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

Sean P. Marrelli, BA; T. David Johnson, PhD; Andrei Khorovets, MD; William F. Childres, MD; Robert M. Bryan, Jr, PhD

Background and Purpose—Several recent studies have demonstrated that inward rectifier potassium channels (K\textsubscript{ir}s) are located on vascular smooth muscle of cerebral arteries in the rat. Activation of the K\textsubscript{ir}s 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 \mu m and 211±5 \mu m [n=6 and n=7], respectively). Activation of the K\textsubscript{ir}s by aboluminal administration of 15 mmol/L KCl to the control MCAs dilated the MCA by 34±4% (n=8). Activation of the K\textsubscript{ir}s in I/R MCAs produced a dilation of only 11±3% (n=8; P<0.001 compared with control). BaCl\textsubscript{2} (75 \mu mol/L), a concentration-selective inhibitor of the K\textsubscript{ir}s, 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 \mu mol/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 K\textsubscript{ir} function is altered after I/R. The K\textsubscript{ir} 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
Selected Abbreviations and Acronyms

I/R = ischemia/reperfusion
K<sub>ATP</sub> = ATP-sensitive K<sup>+</sup> channel
K<sub>Ca</sub> = calcium-activated K<sup>+</sup> channel
K<sub>) = inward rectifier K<sup>+</sup> channel
K<sub>V</sub> = voltage-dependent or delayed rectifier K<sup>+</sup> channel
MCA = middle cerebral artery
PSS = physiological saline solution
TTC = 2,3,5-triphenyltetrazolium chloride
UTP = uridine triphosphate
VSM = vascular smooth muscle

Diabetes, I/R, traumatic brain injury, and subarachnoid hemorrhage, the function of the K<sub>V</sub>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 K<sub>V</sub>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 μm 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 surgically dissected beginning at the circle of Willis and for 15 to 20 mm distally. The left (control) MCA was taken from the nonischemic hemisphere and the right (I/R) MCA from the ischemic hemisphere.

Arteries were placed in an arteriograph (Living Systems Inc), where micropipettes were inserted into both ends of the artery. An arterial segment approximately 1 mm in length and lying between branch points was positioned between the tips of the two micropipettes. The artery was secured to the micropipettes with 11–0 nylon. Each artery was bathed in PSS equilibrated with 20% O<sub>2</sub>, 5% CO<sub>2</sub>, and balance N<sub>2</sub>. The PSS in the bath was maintained at a temperature of 37°C and a pH of 7.4.

Luminal or transmural pressure was maintained at 85 mm Hg by raising reservoirs, connected to the micropipettes by tubing (Tygon), to the appropriate height above each artery. Luminal perfusion was adjusted to 100 μL/min by setting the two reservoirs at different heights. Pressure transducers placed between the micropipettes and the reservoirs provided a measure of perfusion pressure. The luminal perfusate was heated to 37°C and gassed before the lumen of each artery was perfused. Samples of PSS were analyzed for PO<sub>2</sub>, PCO<sub>2</sub>, and pH with the use of a Corning model 280 analyzer.

After they were mounted, MCAs were allowed to equilibrate for 1 hour before any experiments were started. The vessels were magnified (×600) with the use of an inverted microscope equipped with a video camera and monitor. We recorded the experiments on a VCR for post hoc measurement of diameter changes using an 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 abuminally to the vessels and then washed out. After KCl washout, UTP (10 μmol/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<sub>2</sub>, and finally after washout of BaCl<sub>2</sub>. BaCl<sub>2</sub> was added to the extraluminal bath to give a concentration of 75 μmol/L, a concentration that selectively inhibits K<sub>V</sub>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 a 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 lesion by TTC was a prerequisite for all I/R vessels.

