Stroke

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Stroke Association

A Division of American Heart Association

Potassium Channel Activators Protect the N-Methyl-D-Aspartate–Induced Cerebral Vascular Dilation After Combined Hypoxia and Ischemia in Piglets • Editorial Comment

Roland Veltkamp, Ferenc Domoki, Ferenc Bari, David W. Busija and William G. Mayhan

Stroke 1998;29;837-843

Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514

 Print ISSN: 0039-2499. Online ISSN: 1524-4628

Potassium Channel Activators Protect the *N*-Methyl-D-Aspartate–Induced Cerebral Vascular Dilation After Combined Hypoxia and Ischemia in Piglets

Roland Veltkamp, MD; Ferenc Domoki, MD; Ferenc Bari, PhD; David W. Busija, PhD

Background and Purpose—Cerebral arteriolar dilation to *N*-methyl-D-aspartate (NMDA) is a neuronally mediated multistep process that is sensitive to cerebral hypoxia and ischemia (H/I). We tested the hypothesis that topical pretreatment with the selective potassium channel agonists NS1619 and aprikalim preserves the vascular response to NMDA after consecutive H/I.

Methods—Pial arteriolar diameters were measured in anesthetized piglets with the use of a closed cranial window and intravital microscopy. Arteriolar responses to NMDA (10^{-5}, 5×10^{-5}, and 10^{-4} mol/L) were recorded before and 1 hour after 10 minutes of hypoxia (8.5% O_2 in N_2) plus 10 minutes of ischemia (H/I). Ischemia was induced by increasing intracranial pressure. Subgroups were topically pretreated with 10^{-5} mol/L NS1619, 10^{-6} mol/L aprikalim, 10^{-6} mol/L calcitonin gene–related peptide (CGRP), or 10^{-5} mol/L papaverine. We also examined the effects of H/I on vascular responses to kainate (10^{-4} mol/L) to assess specificity of neuronal injury.

Results—Arteriolar responses to NMDA were significantly attenuated after H/I. Baseline compared with post-H/I arteriolar diameters were 9±4% versus 3±2% at 10^{-5} mol/L, 22±4% versus 4±2% at 5×10^{-5} mol/L, and 33±4% versus 7±2% at 10^{-4} mol/L (mean±SE; all $P<.05$, n=7). Pretreatment with NS1619 and aprikalim preserved the arteriolar responses to NMDA after H/I. For NS1619 (n=6), values were as follows: 9±2% versus 6±4% at 10^{-5} mol/L, 19±6% versus 21±5% at 5×10^{-5} mol/L, and 35±3% versus 31±5% at 10^{-4} mol/L. For aprikalim (n=7), values were as follows: 6±2% versus 8±2% at 10^{-5} mol/L, 22±6% versus 15±3% at 5×10^{-5} mol/L, and 41±5% versus 32±6% at 10^{-4} mol/L. In contrast, piglets pretreated with CGRP (n=6) or papaverine (n=5) showed no preservation of the vascular response to NMDA after H/I, although these compounds dilated the arterioles to an extent similar to that with NS1619/aprikalim. Kainate-induced arteriolar dilation (n=6) was largely preserved after H/I compared with preischemic responses.

Conclusions—(1) Vascular responses of cerebral arterioles to NMDA after H/I are preserved by pretreatment with NS1619 or aprikalim, indicating a neuroprotective effect. (2) CGRP and papaverine do not preserve the vascular response to NMDA despite causing vasodilation similar to that with NS1619 or aprikalim. This suggests that activation of potassium channels on neurons accounts for the protective effect of potassium channel agonists. (3) Preserved arteriolar dilation to kainate suggests largely intact functioning of neuronal nitric oxide synthase after H/I. **(*Stroke*. 1998;29:837-843.)**

Key Words: cerebral arterioles ■ neuronal protection ■ *N*-methyl-D-aspartate ■ potassium channels

Glutamate is an important excitatory amino acid neurotransmitter in the central nervous system. It can bind to three different ionotropic glutamate receptor subtypes on neurons named after specific synthetic analogues: NMDA, kainate, and AMPA. Activation of neuronal NMDA and kainate receptors causes cerebral arteriolar dilation in different animal species, which is mediated in part or even totally by NO-dependent mechanisms.[1–6] This sequence may represent one of the mechanisms coupling local cerebral metabolism to blood flow.

See Editorial Comment, page 842

We and others have shown that global H/I severely attenuates or abolishes the arteriolar response to NMDA[7–9] as well as to other vasodilating agents such as CGRP and aprikalim.[10,11] Impaired neuronal-vascular coupling in combination with pathological metabolic and electrophysiological processes may contribute to delayed cerebral damage in brain H/I.[12]

From an experimental point of view, the integrity of the neuronal-vascular axis can be used as a model for the study of

Received October 1, 1997; final revision received December 18, 1997; accepted January 13, 1998.

From the Stroke Research Center (R.V.) and Department of Physiology and Pharmacology (R.V., F.D., F.B., D.W.B.), Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC; and Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary (F.D., F.B.).

