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Mitochondrial Potassium Channel Opener Diazoxide Preserves Neuronal-Vascular Function After Cerebral Ischemia in Newborn Pigs

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Background and Purpose—*N*-Methyl-D-aspartate (NMDA) elicits neuronally mediated cerebral arteriolar vasodilation that is reduced by ischemia/reperfusion (I/R). This sequence has been preserved by pretreatment with the ATP-sensitive potassium (K_{ATP}) channel opener aprikalim, although the mechanism was unclear. In the heart, mitochondrial K_{ATP} channels (mitoK_{ATP}) are involved in the ischemic preconditioning-like effect of K^+ channel openers. We determined whether the selective mitoK_{ATP} channel opener diazoxide preserves the vascular dilation to NMDA after I/R.

Methods—Pial arteriolar diameters were determined with the use of closed cranial window/intravital microscopy in anesthetized piglets. Vascular responses to NMDA were assessed before and 1 hour after 10 minutes of global cerebral ischemia induced by raising intracranial pressure. Subgroups received 1 of the following pretreatments before I/R: vehicle; 1 to 10 μmol/L diazoxide; and coapplication of 100 μmol/L 5-hydroxydecanoic acid (5-HD), a K_{ATP} antagonist with diazoxide.

Results—NMDA-induced dose-dependent pial arteriolar dilation was not affected by diazoxide treatment only but was severely attenuated by I/R. In contrast, diazoxide dose-dependently preserved the NMDA vascular response after I/R; at 10 μmol/L, diazoxide arteriolar responses were unaltered by I/R. The effect of diazoxide was antagonized by coapplication of 5-HD with diazoxide. Percent preservation of 100 μmol/L NMDA–induced vasodilation after I/R was 53±19% (mean±SEM, n=8) in vehicle-treated controls versus 55±10%, 85±5%, and 99±15% in animals pretreated with 1, 5, and 10 μmol/L diazoxide (n=8, n=8, and n=12, respectively) and 60±15% in the group treated with 5-HD+diazoxide (n=5).

Conclusions—The mitoK_{ATP} channel opener diazoxide in vivo preserves neuronal function after I/R, shown by pial arteriolar responses to NMDA, in a dose-dependent manner. Thus, activation of mitoK_{ATP} channels may play a role in mediating the protective effect of other K^+ channel openers. **(*Stroke*. 1999;30:2713-2719.)**

Key Words: cerebral ischemia, global ■ *N*-methyl-D-aspartate ■ potassium channels ■ reperfusion injury ■ pigs

Glutamate elicits cerebral arteriolar vasodilation in piglets via a multistep process, involving activation of neuronal *N*-methyl-D-aspartate (NMDA) receptors, stimulation of nitric oxide (NO) production by neuronal NO synthase, and actions of NO on vascular smooth muscle cells.[1–3] This sequence of events may represent an important mechanism coupling local blood flow to metabolism and neuronal activity.

NMDA-induced vasodilation is attenuated by hypoxia and ischemia/reperfusion (I/R) in a dose- and time-dependent manner.[4–6] For example, 10 minutes of global ischemia followed by reperfusion reduces NMDA-induced vasodilation by ≈50%. However, arteriolar dilator responses to exogenously applied NO are intact,[5,6] thereby implying that the attenuation of the vascular response to NMDA is due to

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effects of ischemia at the level of the neurons. Furthermore, results from other laboratories as well as our own indicate that dysfunction of the NMDA receptor rather than of general neuronal injury is the primary reason for attenuated arteriolar responsiveness to NMDA.[5,7] The mechanisms involved in attenuated arteriolar dilation to NMDA are not known with certainty but appear to involve actions of reactive oxygen species (ROS), such as superoxide anion. Thus, pharmacological agents that prevent production of superoxide anion or that scavenge this radical prevent attenuation of NMDA-induced dilator responses.[4,5,8]

In our laboratory, NMDA-induced vasodilation has been used as a sensitive bioassay to assess the functional integrity

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of the neuronal-vascular axis. For instance, we have shown that activation of ATP-sensitive potassium (K_{ATP}) channels with aprikalim for a short period immediately before combined hypoxic/ischemic stress preserves pial arteriolar dilation to NMDA.[6] Possible mechanisms of action of K_{ATP} activation may be via hyperpolarization of neurons through plasmolemmal K_{ATP} channels, which may result in (1) reduced glutamate release, (2) smaller increases in intracellular Ca^{2+} levels during ischemia, or possibly (3) less ROS production during reperfusion. However, intracellular sites of action of K^+ channel activators have not been considered previously.

