuls test (Figures 4 and 6), or the two-way repeated-measures ANOVA followed by Student-Newman-Keuls test (Figures 4 and 6). Differences were considered significant at \( p < 0.05 \).

Results

Average lesion volume, as determined by TTC staining (see “Materials and Methods”), ipsilateral to the monofilament placement was 154 ± 23 mm\(^3\) (n = 12). In one instance, the TTC stain faded too much for an accurate determination of lesion volume. Lesions were not observed on the side contralateral to the occluder placement.

The initial diameters of control (307 ± 3 \( \mu \)m) and I/R vessels (301 ± 5 \( \mu \)m) immediately after pressurization to 85 mm Hg were similar (Figure 1). This initial diameter before the development of tone is the same as the maximal diameter obtained in Ca\(^{2+}\)-free PSS (S.P.M., unpublished data, 1996). After 1 hour, the control and I/R MCAs developed 29 ± 1% (n = 6) and 30 ± 2% (n = 7) tone, respectively (Figure 1). In three MCAs from the 15 mmol/L group (two control and one I/R MCAs), the initial diameters on pressurization were not recorded and therefore were not included in Figure 1.

Figure 2 shows the dilations of a representative control MCA (top panel) and I/R MCA (bottom panel) after activation of the Kir s by adding 15 mmol/L KCl to the extraluminal bath. Note that the control MCA had a greater dilation that was sustained. The dilation to KCl in the I/R MCA was significantly reduced and appeared to be unstable (oscillations). The oscillations occurred in some but not all I/R
MCAs after the addition of KCl. Figure 3 shows a summary of dilations produced by the addition of 15 mmol/L KCl to the extraluminal bath in both MCA groups. Control and I/R MCAs showed a maintained dilation of $34 \pm 6\%$ (n=8, $P<0.001$) and $11 \pm 3\%$ (n=8, $P<0.01$), respectively (Figure 3). The dilations in response to KCl were significantly different between the two groups ($P<0.001$, unpaired t test).

Previous studies in naïve MCAs have demonstrated that 15 mmol/L KCl is optimal for K$_{ir}$-induced dilations. However, I/R could have produced a shift in the response curve to KCl. To address this possibility, we performed a concentration-response curve to KCl (Figure 4). Control vessels (n=5) produced a concentration-response curve that was maximal at 10 to 15 mmol/L KCl. This response was significantly different from that of the I/R vessels ($P<0.001$, repeated-measures ANOVA). Comparisons of individual concentrations by the Student-Newman-Keuls method revealed that I/R vessels were significantly attenuated compared with control at both 10 and 15 mmol/L KCl ($P<0.05$).

Additional studies were designed to determine the effects of BaCl$_2$, a concentration-selective inhibitor of the K$_{ir}$s, on the dilations produced by the addition of 15 mmol/L KCl in control and I/R MCAs. Three responses to KCl were conducted on each artery of both groups. The bath was washed for 15 minutes with fresh PSS between additions of KCl. Before the second KCl response, 75 μmol/L BaCl$_2$ was added to the extraluminal bath to block the K$_{ir}$s. The results are summarized in Figure 5. In the control MCAs, BaCl$_2$ significantly attenuated the dilation ($P<0.01$, repeated-measures ANOVA followed by Student-Newman-Keuls method for multiple comparisons). The dilation to KCl was restored after the bath was washed with fresh PSS containing no BaCl$_2$. As in the previous study (Figure 3), the response to KCl was significantly attenuated in I/R MCAs (Figure 5; $P<0.05$). Since the dilations in the I/R MCAs were already attenuated, BaCl$_2$ had no significant effect on the response. However, the power of the test was below the desired power of 0.8. Using power analysis, we calculated that an additional six I/R
MCAs would have to be studied to obtain a 50% reduction in the dilation to KCl due to the presence of BaCl2. We believed that the knowledge gained by determining whether BaCl2 could further attenuate the dilation in response to KCl beyond that of I/R alone was not of sufficient importance to conduct further experiments.

Figure 6 shows that the endothelial-mediated dilations produced by the luminal application of 10 μmol/L UTP were not affected by I/R. UTP dilated MCAs by 32±5% (n=4) and 33±2% (n=4) in control and I/R groups, respectively. Thus, the lack of K⁺-induced dilation in I/R MCAs was not simply due to a generalized loss of vasodilatory function.

Discussion

Kᵆs have been found on VSM from several different cerebral vessels in the rat.⁵⁻⁸,¹¹ The evidence is based on hyperpolarizations and dilations of cerebral vessels due to modest increases in extracellular K⁺. These hyperpolarizations and dilations could be blocked by Ba²⁺ (at concentrations known to selectively inhibit the Kᵆs) and Cs⁺, another inhibitor of the channel.⁵⁻⁸,¹¹

From the present study we conclude that the response of Kᵆs to extracellular K⁺ was significantly attenuated after 2 hours of ischemia and 24 hours of reperfusion in the rat MCA (Figures 2 to 5). In contrast, I/R MCAs developed normal spontaneous tone (Figure 1) and showed no attenuation in the vasodilation to luminal UTP, an endothelial-mediated dilator (Figure 6). UTP dilates the rat MCA through the stimulation of P2Y2 purinoceptors (formerly P₂y), which results in the release of nitric oxide and another relaxing factor, which may be endothelium-derived hyperpolarizing factor.²⁸ Thus, the attenuated dilation to extracellular K⁺ appears to be specific and not due to a general vasodilatory dysfunction.