Correspondence to Roland Veltkamp, MD, Stroke Research Center, Bowman Gray School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157-1083. E-mail veltkamp@bgsm.edu

Selected Abbreviations and Acronyms

aCSF = artificial cerebrospinal fluid
AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
CGRP = calcitonin gene–related peptide
H/I = hypoxia/ischemia
K_{ATP} = ATP-dependent potassium channels
K_{Ca} = calcium-dependent potassium channels
NMDA = *N*-methyl-D-aspartate
NO = nitric oxide

potential neuroprotective agents. The rationale of this model is based on the observation that the arteriolar response to a neuron-mediated vasodilator (eg, NMDA) is attenuated after ischemia compared with preischemic baseline measurements. An experimental intervention is considered neuroprotective if it preserves the original vascular responsiveness to NMDA after ischemia.

Recent advances in the molecular biology and pharmacology of potassium channels[13] have enabled the investigation of potential therapeutic effects of K^+ channel agonists. Different types of ligand-operated channels have been characterized according to their primary regulatory or gating mechanism. Opening of K^+ channels leads to efflux of K^+ ions, which is a mechanism for recovering (repolarization) and/or enhancing (hyperpolarization) the membrane potential of a cell.[14] Potassium channel activation may therefore counteract ischemia-induced depolarizations. In previous studies K^+ channel agonists blocked ischemia-induced glutamate release in rat hippocampal slices[15] and prevented the ischemia-induced expression of immediate early genes in a rat model of global ischemia.[16]

The purpose of the present study was to test the hypothesis that potassium channel agonists preserve the NMDA-induced cerebral arteriolar dilation after consecutive H/I. Specifically, we examined the effects of NS1619 and aprikalim, putatively specific openers of calcium-dependent potassium channels (K_{Ca}) and ATP-dependent potassium channels (K_{ATP}), respectively. We also examined the effects of CGRP and papaverine to determine whether protective effects were independent of arteriolar dilation. Furthermore, we investigated whether the combined H/I protocol attenuated the kainate-induced arteriolar dilation, which had been resistant to ischemia alone in an earlier study.[6] Kainate-induced arteriolar dilation shares features of NMDA-induced arteriolar responses in that at least one half of the vascular response is NO dependent.

Materials and Methods

Surgical Preparation

Experiments were performed on newborn pigs (1 to 7 days) of either sex weighing 1 to 2 kg. The procedures used in the study were approved by the Institutional Animal Care and Use Committee. The piglets were initially anesthetized with sodium thiopental (30 mg/kg IP) and later with α-chloralose (75 mg/kg IV). Additional amounts of α-chloralose were given as needed to maintain a stable level of anesthesia. The piglets were intubated by a tracheotomy and artificially ventilated. A femoral artery and vein were cannulated with polyethylene tubing (PE-90). Arterial blood gases and pH were repetitively measured, and rectal temperature was continually monitored. These parameters were kept within the normal physiological range. The head of each piglet was fixed in a stereotaxic apparatus. Approximately 3 mL of CSF was withdrawn from the cisterna magna. The scalp was cut, and the connective tissue over the parietal bone was removed. A circular craniectomy (19 mm in diameter) was made in the left parietal bone. The dura was cut and reflected over the skull. A stainless steel and glass cranial window with three ports was put into the opening, sealed with bone wax, and cemented with cyanoacrylate ester (SuperGlue) followed by one or two layers of dental acrylic. The closed window was filled with aCSF that was warmed to 37°C and equilibrated with 6% O_2, 6.5% CO_2, balance N_2. Arterioles were observed with a microscope (Wild M36) equipped with a television camera (Panasonic), and arteriolar diameter was measured with a video microscaler (IV-550, For-A Co).

Cerebral H/I

Cerebral hypoxia was induced by artificial ventilation with 8.5% O_2, balance N_2 over 10 minutes. Arterial blood gases were measured 8 minutes after hypoxia was started. Ten minutes of hypoxia were immediately followed by 10 minutes of global cerebral ischemia.

Cerebral ischemia was produced by implantation of a hollow brass bolt in the left parietal cranium 20 mm rostral to the cranial window. A 3-mm hole was drilled in the skull with an electric drill with a toothless bit, and the dura was exposed. The hollow bolt was inserted and secured in place with cyanoacrylate ester and dental acrylic. After implantation of the window and the bolt, aCSF was allowed to equilibrate with the periarachnoid CSF for 20 minutes. To induce ischemia, aCSF was infused to maintain intracranial pressure above mean arterial pressure so that blood flow through pial vessels was stopped. Venous blood was withdrawn as necessary to maintain mean arterial pressure near normal values. At the end of the 10-minute period of ischemia, the infusion tube was clamped, and the intracranial pressure was allowed to return to preischemia values. The heparinized blood was reinfused intravenously.