Mitochondrial K_{ATP} (mitoK_{ATP}) channels have been found in the inner membrane of mitochondria[9] and represent a pharmacologically distinct population of K_{ATP} channels.[10] There is increasing evidence about the diverse functions of mitoK_{ATP} channels in the regulation of mitochondrial matrix volume, ATP production, and Ca^{2+} homeostasis in mitochondria, essential factors determining the outcome of ischemic stress on cellular function and survival.[11–14] In fact, several K^+ channel openers can mimic ischemic preconditioning (IPC) in the heart,[15] and mitoK_{ATP} channels are certainly involved in mediating these effects.[16–18] However, no study has investigated the possible beneficial role of mitoK_{ATP} channel activation in vivo in the brain and the cerebral circulation.

In this study our purpose was to determine whether diazoxide, a selective mitoK_{ATP} channel opener, would preserve the NMDA-induced arteriolar dilation 1 hour after 10 minutes of global cerebral ischemia. Additionally, we investigated whether 5-hydroxydecanoic acid (5-HD), a relatively selective inhibitor of mitoK_{ATP} channels, would reduce the effect of diazoxide.

Materials and Methods

Animals

Newborn piglets of either sex (age, 1 to 7 days; body weight, 1 to 2 kg) were used. All procedures were approved by the Institutional Animal Care and Use Committee. The animals were anesthetized with sodium thiopental (30 to 40 mg/kg IP) followed by injection of α-chloralose (75 mg/kg IV). Supplemental doses of α-chloralose were given to maintain a stable level of anesthesia. The right femoral artery and vein were catheterized to record blood pressure and to administer drugs and fluids, respectively. The piglets were intubated via tracheotomy and artificially ventilated with room air. The ventilation rate ($\approx$20/min) and tidal volume ($\approx$20 mL) were adjusted to maintain arterial blood gas values and pH in the physiological range. Body temperature was maintained at 37°C to 38°C by a water-circulating heating pad. Body temperature, arterial pH, and blood gases were also in the normal ranges and did not vary significantly among different groups. For instance, in group 5, the values were as follows: body temperature, 37.9$\pm$0.2°C; pH, 7.51$\pm$0.03; P_{CO_2}, 33.3$\pm$1.9 mm Hg; and P_{O_2}, 97$\pm$4 mm Hg.

The head of the piglet was fixed in a stereotaxic frame. The scalp was incised and removed along with the connective tissue over the calvaria. A circular (19 mm in diameter) craniotomy was made in the left parietal bone. The dura was cut and reflected over the skull. A stainless steel cranial window with 3 needle ports was placed into the craniotomy, sealed with bone wax, and cemented with cyanoacrylate ester (Super Glue) and dental acrylic.

The closed window was filled with artificial cerebrospinal fluid (aCSF) warmed to 37°C and equilibrated with 6% O_2 and 6.5% CO_2 in balance N_2 to give pH=7.33, P_{CO_2}=46 mm Hg, and P_{O_2}=43 mm Hg. The aCSF consisted of the following (mmol/L): NaCl 132, KCl 2.9, $CaCl_2$ 1.2, $MgCl_2$ 1.4, $NaHCO_3$ 24.6, urea 6.7, and glucose 3.7. Diameters of pial arterioles were measured with a microscope (Wild M36) equipped with a video camera (Panasonic) and a video micro scaler (IV-550, For-A-Co). After surgery, the cranial window was gently perfused with aCSF until a stable baseline was obtained. At the end of the experiments, the animals were killed while anesthetized with an intravenous bolus of KCl.

Cerebral Ischemia

To induce global cerebral ischemia, a 3-mm hole was made by an electric drill with a toothless bit, and the dura was exposed. A hollow brass bolt was inserted in the left frontal cranium rostral to the cranial window and secured in place with cyanoacrylate ester and dental acrylic. Cerebral ischemia was produced by infusion of aCSF to raise intracranial pressure above arterial pressure. Ischemia was verified by the cessation of blood flow in the observed vessels. Previously, we have shown using microspheres that cerebral blood flow is virtually zero in all brain areas examined during the ischemic period.[19] Venous blood was withdrawn as necessary to maintain mean arterial blood pressure near normal values. At the end of the ischemic period, the infusion tube was clamped, and the intracranial pressure returned to preischemic values. The heparinized blood was reinfused intravenously.