The PSS consisted of the following (mmol/L): NaCl 119, NaHCO<sub>3</sub> 24, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.18, MgSO<sub>4</sub> 1.17, CaCl<sub>2</sub> 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: %Change=[(D<sub>t</sub>−D<sub>i</sub>)/D<sub>i</sub>]·100, where D<sub>t</sub> is the diameter after stimulation and D<sub>i</sub> is the initial diameter. Statistical comparisons between groups were made with the paired t test (Figure 1), the unpaired t test (Figures 3 and 6), or the two-way repeated-measures ANOVA followed by Student-Newman-Keuls test (Figures 4 and 5). 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<sup>3</sup> (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 μm) and I/R vessels (301±5 μm) 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<sup>2+</sup>-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 K<sub>V</sub>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 ± 4% (n = 8, P < 0.001) and 11 ± 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 naive MCAs have demonstrated that 15 mmol/L KCl is optimal for K^+-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 were necessary to achieve the desired power.
altered with changes in the concentration of extracellular K⁺ would not oppose the action potential. However, in cells where action potentials are produced by the luminal application of 10 μmol/L UTP, an endothelial-mediated dilator (Figure 6). UTP dilates the rat MCA through the stimulation of P2Y₂ purinoceptors (formerly P₂u), which results in the release of nitric oxide and another relaxing factor, which may be endothelium-derived hyperpolarizing factor. Thus, the attenuated dilation to extracellular K⁺ appears to be specific and not due to a general vasodilatory dysfunction.

### Discussion

Kᵣs represent a diverse and heterogeneous class of potassium channels that have two distinguishing characteristics. First, they are voltage sensitive, with the open state probability decreasing with depolarization. Second, the open state probability is increased with modest increases in extracellular K⁺ (7 to 20 mmol/L). Interestingly, the voltage dependency is altered with changes in the concentration of extracellular K⁺. In the presence of increased extracellular K⁺, the open state probability is shifted to more positive potentials (ie, depolarization).

The Kᵣs are thought to play a significant role in excitable tissues such as neurons, where they can stabilize the membrane potential until a threshold potential is reached. After the threshold is reached, the Kᵣs would then close so that they would not oppose the action potential. However, in cells such as cerebrovascular smooth muscle that do not typically exhibit action potentials, their purpose is not as readily apparent. We speculate that their purpose in the cerebrovascular circulation is to link increased function and metabolism with flow (see below).

Kᵣs have been found on VSM from several different cerebral vessels in the rat. 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 Kᵣs) and Cs⁺, another inhibitor of the channel.

From the present study we conclude that the response of Kᵣs 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 P₂u), which results in the release of nitric oxide and another relaxing factor, which may be endothelium-derived hyperpolarizing factor. 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, traumatic brain injury, diabetes, and ischemia. In contrast, KₐTP function appears to be augmented after subarachnoid hemorrhage. Kᵣ channel function was reported to be diminished after traumatic brain injury, enhanced during hypertension, or not affected by ischemia. However, pathology-associated changes in cerebrovascular Kᵣ 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 Kᵣs.

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>ir</sub> blockers is likely a result of proportionally more open channels in vessels of hypertensive rats compared with normotensives. The greater proportion of open K<sub>ir</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>ir</sub>. Regardless of whether the K<sub>ir</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, and dilate cerebral pial vessels in vivo, Maximum dilations of pial vessels. 31–34 and 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> 32.33 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. The latter mechanism depends on the detection of elevated K<sup>+</sup> in the extracellular fluid by astrocytes and the subsequent release of K<sup>+</sup> at astrocytic endfeet surrounding cerebral vessels. 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>ir</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>ir</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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References
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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 K<sub>ATP</sub> but not K<sub>Ca</sub> channels in pial arterioles 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 K<sub>i</sub> 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 K<sub>ATP</sub> and K<sub>i</sub> occurred while normal responsiveness was present to other stimuli working through other mechanisms. What is unclear from this study, however, is whether K<sub>i</sub> function is permanently affected or whether normal responsiveness reappears at a later time. Also unknown is the mechanism of impaired K<sub>i</sub> 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 resistance vessels are probably present after ischemia. 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.

**References**

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