Experimental Design

At the beginning of each experiment the cranial window was flushed several times with aCSF until a stable baseline was observed. Then arteriolar responses to NMDA (10^{-5}, 5×10^{-5}, 10^{-4} mol/L) were determined. Each dose of NMDA was introduced into the window, the infusion was stopped, and arteriolar diameter was recorded over the next 5 to 10 minutes. Afterward the window was flushed with aCSF. The arteriolar diameter returned to baseline within 15 to 20 minutes.

Animals were divided into five experimental groups. In group 1 (n=7) arteriolar responses to NMDA were recorded before (see above) and 1 hour after 10 minutes of hypoxia plus 10 minutes of global ischemia. In addition to this protocol, the other four groups were pretreated with a topical infusion 10 minutes before H/I of either NS1619 10^{-5} mol/L (group 2, n=6); aprikalim 10^{-6} mol/L (group 3, n=7); CGRP 10^{-6} mol/L (group 4, n=6); or papaverine, 10^{-5} mol/L (group 5, n=5). Arteriolar responses to these drugs were recorded during the infusion period. The drugs were washed away just before the beginning of hypoxia. In groups 1 and 4, 10^{-4} mol/L sodium nitroprusside was applied topically after the NMDA responses 1 hour after H/I were measured to examine the vascular responsiveness to exogenous NO.

In another experiment, the cerebral arteriolar responses to 10^{-4} mol/L kainate (group 5, n=6) before and 1 hour after H/I were determined.

Drugs

We used NMDA (Sigma), kainate (Sigma), sodium nitroprusside (Sigma), NS1619 (Research Biochemicals International), aprikalim (Rhone-Roulenc Rorer), CGRP (Research Biochemicals International), and papaverine (Sigma).

Statistical Analysis

Data are expressed as mean±SEM. A paired *t* test was used for comparing data between two groups. For repeated-measurement analysis, ANOVA was used, and the Student-Newman-Keuls test was then performed. Data analyses were performed on absolute and

Arteriolar Dilation to NMDA Before and After H/I

	NMDA Application Before H/I				NMDA Application 1 h After H/I			
	Baseline	10^{-5} mol/L	5×10^{-5} mol/L	10^{-4} mol/L	Baseline	10^{-5} mol/L	5×10^{-5} mol/L	10^{-4} mol/L
Group 1 (n=7): none								
Diameter	101±2	109±3	123±6†	134±5†	104±4	105±6	109±6*	111±6*
Δ Diameter		9±1	22±4†	33±4†		3±2	5±2*	7±2*
Group 2 (n=6): NS1619								
Diameter	103±3	112±3	123±7†	139±3†	104±3	109±3	125±5†	135±5†
Δ Diameter		9±1	20±6†	36±3†		6±2	25±5†	32±5†
Group 3 (n=7): aprikalim								
Diameter	101±4	107±5	123±8†	142±9†	104±4	113±6	120±6†	138±9†
Δ Diameter		6±2	22±6†	41±6†		9±2	15±3†	34±7†
Group 4 (n=6): CGRP								
Diameter	104±4	110±5	127±7†	133±8†	112±7	113±7	114±8*	118±9*
Δ Diameter		6±2	23±5†	29±7†		1±1	2±1*	3±1*

Values are mean±SEM, expressed in micrometers.
*Significantly different from respective preischemic value ($P<.05$).
†Significantly different from respective baseline.

percent change data. A P value $<.05$ was regarded as statistically significant.

Results

Before and after H/I, mean arterial blood pressures were stable and within normal limits for piglets. For example, in the NS1619 group, during baseline conditions arterial blood pressures were 64±4 mm Hg before H/I and 63±2 mm Hg 1 hour after H/I. Arterial blood pressures were not affected by the topical application of the drugs used in these experiments.

Arterial blood gases and pH were monitored regularly during the experiments and were generally kept within the physiological range. At baseline, pH was 7.45±0, P_{CO_2} was 32±1 mm Hg, and P_{O_2} was 106±4 mm Hg (n=32). After 8 minutes of hypoxia, pH was 7.40±0, P_{CO_2} was 32±2 mm Hg, and P_{O_2} was 27±1 mm Hg (n=24). Arterial blood gases were similar to baseline after recovery from H/I. Blood gases and pH did not differ significantly among groups.

Application of NMDA before H/I caused a reproducible, dose-dependent cerebral arteriolar dilation (Table, Fig 1). After H/I, however, arteriolar responses to NMDA were markedly reduced (Table, Fig 1). Subsequent administration of sodium nitroprusside (10^{-4} mol/L) caused consistent dilation of the same vessels by 25±2% compared with baseline.

Topical administration of the drugs 10 minutes before H/I caused arteriolar dilation by 15±4% for NS1619, 26±7% for aprikalim, 15±4% for CGRP, and 20±2% for papaverine.