Experimental Design

After obtaining stable baseline arteriolar diameters, we examined the responses of cerebral arterioles to NMDA (10, 50, 100 μmol/L, except in group 7). NMDA and all other drugs were dissolved in aCSF and administered topically through the injectable ports of the cranial window onto the brain surface with single application. Arteriolar diameters were measured continuously for 5 to 7 minutes for each dose of NMDA. Then the window was flushed with aCSF, and the arteriolar diameters returned to baseline values. Instrumented piglets (n=49) were then divided into 7 groups, as follows.

Group 1 (n=4)

To assess whether diazoxide may have direct effect on NMDA-induced vasodilation, in the first group the animals were treated with 10 μmol/L diazoxide for 10 minutes but did not undergo ischemia. NMDA challenge was repeated 1 hour after treatment with diazoxide.

Group 2 (n=8)

To repeat our previous findings on attenuation of NMDA-induced vasodilation by I/R, in this group the piglets received vehicle (aCSF) and were exposed to 10 minutes of global cerebral ischemia followed by reperfusion. In all ischemia groups, NMDA-induced changes in pial arteriolar diameters were reexamined after the first hour of reperfusion. We have shown that attenuation of cerebral vasodilation to NMDA is greatest 1 hour after I/R (1 hour is also the shortest time after I/R at which the measurements are technically feasible).

Groups 3 to 5 (n=8, n=8, and n=12, Respectively)

To investigate the effect of diazoxide on preservation of NMDA-induced vasodilation, in these groups the piglets were pretreated with 1, 5, and 10 μmol/L diazoxide, respectively, for 10 minutes before the initiation of 10 minutes of global cerebral ischemia. The diazoxide was removed by flushing the window with aCSF just before the initiation of ischemia.

Group 6 (n=5)

To investigate the inhibitory effect of 5-HD on K_{ATP} channels activated by diazoxide, the piglets were pretreated with 100 μmol/L 5-HD for 5 minutes, followed by coapplication of 100 μmol/L 5-HD and 10 μmol/L diazoxide for 10 minutes before 10 minutes of ischemia. The diazoxide and 5-HD were removed by flushing the window with aCSF just before the initiation of ischemia.

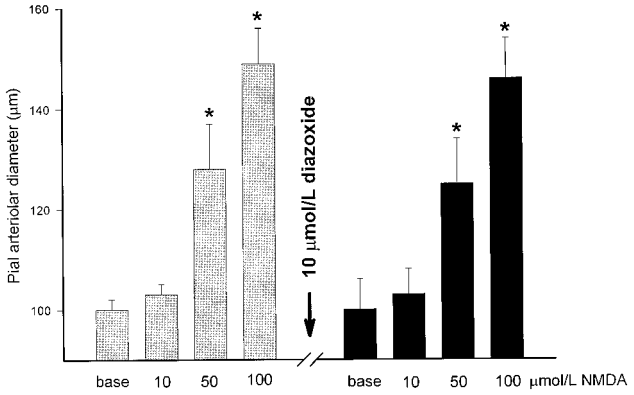

Figure 1. Effect of 10 μmol/L diazoxide on pial arteriolar responses to NMDA. NMDA induced dose-dependent vasodilation that was unaffected 1 hour after topical application of diazoxide for 10 minutes (n=4). *$P<0.05$, significantly different from corresponding baseline diameter (base).

Group 7 (n=4)

To study the effect of 5-HD on the sarcolemmal K_{ATP} channels and the vascular response to NMDA, we examined the cerebral arteriolar responses to the nonselective K_{ATP} channel opener aprikalim (10 μmol/L) followed by 100 μmol/L NMDA. Then we coapplied 10 μmol/L aprikalim and 100 μmol/L 5-HD for 10 minutes. We repeated the NMDA challenge 1 hour after pretreatment with 5-HD+aprikalim. Previously we have shown that aprikalim treatment does not affect the vascular response to NMDA.[6] Between each drug application we flushed the window several times with aCSF, until arteriolar diameters returned to baseline values.

Drugs

The drugs used in this study were NMDA (Sigma), diazoxide (Sigma), 5-HD (H135, Research Biochemicals International), and aprikalim (Rhone-Roulenc-Rohrer).