Altered potassium channel function has been reported for multiple pathological conditions. Diminished KₐTP channel function has been reported in cerebral vessels as a result of chronic hypertension,²³ traumatic brain injury,²² diabetes,¹⁸,¹⁹ and ischemia.¹⁶ In contrast, KₐTP function appears to be augmented after subarachnoid hemorrhage.¹⁷ Kᵆs channel function was reported to be diminished after traumatic brain injury,²¹ enhanced during hypertension,¹⁴ or not affected by ischemia.²⁴ However, pathology-associated changes in cerebrovascular Kᵆ channel function have received little attention in the literature. To our knowledge, only one study has been published. Similar to the finding of the present study, those authors reported that posterior cerebral arteries isolated from hypertensive rats no longer dilated in response to extracellular K⁺ concentrations that activated the Kᵆs.²⁵

While I/R produces conditions that might be deleterious to potassium channel function, it must also be considered that changes in potassium channel function during pathological conditions may not necessarily reflect channel dysfunction per se. Changed function of the channel could represent altered cellular conditions produced by the pathological state that affect the channel directly. For example, increased constriction of the basilar artery of the rat after administration
of K<sub>i</sub> blockers is likely a result of proportionally more open channels in vessels of hypertensive rats compared with normotensives. The greater proportion of opened K<sub>i</sub>S is likely a result of increased Ca<sup>2+</sup> concentrations in the VSM of the hypertensive rats.

In a similar manner, the diminished dilation to extracellular K<sup>+</sup> after I/R may not be a result of channel dysfunction per se, but rather cellular conditions (such as a change in membrane potential) that influence an otherwise normal K<sub>i</sub>. Regardless of whether the K<sub>i</sub>S are damaged after I/R, the response to extracellular K<sup>+</sup> is altered. The consequences of this effect could exacerbate injury due to the pathological condition.

K<sup>+</sup> appears to be one of several factors that link increases in cerebral blood flow with increased metabolism in the brain. Extracellular K<sup>+</sup>, which is normally approximately 3 mmol/L, can increase to 10 to 12 mmol/L during activation of neurons, dilate cerebral pial vessels in vivo, and increase cerebral blood flow. Maximum dilations of pial arteries in vivo occur at approximately 10 mmol/L K<sup>+</sup> and increase with further increases in K<sup>+</sup> diminishing the magnitude of the dilation until the arteries are no longer dilated.

Local increases in extracellular K<sup>+</sup> can either diffuse to nearby arteries and arterioles or can be aided by astrocytic glia through a process termed “potassium siphoning.” Potassium siphoning can raise the K<sup>+</sup> concentration at the resistance arteries more quickly and to a higher level than simple diffusion alone. Since the glial endfeet project to the pial surface, increases of K<sup>+</sup> in the brain parenchyma can theoretically be transmitted to the pial arteries and arterioles on the surface of the brain, however, the magnitude of K<sup>+</sup> increase at the pial surface may not be the same magnitude as the K<sup>+</sup> increase in parenchymal interstitial fluid.

If indeed increased concentrations of extracellular K<sup>+</sup> link function and metabolism to blood flow, then the attenuation of vessel response to extracellular K<sup>+</sup> could be responsible for the uncoupling of flow and metabolism that occurs after ischemia. However, this concept remains speculative. More studies are clearly needed.

In summary, we have demonstrated that the stimulation of K<sub>i</sub>S on the rat MCA is significantly attenuated (by 70%) after 2 hours of ischemia and 24 hours of reperfusion. Given the potential importance of the K<sub>i</sub>S in the control of cerebral blood flow, their altered function is likely to exacerbate the brain injury that occurs after I/R.

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Kᵋ Channels in Cerebrovascular Smooth Muscle After I/R

Recent evidence indicates that potassium channels are a critical component of normal cerebrovascular responsiveness. In addition, potassium channels in cerebral arteries and arterioles are a target of disease processes such as diabetes, ischemia, subarachnoid hemorrhage, and head trauma. For example, we have shown that Kᵋ ATP but not Kᵋ Ca channels in cerebral arteries are transiently (1 to 2 hours) inhibited by even a short duration (10 minutes) of global ischemia. In the accompanying article, the authors have extended these findings in several ways and shown that ischemia of 2 hours' duration is able to disrupt Kᵋ function in rat MCA for at least 24 hours of reperfusion. In this and previous reports, ischemia-induced changes in cerebrovascular responsiveness were selective, so that reduced arterial and arteriolar dilation to activators of Kᵋ ATP and Kᵋ s occurred while normal responsiveness was present to other stimuli working through other mechanisms. What is unclear from this study, however, is whether Kᵋ function is permanently impaired or whether normal responsiveness reappears at a later time. Also unknown is the mechanism of impaired Kᵋ function in cerebral arteries after ischemia, although oxygen free radicals may be involved. Nonetheless, the accompanying article provides important information on the extent and duration of potassium channel dysfunction in cerebral arteries after transient ischemic episodes.

Several severe consequences of altered potassium channel function in cerebral arteries after ischemia are probably present in the clinical situation. First, cerebrovascular responsiveness to neurotransmitters such as calcitonin gene–related peptide as well as to other stimuli is lost or reduced after ischemia. Thus, the ability of the cerebral circulation to respond appropriately to secondary insults after ischemia is impaired. Second, coupling between metabolic stimuli such as potassium and blood flow in the brain is disrupted. Thus, the cerebral circulation may not be able to respond appropriately to basal or elevated levels of brain metabolism after ischemia so that nutrient delivery is inadequate. Third, functional interrelationships between endothelium and smooth muscle layers of cerebral arteries and arterioles are altered. Thus, substances such as prostacyclin released from endothelium may not dilate cerebral arteries after ischemia. Future studies should be directed at developing pharmacological approaches for minimizing potassium channel dysfunction during the reperfusion period after ischemia.

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