Pretreatment with NS1619 (10^{-5} mol/L) resulted in an almost complete preservation of the arteriolar dilation to the different concentrations of NMDA 1 hour after H/I (Table, Fig 2), indicating no statistically significant difference between the preischemic and postischemic arteriolar responses. Similarly, administration of aprikalim (10^{-6} mol/L) preserved the post-H/I vascular response to NMDA (Table). Percent changes in vascular diameter to NMDA before and after H/I were as follows: 6±2% versus 8±2% at 10^{-5} mol/L, 22±6% versus 15±3% at 5×10^{-5} mol/L, and 41±5% versus 32±6% at 10^{-4} mol/L (n=7, $P>.05$). In contrast, piglets pretreated with CGRP (10^{-6} mol/L) showed a severe attenuation of the vascular response to NMDA comparable to the group that did not receive any pretreatment (Table). Vascular diameter increased by 6±2% versus 0±0% at 10^{-5} mol/L, 22±5% versus 2±1% at 5×10^{-5} mol/L, and 28±7% versus 2±1% at 10^{-4} mol/L of NMDA. In addition, vascular responses to NMDA were markedly attenuated after pretreatment with papaverine (10^{-5} mol/L). Arteriolar dilation before compared with after H/I was 7±1% versus 3±1% at 10^{-5} mol/L NMDA and 38±4% versus 16±6% at 5×10^{-5} mol/L NMDA (n=5, $P<.05$).

Administration of kainate (10^{-4} mol/L) before H/I dilated cerebral arterioles from a baseline of 106±3 μm by 27±4%. One hour after H/I kainate induced a 21±3% dilation from a baseline of 110±6 μm ($P>.05$).

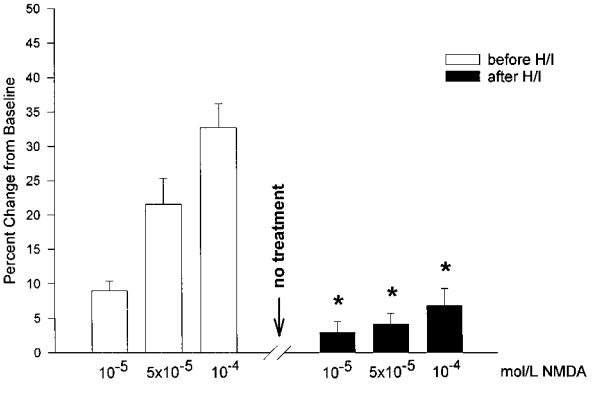

Figure 1. Effects of H/I on cerebral arteriolar dilation to NMDA. Cerebral arteriolar dilator responses to NMDA were significantly decreased 1 hour after 10 minutes of hypoxia plus 10 minutes of ischemia. Values are mean±SEM for 7 piglets. *Significantly different from preischemic dilation ($P<.05$).

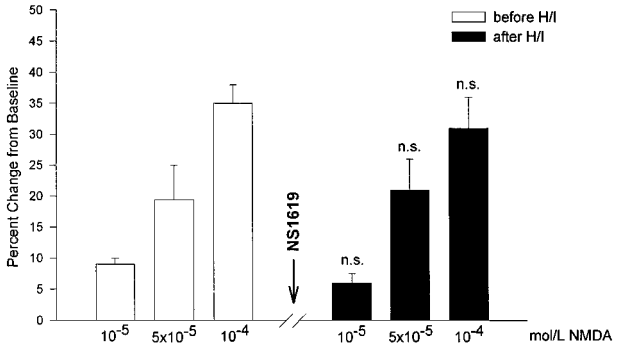

Figure 2. Effects of NS1619 pretreatment on cerebral arteriolar dilation to NMDA after H/I. Cerebral arteriolar responses to NMDA were preserved 1 hour after 10 minutes of hypoxia plus 10 minutes of ischemia by NS1619 (10^{-6} mol/L). Values are mean±SEM for 6 piglets. n.s. indicates not significantly different from preischemic dilation ($P>.05$).

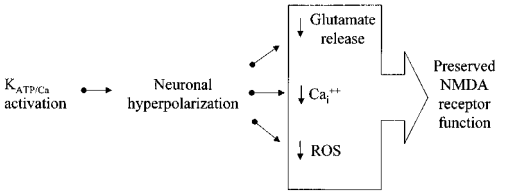

Figure 3. Potential mechanisms by which potassium channel activators may protect the NMDA-induced cerebral arteriolar dilation during and after H/I. Efflux of K^+ through activated K^+ channels leads to hyperpolarization of neurons. $K_{ATP/Ca}$ indicates ATP-dependent/calcium-dependent potassium channels; Ca_i^{++}, intracellular calcium concentration; and ROS, reactive oxygen species.

Discussion

There are three major new findings from these experiments: First, the vascular responsiveness of cerebral arterioles to NMDA, which is attenuated after consecutive H/I, was preserved by topical pretreatment with either NS1619 or aprikalim. Second, CGRP and papaverine did not preserve the vascular response to NMDA, although they caused dilation of the cerebral arterioles to an extent similar to that of the potassium channel agonists before H/I. Third, the kainate-induced arteriolar dilation was largely preserved after H/I.