Statistical Analysis

Data are expressed as mean±SEM. Pial arteriolar diameter data were analyzed with repeated-measures ANOVA, followed by pairwise comparisons using the Student-Newman-Keuls test when appropriate. Percent preservations of preischemic vasodilation data were analyzed with 1-tailed *t* test. *P* values of <0.05 were considered statistically significant.

Results

Arterial blood pressure was in the normal range and was not significantly different before and 1 hour after ischemia; for instance, in group 5 arterial pressure was 70±4 mm Hg before and 68±4 mm Hg after I/R (n=12).

Topical application of diazoxide did not affect pial vascular diameters significantly. Typically, there was only a transient dilation immediately on application of diazoxide. Percent changes from baseline diameters were as follows: group 3, no vasoactivity was observed; group 4, 2±1%; and group 5, 9±3%. Vascular diameters quickly returned to baseline values in 2 to 3 minutes, and none of these changes were significantly different from baseline values.

NMDA elicited dose-dependent pial arteriolar vasodilation (Figures 1 and 2). In group 1, 10 μmol/L diazoxide did not potentiate or attenuate vascular dilations to NMDA 1 hour after diazoxide treatment (Figure 1). Baseline arteriolar diameters were 100±2 μm before and 100±6 μm 1 hour after diazoxide treatment. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after diazoxide treatment) were 3±1% versus 4±1%, 28±7% versus 26±9%, and 50±8% versus 47±8%, respectively.

Global cerebral ischemia (10 minutes) followed by reperfusion significantly reduced pial arteriolar responses to NMDA (Figure 2). In group 2, baseline arteriolar diameters were 100±3 μm before and 103±4 μm 1 hour after ischemia. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after ischemia) were 6±2% versus 2±1%, 28±5% versus 9±3%, and 38±5% versus 16±4%, respectively. Thus, vascular dilations to 100 μmol/L NMDA were diminished by ≈50% (Figure 3).

Diazoxide exhibited a dose-dependent effect on preservation of NMDA-induced vasodilation after I/R. In group 3, decreases in pial arterial responsiveness to NMDA were similar to those observed in group 2 (Figures 2 and 3). In group 3, baseline arteriolar diameters were 102±3 μm before and 104±3 μm 1 hour after ischemia. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after ischemia) were 5±2% versus 3±1%, 20±7% versus 8±2%, and 38±5% versus 19±3%, respectively. In contrast, in groups 4 and 5 we found a dose-dependent preservation of pial vascular responses to NMDA (Figures 2 and 3). More specifically, in group 4, baseline arteriolar diameters were 95±3 μm before and 95±4 μm 1 hour after ischemia. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after ischemia) were 4±0% versus 7±2%, 30±10% versus 23±6%, and 45±6% versus 37±3%, respectively. In group 5, baseline arteriolar diameters were 102±6 μm before and 106±5 μm 1 hour after ischemia. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after ischemia) were 7±1% versus 6±2%, 28±5% versus 24±4%, and 36±5% versus 32±4%, respectively. Therefore, pretreatment with 10 μmol/L diazoxide resulted in virtually full preservation of pial arteriolar responses to NMDA 1 hour after I/R compared with preischemic values.

Topical application of the K_{ATP} channel antagonist 5-HD and coapplication of 5-HD with diazoxide did not alter pial arteriolar diameters. In addition, 5-HD treatment did not affect pial arteriolar responses to NMDA. In group 7, baseline arteriolar diameters were 105±9 μm before and 103±7 μm 1 hour after pretreatment with 5-HD. Percent changes in pial arteriolar diameter from baseline to 100 μmol/L NMDA (before versus 1 hour after 5-HD treatment) were 52±3% versus 56±7%. However, pretreatment with 5-HD and diazoxide abolished the protection on NMDA-induced vasodilation achieved by diazoxide alone (Figures 2 and 3). In group 6, baseline arteriolar diameters were 90±6 μm before and 92±6 μm 1 hour after ischemia. Percent changes in pial arteriolar diameter from baseline to 10, 50, and 100 μmol/L NMDA (before versus 1 hour after ischemia) were 3±1% versus 0±0%, 40±12% versus 19±6%, and 61±7% versus 33±5%, respectively. Interestingly, coapplication of 5-HD with aprikalim did not block the vasodilation elicited by aprikalim. In group 7, pial baseline arteriolar diameters were