Cerebral arterioles do not possess NMDA receptors.[17,18] Consequently, the NMDA-induced cerebral arteriolar dilation can only take place through an indirect pathway involving production, release, and action of NO. While the complete sequence of mechanisms involved is currently unknown, NMDA mainly causes cerebral arteriolar dilation through the sequential production of neuronal NO and vascular smooth muscle cGMP.[1–5,19]

In the present study the combined stress of H/I markedly attenuated the response of cerebral arterioles to NMDA as measured 1 hour after the insult. Previously, we have shown that a period of 5 to 15 minutes of either ischemia, asphyxia, or hypoxia is able to attenuate the NMDA-induced arteriolar dilation.[7,8,20] Administration of either NS1619 or aprikalim, putatively selective agonists of K_{Ca} channels[13,21,22] and K_{ATP} channels,[23–25] respectively, essentially preserved the response of cerebral arterioles to NMDA. The mechanism leading to this unprecedented finding is as yet unclear. NS1619 and aprikalim can dilate cerebral arterioles by the direct stimulation of potassium channels on vascular smooth muscle cells.[25–28] Therefore, hypothetically, the preservation of the NMDA-induced vascular response may take place as a consequence of vasodilation before ischemia. This hypothesis, however, is hard to reconcile with the observation that the similar dilation of cerebral arterioles to CGRP and papaverine before ischemia had little or no effect on the postischemic vascular response to NMDA.

Several different subtypes of potassium channels (eg, voltage-gated, ATP-dependent, calcium-dependent, inward rectifier potassium channels) have been identified on cells,[14] and all subtypes are present on neurons.[29] The interaction of NS1619 and aprikalim with their respective potassium channels on neurons therefore represents an alternative mechanism through which their preservative effect on the NMDA-mediated neuronal-vascular coupling may have taken place. Although the gating mechanism and conduction properties differ between subtypes, opening of potassium channels generally leads to hyperpolarization of excitable cells by increasing the efflux of potassium ions from the relatively negatively charged intracellular compartment into the extracellular space. Current concepts of H/I brain damage include the deleterious effects of excessive secretion of neurotoxic excitatory neurotransmitters[30] and the intracellular accumulation of calcium.[31] Theoretically, potassium channel openers may counteract these effects by hyperpolarizing presynaptic and postsynaptic neurons.[14,29] Potassium channel openers also may inhibit the release of calcium from intracellular stores.[14] This may lead to decreased glutamate secretion, slowing of the depolarization rate, diminished intracellular calcium accumulation, lower energy consumption, and reduced production of free oxygen radicals (Fig 3).

Experimental data supporting this attractive neuroprotective concept are sparse. Wind et al[32] found protection of cultured neurons against chemically induced hypoxia by bimakalim, a K_{ATP} activator. Suzuki and coworkers[33] showed that pretreatment with pinacidil, a partial K_{ATP} agonist, shortened the recovery time of spinal reflexes after spinal ischemia in cats. When glibenclamide, an inhibitor of K_{ATP}, was coadministered, the protective effect was absent. Similarly, in a study by Riepe et al,[34] glibenclamide partly reversed increased hypoxic tolerance after chemical inhibition of oxidative phosphorylation in hippocampal slices. Zini et al[15] reported that K^+ channel agonists blocked ischemia-induced glutamate release in rat hippocampal slices. Using a rat model of global ischemia, Heurteaux et al[16] demonstrated that several K_{ATP} openers administered intracerebroventricularly blocked the expression of immediate early genes in the hippocampus. The same and a similar study by these authors[16,35] also showed a decrease in delayed neuronal death in hippocampal CA1 neurons. We are not aware of other studies examining the

neuroprotective effect of K_{Ca} openers in cerebral hypoxia and ischemia.

The methods in the present study do not allow us to identify the precise molecular site at which NS1619 and aprikalim preserved the NMDA-mediated neuronal-vascular coupling after H/I. We speculate that both types of potassium channel openers preserved the NMDA-mediated response by indirectly protecting the NMDA receptor complex. Based on the results of previous work, we suggested that the affected step in the NMDA-vasodilating sequence may be the neuronal NMDA receptor rather than NO synthesis or the action of subsequent metabolites.[8,20] The present study partly supports this concept since the arterioles, which did not respond to NMDA after H/I, still dilated to sodium nitroprusside, an NO donor. Indeed, the postischemic dilation to sodium nitroprusside (25±2%) was of an extent similar to that of previous preischemic control measurements in the same experimental setup. Moreover, the partially (approximately 80%) preserved arteriolar response to kainate, which has been shown to be mediated equally by NO and prostaglandins[6] in this model, suggests that neurons carrying glutamate receptors maintain some signaling mechanisms including stimulus-dependent NO synthesis. However, we cannot rule out a compensatory role of prostaglandins in kainate-induced arteriolar dilation after H/I.

Holland et al[36] demonstrated a neuroprotective effect of intravenous CGRP in a rat model of focal ischemia. Since CGRP does not easily cross the blood-brain barrier, the authors speculated that the recorded increase in cerebral blood flow may account for their findings. In contrast, CGRP failed to preserve the NMDA-mediated neuronal-vascular coupling in our experiments, although it dilated cerebral arterioles before ischemia. CGRP exerts its vasodilating effect largely through activation of K_{ATP} on smooth muscle cells.[37] In contrast to direct activators of potassium channels (eg, aprikalim, NS1619), which are known to hyperpolarize neurons, the effect of CGRP on neurons is probably more complex and may depend on the neuronal circuit involved. CGRP receptors have been shown to be present in the porcine cortex,[38] but we are not aware of any study investigating the receptor density in certain cortical layers or even colocalization with NMDA receptor–positive neurons. To our knowledge the relationship of CGRP to neuronal potassium channels has never been studied. From our experiments we can only conclude that CGRP does not have a sufficient (if any) effect on neuronal K^+ channels to preserve the NMDA-mediated vasodilating sequence. The modest preservation of NMDA-induced dilation by papaverine may be due to effects on neuronal K^+ channels or to other yet undisclosed neuroprotective effects.

Even after the severe consecutive H/I stress, the vascular responses to kainate were only attenuated by approximately 20%. This confirms the remarkable resistance of the kainate-mediated neuronal-vascular sequence in general and of the kainate receptor in particular, which we reported recently.[6] It also illustrates the selectivity of damage caused by cerebral H/I affecting the response linked to one glutamate receptor subtype while in part sparing another.

We cautiously interpret our findings as indicative of a neuroprotective effect of potassium channel agonists on the postischemic function of neurons carrying the NMDA receptor. The immediate implications of this specific preservative effect for the overall protection of the brain against ischemia are currently unknown. We suggest, however, that the protective mechanisms of potassium channel openers are unlikely to be confined to the NMDA receptor but rather express their beneficial effect on another, more general pathophysiological mechanism in H/I.

The presented results may have clinical implications for focal ischemic stroke or cerebral global ischemia after heart arrest. Our experimental paradigm circumvents some of the problems associated with the systemic administration of central nervous system drugs.[39] In addition, postischemic treatment may encounter altered receptor interfaces favoring or disfavoring particular therapeutic strategies. In the present study the drugs were applied before H/I when receptors and signaling mechanisms were evidently intact. Our recent work has shown that postischemic vasodilation to K_{Ca} openers[40] but not to K_{ATP} agonists is resistant to ischemia.[11] Although the vasodilation induced by potassium channel openers is probably caused mainly by immediate interaction with potassium channels on vascular smooth muscle cells, the attenuated response to K_{ATP} agonists may indicate the vulnerability and transient nonresponsiveness of the targeted receptor and pathway. If this also involved neuronal K_{ATP} channels, they would become a less attractive target for neuroprotection than K_{Ca} channels for treatment started in the early postischemic period.

In summary, our findings show that topical pretreatment with the selective potassium channel openers NS1619 and aprikalim preserves the NMDA-mediated neuronal-vascular coupling after H/I in newborn piglets. This finding suggests considerable neuroprotective potential of these agents.

Acknowledgments

This study was supported by grants HL-30260, HL-46558, and HL-50587 from the National Institutes of Health and a grant from the Hungarian Ministry of Education (FKFP 0713/1997). We gratefully acknowledge that aprikalim was a gift from Rhone-Poulenc-Rohrer.

References

1. Busija DW, Leffler CW. Dilator effects of amino acid neurotransmitters on piglet pial arterioles. *Am J Physiol*. 1989;257:H1200–1203.
2. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature*. 1988;336:385–388.
3. Armstead WM, Mirro R, Zuckerman S, Busija DW, Leffler CW. The influence of opioids on local cerebral glucose utilization in newborn pig. *Brain Res*. 1992;571:97–102.
4. Faraci FM, Breese KR. Nitric oxide mediates vasodilation in response to activation of *N*-methyl-D-aspartate receptors in brain. *Circ Res*. 1993;72:476–480.
5. Faraci FM, Breese KR, Heistead DD. Responses of cerebral arterioles to kainate. *Stroke*. 1994;25:2080–2084.
6. Bari F, Louis TM, Busija DW. Kainate-induced cerebrovascular dilation is resistant to ischemia in piglets. *Stroke*. 1997;28:1272–1277.