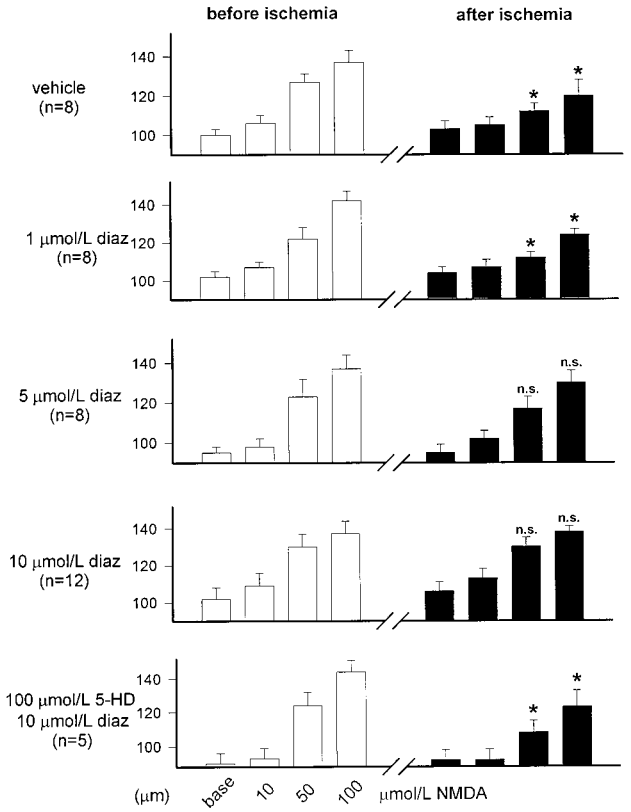

Figure 2. Changes in pial arteriolar diameters in response to NMDA 1 hour after 10 minutes of cerebral ischemia. Baseline diameters (base) did not change significantly in any groups after I/R. However, in the nontreated animals arteriolar responses to 50 and 100 μmol/L NMDA were severely reduced by ≈50%. Pretreatment with 1 μmol/L diazoxide (diaz) did not affect the reduction in NMDA-induced vascular dilation by I/R. In contrast, pretreatment with 5 or 10 μmol/L diazoxide resulted in preserved vascular responses; the changes in pial arteriolar diameters were not significantly different compared with preischemic values. Coapplication of 100 μmol/L 5-HD, a relatively specific inhibitor of mitoK$_{ATP}$ channels with 10 μmol/L diazoxide attenuated the protective effect of diazoxide. *$P<0.05$, significantly different from corresponding preischemic value.

102 ± 8 μm before application of aprikalim alone and 101 ± 7 μm before coapplication of aprikalim and 5-HD. Percent changes in pial arteriolar diameter from baseline to 10 μmol/L aprikalim were $65\pm6\%$ versus $65\pm6\%$ (aprikalim alone versus aprikalim+5-HD, respectively).

Discussion

The major finding of the present study is that the selective mitoK$_{ATP}$ channel opener diazoxide dose-dependently preserves NMDA-induced cerebral arteriolar vasodilation after I/R in piglets. Since NMDA-induced vasodilation is dependent on intact neuronal function, we present evidence for the first time showing an in vivo protective effect of diazoxide after I/R in the central nervous system.

Previously, we found that the nonselective K$_{ATP}$ channel opener aprikalim protected NMDA-induced vasodilation after combined hypoxia-ischemia.[6] The protective effect of aprikalim was shown to be mediated by neuronal rather than vascular K$_{ATP}$ channels and was independent of the vasodilation elicited by aprikalim. Our present data confirm that the protective effect of pretreatment with K^+ channel openers is independent of vasodilation accompanied by the administration of such drugs: diazoxide showed no significant vasoactivity but preserved NMDA-induced dilation. The beneficial effects of K$_{ATP}$ channel openers reducing injury by I/R have been most extensively studied in the heart. K$_{ATP}$ channels serve as the final common pathway in the event of IPC, a phenomenon in which short periods of ischemia protect the heart from subsequent exposure of a more prolonged period of ischemia. K^+ channel openers mimic IPC,[15–18] and the protection by IPC is blocked by K$_{ATP}$ channel inhibitors.[20–22] The exact mechanism of this remarkable effect has not been elucidated.