7. Busija DW, Meng W. Altered cerebral vascular responsiveness to *N*-methyl-D-aspartate after asphyxia in piglets. *Am J Physiol*. 1993;265: H389–H394.
8. Busija DW, Meng W, Bari F, McGough PS, Errico RA, Tobin JR, Louis TM. Effects of ischemia on cerebrovascular responses to *N*-methyl-D-aspartate in piglets. *Am J Physiol*. 1996;270:H1225–1230.
9. Hoffman DJ, McGowan JE, Marro PJ, Mishra OP, Delivoria-Papadopoulos M. Hypoxia-induced modification of the *N*-methyl-D-aspartate receptor in the brain of the newborn piglet. *Neurosci Lett*. 1994;167:156–160.
10. Louis TM, Meng W, Bari F, Errico RA, Busija DW. Ischemia reduces CGRP-induced cerebral vascular dilation in piglets. *Stroke*. 1996;27: 134–139.
11. Bari F, Louis TM, Meng W, Busija DW. Global ischemia impairs ATP-sensitive K^+ channel function in cerebral arterioles in piglets. *Stroke*. 1996;27:1874–1881.
12. Hossmann KA. Periinfarct depolarizations. *Cerebrovasc Brain Metab Rev*. 1996;8:195–208.
13. Garcia ML, Hanner M, Knaus HG, Koch R, Schmalhofer W, Slaughter RS, Kaczorowski GJ. Pharmacology of potassium channels. *Adv Pharmacol*. 1997;39:425–471.
14. Lawson K. Potassium channel activation: a potential therapeutic approach? *Pharmacol Ther*. 1996;70:39–63.
15. Zini S, Roisin AAP, Armengaud C, Ben-Ari Y. Effect of potassium channel modulators on the release of glutamate induced by ischaemic-like conditions in rat hippocampal slices. *Neurosci Lett*. 1993;153:202–205.
16. Heurteaux C, Bertaina V, Widmann C, Ladzunski M. K^+ channel openers prevent global ischemia-induced expression of c-fos, c-jun, heat shock protein, and amyloid β-protein precursor genes and neuronal death in rat hippocampus. *Proc Natl Acad Sci U S A*. 1993;90:9431–9435.
17. Small D, Morley P, Lafferiere N, Mealing G, Poulter M, Durkin J, Stanimirovic D. Evidence that glutamate receptors are not expressed on rat or human cerebromicrovascular endothelial cells. *Stroke*. 1997;28:261. Abstract 152.
18. Beart PM, Sheehan KAM, Manallack DT. Absence of *N*-methyl-D-aspartate receptors on ovine cerebral microvessels. *J Cereb Blood Flow Metab*. 1988;8:879–882.
19. East SJ, Garthwaite J. NMDA receptor activation in rat hippocampus induces cyclic GMP formation through the L-arginine-nitric oxide pathway. *Neurosci Lett*. 1991;123:17–19.
20. Bari F, Errico RA, Louis TM, Busija DW. Differential effects of short-term hypoxia and hypercapnia on *N*-methyl-D-aspartate–induced cerebral vasodilatation in piglets. *Stroke*. 1996;27:1634–1640.
21. Olesen SP, Munch E, Moldt P, Drejer J. Selective activation of Ca^{2+}-dependent K^+ channels by novel benzimidazolone. *Eur J Pharmacol*. 1994;251:53–59.
22. Edwards G, Niederste-Hollenberg A, Schneider J, Noack T, Weston AH. Ion channel modulation by NS1619, the putative BKCa channel opener in vascular smooth muscle. *Br J Pharmacol*. 1994;113:1538–1547.
23. Aloup JC, Farge D, James C, Mondot S, Cavero I. 2-(3-Pyrridyl)-tetrahydrothiopyran-2-carbonthianmide derivatives and analogues: a novel family of potent potassium channel openers. *Drugs Future*. 1990; 15:1097–1108.
24. Richer J, Pratz J, Mulder P, Mondot S, Giudicelli JF, Cavero I. Cardiovascular and biological effects of K^+ channel openers, a class of drugs with vasorelexant and cardioprotective properties. *Life Sci*. 1990;47: 1693–1705.
25. Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*. 1989;245:177–180.
26. Quast U, Guillon JM, Cavero I. Cellular pharmacology of potassium channel openers in vascular smooth muscle. *Cardiovasc Res*. 1994;28: 805–810.
27. Faraci FM, Sobey CG. Potassium channels and the cerebral circulation. *Clin Exp Pharmacol Physiol*. 1996;23:1091–1095.
28. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol*. 1995;268:C799–C822.
29. Obrenovitch TP. Sodium and potassium channel modulators: their role in neuroprotection. *Int Rev Neurosci*. 1997;40:109–133.
30. Choi DW. Glutamate receptors and the induction of excitotoxic neuronal death. *Prog Brain Res*. 1994;100:47–51.
31. Kristian T, Siesjo BK. Calcium related damage in ischemia. *Life Sci*. 1996;59:357–367.
32. Wind T, Prehn JH, Peruche B, Krieglstein J. Activation of ATP-sensitive potassium channels decreases neuronal injury caused by chemical hypoxia. *Brain Res*. 1997;751:295–299.
33. Suzuki T, Sekikawa T, Nemoto T, Mriya H, Nakaya H. Effects of nicorandil on the recovery of reflex potentials after spinal cord ischaemia in cats. *Br J Pharmacol*. 1995;116:1815–1820.
34. Riepe MW, Esclaire F, Kaischke K, Schreiber S, Nakase H, Kempski O, Ludolph AC, Dirnagl U, Hugon J. Increased hypoxic tolerance by chemical inhibition of oxidative phosphorylation: 'chemical preconditioning.' *J Cereb Blood Flow Metab*. 1997;17:257–264.
35. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M. Essential role of adenosine, adenosine A1 receptors and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. *Proc Natl Acad Sci U S A*. 1995;92: 4666–4670.
36. Holland JP, Sydserff SGC, Taylor WAS, Bell BA. Calcitonin gene–related peptide reduces brain injury in a rat model of focal ischemia. *Stroke*. 1994;25:2055–2059.
37. Kitazono T, Heistad DD, Faraci FM. Role of ATP-sensitive K^+ channels in CGRP induced dilatation of basilar artery in vivo. *Am J Physiol*. 1993;265:H581–H585.
38. Wimalawansa SJ, El-Kholy AA. Comparative study of distribution and biochemical characterization of brain calcitonin gene-related peptide receptors in five different species. *Neuroscience*. 1993;54:513–519.
39. Pardridge WM. Drug delivery to the brain. *J Cereb Blood Flow Metab*. 1997;17:713–731.
40. Bari F, Louis TM, Busija DW. Calcium-activated K^+ channels in cerebral arterioles in piglets are resistant to ischemia. *J Cereb Blood Flow Metab*. 1997;17:1152–1156.