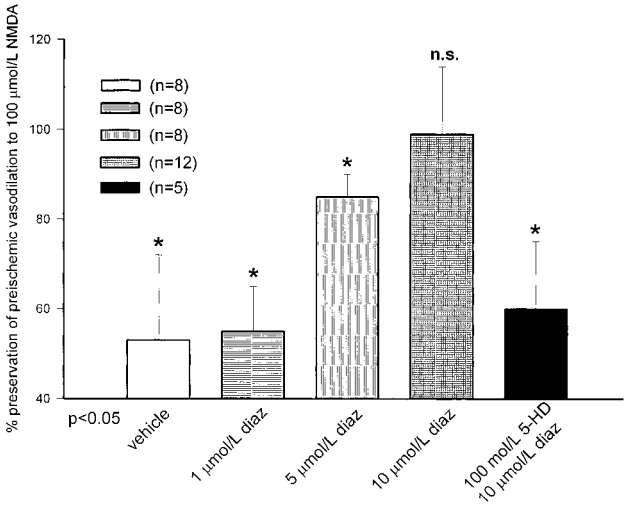

Figure 3. Protective effect of diazoxide (diaz) on 100 μmol/L NMDA–induced pial arteriolar dilation. Data are expressed as percent preservation of dilation compared with preischemic values. Note the dose-dependent preservation of vascular responses in the diazoxide-treated groups; at 10 μmol/L diazoxide the vascular response was virtually identical to preischemic value. 5-HD antagonized the effect of diazoxide. *Significantly different from preischemic value.

The discovery of mitoK$_{ATP}$ channels added further complexity to the interpretation of experimental data from pharmacological interventions on these channels. Unfortunately, there are no absolutely selective pharmacological tools to assess the mitoK$_{ATP}$ channels in vivo. However, a consistent and unique feature of these channels is their remarkably selective sensitivity to opening by diazoxide. The mitoK$_{ATP}$ channel was found to be >2000-fold more sensitive to diazoxide than the sarcolemmal K$_{ATP}$ channel in bovine cardiac myocytes ($K_{1/2}$ was 0.4 μmol/L for mitoK$_{ATP}$ channel versus 855 μmol/L for sarcolemmal K$_{ATP}$ channel). In contrast, cromakalim was an equally potent opener of both mitochondrial and plasma membranes.[10] Subsequently, mitoK$_{ATP}$ channel selective concentrations (5 to 20 μmol/L) of diazoxide have been demonstrated to improve functional recovery in isolated rat hearts after I/R in a manner similar to that of a nonselective K$_{ATP}$ channel opener, cromakalim. The cardioprotection by diazoxide was inhibited by K$_{ATP}$ channel inhibitors glibenclamide and 5-HD, confirming the effect of diazoxide via K$_{ATP}$ channels.[17] In a different study, in intact

rabbit ventricular myocytes, diazoxide induced mitochondrial depolarization, demonstrated by flavoprotein fluorescence with a $K_{1/2}$ of 27 μmol/L, but did not affect the simultaneously measured sarcolemmal K_{ATP} channel current.[16] These findings and others in the literature (for recent review, see Reference 15) strongly indicate the involvement of mitoK_{ATP} channels in the development of acute and perhaps delayed IPC in the heart.

In our present experiments we used topical diazoxide (1 to 10 μmol/L) in the mitoK_{ATP} channel–selective dose range. We did not test directly whether only mitoK_{ATP} channels were activated by diazoxide, but fortunately a good indication of selective activation was the absence of significant vasodilation accompanied by application of diazoxide. The vasodilatory effect of K^+ channel openers on cerebral arterioles was directly mediated by the sarcolemmal K^+ channels. Administration of 5 to 10 μmol/L diazoxide elicited only 2% to 9% arteriolar dilation, and the response was transient, ie, it did not last for >1 to 2 minutes. In contrast, we found that the nonselective K_{ATP} channel opener aprikalim (10 μmol/L) elicits ≈60% to 70% increases in vascular diameters, and the vasodilation does not wane. Moreover, the dose-dependent effect of diazoxide on preservation of the NMDA-induced vasodilation after I/R was inhibited by the selective K_{ATP} channel antagonist 5-HD, and 5-HD was found to be selective for mitoK_{ATP} channels, at least in some experimental designs.[16,17,23] Additionally, in our experimental model 5-HD did not inhibit the vasodilation induced by aprikalim, suggesting minor effects on plasmolemmal K_{ATP} channel channels. These observations, together with those of the literature, lead us to conclude that the protective effect of diazoxide on neuronal-vascular function after I/R is probably mediated by activation of mitoK_{ATP} channels.

The mechanism by which activation of mitoK_{ATP} channels may lead to increased resistance to I/R remains to be clarified. In our experimental model, NMDA-induced vascular response is severely attenuated at 1 hour after I/R, and responsiveness gradually returns over the time course of 2 to 4 hours.[5,24] The duration of global cerebral ischemia (10 minutes) used in the present study has been thought to cause only reversible mitochondrial alterations, ie, mitochondria have been shown to recover full function 1 to 2 hours after reperfusion.[25,26] Thus, the attenuation of the NMDA-mediated cerebral arteriolar response is not likely due to energy failure by inhibited mitochondrial function. This statement is further supported by our previous findings that kainate-induced vasodilation is resistant to ischemia in the same experimental model.[27] Additionally, neuronal NO synthase levels and activity are unchanged by I/R, and cerebral arterioles show normal responses to exogenous NO donors such as sodium nitroprusside after ischemia.[5,6] Therefore, the primary target of I/R may be the NMDA receptor itself. The acute effect of ischemia on NMDA-induced pial arteriolar vasodilation has been amply demonstrated to be mediated by ROS (Figure 4). Thus, NMDA-induced vascular response has been found to be preserved by ROS scavengers and inhibitors of cyclooxygenase (COX) activity,[4,5,8,24] a major source of ROS after I/R.[28] Our recent observations on the preservation of neural function with K^+ channel openers after hypoxia-ischemia were somewhat at odds with the general scheme of the pathological mechanism of the effect of I/R on NMDA-induced neuronal-vascular sequence. However, our present results may link the beneficial effect of K^+ channel openers on preservation of NMDA-induced vasodilation to reducing oxidative stress on the neurons involved in this response. We speculate that activation of mitoK_{ATP} channels by K^+ channel openers may reduce mitochondrial ROS production.

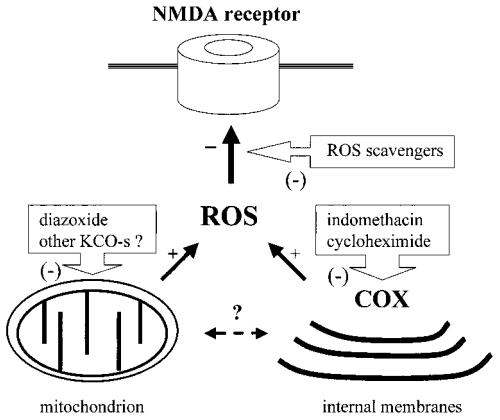

Figure 4. Preservation of NMDA-induced cerebral vasodilation after I/R. The most sensitive component of this neuronal-vascular sequence to I/R may be the NMDA receptor itself. ROS produced by mitochondria and COX seem to play a pivotal role in attenuating the NMDA-induced vasodilation. Thus, pretreatment with ROS scavengers, the COX inhibitor indomethacin, or the protein (including COX) synthesis inhibitor cycloheximide shortly before I/R results in preserved vascular responsiveness to NMDA. NMDA-induced dilation is also preserved by pretreatment with diazoxide, a selective mitoK_{ATP} channel opener, and other K^+ channel openers (KCO-s). Activation of mitoK_{ATP} channels may reduce generation of ROS in reperfusion. Reduction in either COX-derived or mitochondrial ROS production may be sufficient to preserve the vascular response to NMDA after I/R. However, a potentiating interaction between these sources of ROS is conceivable but unknown.

Currently, the physiological role of mitoK_{ATP} channels is still debated and mostly speculative. Briefly, mitoK_{ATP} channels seem to control the activity of the electron transport chain via regulating mitochondrial matrix volume by regulated K^+ uptake. The physiological patterns of activation and inhibition of these channels are largely unknown, but ironically the physiological role of ATP as a regulator is unlikely.[29] In isolated mitochondria, K^+ channel openers induce slight swelling, partially dissipate the transmembrane potential ($\Delta\Psi$, negative inside), but increase the activity of electron transport chain and hence the chemical proton gradient (ΔpH, alkaline inside); thus, the total protonmotive hardly changes.[10–14] However, the activity of numerous important transport mechanisms depends on either $\Delta\Psi$ or ΔpH. One such possibly crucial "metabolite" may be Ca^{2+}. Mitochondria readily uptake Ca^{2+} when intracellular levels increase above a so-called mitochondrial buffer concentration. Ca^{2+} is transported through the mitochondrial inner membrane via the electrogenic Ca^{2+} uniporter down its electrochemical gradient, and thus the rate of this transport is dependent on $\Delta\Psi$.[30] Mitochondrial Ca^{2+} overload substantially influences the recovery of mitochondrial function after ischemic stress: for example, increased mitochondrial Ca^{2+} sequestration has

been demonstrated to increase production of ROS.[31,32] Opening of mitoK$_{ATP}$ channels should decrease mitochondrial Ca^{2+} uptake by decreasing $\Delta\Psi$, and in fact K^+ channel openers induce release of Ca^{2+} from Ca^{2+}-preloaded mitochondria in vitro.[11]