Editorial Comment

Acute episodes of cerebral ischemia followed by reperfusion produce reactive hyperemia[1] with subsequent reductions in cerebral blood flow,[2] disruption of the blood-brain barrier,[3] damage to cerebrovascular endothelium,[4] and impairment of NO synthase–dependent reactivity of cerebral arteries.[5] Mechanisms that contribute to impaired NO synthase–dependent reactivity of cerebral arteries after cerebral ischemia are unclear.

Recent evidence suggests that activators of K^+ channels may have potential therapeutic implications. It is possible that K^+ channel activators, by stimulating the efflux of potassium ions from cellular compartments, may counteract ischemia-induced depolarizations and thus protect the cerebral circulation. Glutamate is an important neurotransmitter in the brain and produces dilation of cerebral blood vessels, in part, through activation of NMDA receptors and synthesis/release of NO. Previous studies by this group[6] and others[7] have shown that cerebral H/I impairs or abolishes dilation of cerebral arterioles in response to NMDA. The purpose of the present study was to determine whether treatment with K^+ channel activators before cerebral H/I preserves NMDA-induced arteriolar dilation.

These investigators measured in vivo responses of piglet cerebral arterioles to topical application of NMDA before and after periods of cerebral H/I. The authors report impaired responses of cerebral arterioles to NMDA after H/I. Pretreatment with K^+ channel activators (aprikalim and NS1619), however, preserved arteriolar dilation to NMDA after cerebral H/I. Restoration in responses to NMDA after treatment with K^+ channel activators was specific since CGRP did not alter responses to NMDA.

Thus, it appears that activation of K^+ channels, presumably on neurons, accounts for preservation in cerebrovascular responses to NMDA after cerebral H/I. These findings suggest a potentially important therapeutic role for activation of K^+ channels after cerebral ischemia.

William G. Mayhan, PhD, *Guest Editor*
Department of Physiology and Biophysics
University of Nebraska Medical Center
Omaha, Nebraska

References

1. Gourley J, Heistad DD. Characteristics of reactive hyperemia in the cerebral circulation. *Am J Physiol*. 1984;246:H52–H58.
2. Levy DE, van Uitert RL, Pike CL. Delayed postischemic hypoperfusion: a potentially damaging consequence of stroke. *Neurology*. 1979;29: 1245–1252.
3. Sage JI, van Uitert RL, Duffy TE. Early changes in blood-brain barrier permeability to small molecules after transient cerebral ischemia. *Stroke*. 1984;15:46–50.
4. Paljarvi L, Rehncrona S, Soderfeldt B, Olsson Y, Kalimo H. Brain lactic acidosis and ischemic cell damage: quantitative ultrastructural changes in capillaries of rat cerebral cortex. *Acta Neuropathol (Berl)*. 1983;60: 232–240.
5. Mayhan WG, Amundsen SM, Faraci FM, Heistad DD. Responses of cerebral arteries after ischemia and reperfusion in cats. *Am J Physiol*. 1988;255:H879–H884.
6. Busija DW, Meng W, Bari F, McGough PS, Errico RA, Tobin JR, Louis TM. Effects of ischemia on cerebrovascular responses to *N*-methyl-D-aspartate in piglets. *Am J Physiol*. 1996;270:H1225–H1230.
7. Hoffman DJ, McGowan JE, Marro PJ, Mishra OP, Delivoria-Papadopoulos M. Hypoxia-induced modification of the *N*-methyl-D-aspartate receptor in the brain of the newborn piglet. *Neurosci Lett*. 1994; 167:156–160.