In summary, we conclude that diazoxide in a mitoK$_{ATP}$ channel–selective range dose-dependently preserves neuronal function demonstrated by NMDA-induced arteriolar dilation after I/R. This acute effect of mitoK$_{ATP}$ channel openers may be mediated by decreasing mitochondrial ROS production in the immediate reperfusion. This effect may be important in the protective effect of other nonspecific K^+ channel openers as well. Our findings may offer the development of new therapies to reduce neuronal injury after global hypoxic-ischemic stress in the newborn.

Acknowledgments

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Editorial Comment

The protection of tissues and organs, after I/R and other pathological conditions, is an extremely important but often disappointing area of investigation. Such protection is of particular importance in heart and brain, vital organs that are most vulnerable to I/R. Interestingly, a brief activation of K$_{ATP}$ before an ischemic event has been reported to provide a reasonable degree of tissue protection.[1] Although the mechanism behind this protective effect of K$_{ATP}$ stimulation was

not known, a reasonable hypothesis stated that protection occurred through hyperpolarizing the plasma membrane. Hyperpolarization would reduce the probability and/or the duration of an action potential. Protection would be afforded in 2 ways. First, the energy requirement would be reduced at a time when energy supply in the form of ATP is likely to be compromised. Second, a decrease in the number and/or duration of action potentials would reduce influx of Ca^{+2} into the cell and help to prevent intracellular Ca^{+2} concentrations from reaching toxic levels.

However, several studies in heart demonstrated that the abbreviation of action potentials could be dissociated from protection conferred by K_{ATP} channel openers.[2–6] With this knowledge in mind and the discovery of mitochondrial K_{ATP} channels, attention was turned away from the sarcolemma to K_{ATP} channels in the mitochondria. Indeed, a number of studies in heart strongly support the hypothesis that K_{ATP} openers confer protection through K_{ATP} channels located not on the sarcolemma membrane but on the inner mitochondrial membrane.[2,4]

Would the same hold true for cerebral protection after I/R? Could this be demonstrated in vivo? Domoki and colleagues asked these questions in the study in the accompanying article. Convincingly, these authors showed that prior stimulation of mitochondrial K_{ATP} channels, not plasma membrane K_{ATP} channels, are involved with cerebral protection (as measured by preservation of dilator responses to NMDA) after I/R in the newborn pig. In their studies the authors reported that the selective mitochondrial K_{ATP} opener diazoxide restored dilator responses to NMDA that had been diminished by I/R. Furthermore, the selective mitochondrial K_{ATP} blocker 5-HD antagonized the protective effects of diazoxide. Although selectivity of these 2 agents had been demonstrated in heart,[1] there was no guarantee that this selectivity automatically transferred to brain. In a series of cleverly designed studies, Domoki et al demonstrated that diazoxide produced only minor and transient dilations (5% dilation that lasted 2 to 3 minutes) of the pial arteries, whereas stimulation of K_{ATP} channels in the plasma membrane (by aprikalim) produced large (65% dilation) and sustained dilations. Hence, diazoxide had practically no effect on dilation and thus plasma membrane K_{ATP} channels. Second, 5-HD had no effect on dilations elicited by opening K_{ATP} channels in the plasma membrane with aprikalim. If 5-HD was not blocking K_{ATP} channels in the plasma membrane, then it was reasonable to conclude that it was selectively blocking K_{ATP} channels in the mitochondria.

This study by Domoki and colleagues has 2 important implications. First, it demonstrates the importance of mitochondria in the developing pathophysiology after I/R. Second, this study provides a potentially important therapy for treatment of stroke in humans. Cerebral protection by openers of mitochondrial K_{ATP} channels and the study of mitochondria in the pathophysiology of cerebral insults promise to be important frontiers for future research.

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