Role of Endothelial Nitric Oxide and Smooth Muscle Potassium Channels in Cerebral Arteriolar Dilation in Response to Acidosis

Tetsuyoshi Horiuchi, MD; Hans H. Dietrich, PhD; Kazuhiro Hongo, MD; Tetsuya Goto, MD; Ralph G. Dacey, Jr, MD

**Background and Purpose**—Potassium channels or nitric oxide or both are major mediators of acidosis-induced dilation in the cerebral circulation. However, these contributions depend on a variety of factors such as species and vessel location. The present study was designed to clarify whether potassium channels and endothelial nitric oxide are involved in acidosis-induced dilation of isolated rat cerebral arterioles.

**Methods**—Cerebral arterioles were cannulated and monitored with an inverted microscope. Acidosis (pH 6.8 to 7.4) produced by adding hydrogen ions mediated dilation of the cerebral arterioles in a concentration-dependent manner. The role of nitric oxide and potassium channels in response to acidosis was examined with several specific inhibitors and endothelial damage.

**Results**—The dilation was significantly inhibited by potassium chloride (30 mmol/L) and glibenclamide (3 μmol/L; ATP-sensitive potassium channel inhibitor). We found that 30 μmol/L BaCl₂ (concentration-dependent potassium channel inhibitor) also affected the dilation; however, an additional treatment of 3 μmol/L glibenclamide did not produce further inhibition. Tetraethylammonium ion (1 mmol/L; calcium-activated potassium channel inhibitor) and 4-aminopyridine (100 μmol/L; voltage-dependent potassium channel inhibitor) as well as ouabain (10 μmol/L; Na-K ATPase inhibitor) and N-methylsulphonyl-6-(2-proparglyoxyphenyl) hexanamide (1 μmol/L; cytochrome P450 epoxygenase inhibitor) did not alter acidic dilation. Nω-Monomethyl-L-arginine (10 μmol/L) and Nω-nitro-L-arginine (10 μmol/L) as nitric oxide synthase inhibitor blunted the dilation. Furthermore, the dilation was significantly attenuated after the endothelial impairment. Additional treatment with glibenclamide (3 μmol/L) further reduced the dilation in response to acidosis.

**Conclusions**—Endothelial nitric oxide and smooth muscle ATP-sensitive potassium channels contribute to acidosis-induced dilation of rat cerebral arterioles. Endothelial damage caused by pathological conditions such as subarachnoid hemorrhage or traumatic brain injury may contribute to reduced blood flow despite injury-induced cerebral acidosis. 

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**Key Words:** acid-base equilibrium ■ cerebral circulation ■ hydrogen-ion concentration ■ microcirculation ■ rats

It is well known that hypercapnic and normocapnic acidosis cause vasodilation in cerebral blood vessels.1–4 Decrease in the extracellular pH plays an important role in acidosis-induced dilation.5,6 As such, the hydrogen-ion concentration is one of the regulators of this response in the cerebral microcirculation.3,4

In the cerebral circulation, neuronal and/or endothelial nitric oxide (NO) is known as the regulator of acidosis-induced dilation.7–10 Recently, Lindauer et al11 found that in isolated rat middle cerebral artery, denudation did not alter the vessel response to acidosis, favoring neuronal but not endothelial NO as a modulator of pH-dependent vasoactivity in this preparation. In addition, ATP-sensitive potassium (K_{ATP}) channels in vascular smooth muscle have received attention as major contributors to acidosis-induced dilation in pial vessels and basilar artery.7,9

While there are numerous reports studying pH-dependent vasomotor mechanisms in cerebral arteries and pial vessels,1,2,5,7 only few studies concerning such mechanisms in cerebral arterioles exist. These arterioles are in close contact with the supplied tissue and are strong regulators of cerebrovascular tone, representing approximately 23% of the total arterial cerebrovascular resistance.12 Previously, our laboratory reported that rat isolated cerebral arterioles were very sensitive to the extracellular acidosis, produced by adding hydrogen ions, that caused dilation and hyperpolarization of

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vascular smooth muscle cell. Furthermore, we also speculated that endothelial NO may be involved in part in acidosis-mediated dilation. However, the contribution of the endothelium or potassium channels to acidic vasodilatation in these microvessels is not known. With the use of specific inhibitors for various potassium channel types, NO synthase (NOS), cyclooxygenase, cytochrome P450 epoxygenase, Na-K ATPase, and endothelial damage, the present study was designed to determine the mechanisms and cell type involved in acidosis-induced dilation in isolated rat cerebral arterioles. Implications of our findings on the physiology and pathophysiology of cerebrovascular regulation are discussed.

**Materials and Methods**

**Animal Preparation**

All animal experimentation was approved by the Institutional Animal Care and Use Committee. Sprague-Dawley rats (weight, 450-628; n = 48; Harlan, Indianapolis, Ind) were anesthetized with injection of pentobarbital sodium (65 mg/kg IP) and killed. The brain was carefully removed and placed in a cold (4°C) dissection chamber, filled with 3-(N-morpholino)propanesulfonic acid (MOPS)-buffered saline (see below for composition) containing 1% diazyl bovine serum albumin.

**Isolation and Cannulation of Arterioles**

The technique for isolation of cerebral penetrating arteriole was previously described in detail. Briefly, unbranched penetrating arterioles were obtained from the middle cerebral artery and transferred to an organ bath. The arteriole was cannulated at one end and occluded at the other end with microproppets. The internal diameter of arteriole was continuously monitored under an inverted microscope (Daphot, Nikon) coupled to a charge-coupled device camera (Dage-MTI). Each arteriole was equilibrated with room air. The organ bath was heated from room temperature to 37.5°C and perfused with a peristaltic pump at a rate of 0.5 mL/min. Within 30 minutes, the arteriole developed spontaneous tone with a pH of 7.4. We discarded vessels with poor tone (<20% decrease from the maximum diameter) for further studies.

**Chemicals**

MOPS-buffered saline was used with the following composition (in mmol/L): NaCl 144, KCl 3.0, CaCl2 2.5, MgSO4 1.4, pyruvate 2.0, glucose 5.0, EDTA 0.02, NaH2PO4 1.21, and MOPS 2.0. The following saline (30 mmol/L) was used to negate any effects of K+ channels, 100 μmol/L 4-AP to inhibit voltage-dependent K+ (Kv) channels, and 30 μmol/L BaCl2 specific for inward rectifier K+ (Kir) channels. BaCl2 is a dose-dependent potassium channel inhibitor, and we used concentrations established previously.

Either 10 μmol/L L-NMMA (NOS inhibitor) or 10 μmol/L indomethacin (a cyclooxygenase inhibitor) was administered extraluminally to test whether NO or prostaglandins contribute to the acidosis-induced dilation, respectively.

The effects of functional disruption of endothelium on the acidosis-induced responses were investigated. After a control dilation in response to acidosis was obtained, the endothelium was impaired by passing air through the lumen of the arteriole at 60 mm Hg. This method has been previously reported in detail. To ensure the function of the arteriolar smooth muscle, we obtained a dose-dependent dilation of the arterioles in response to sodium nitroprusside (SNP) (0.1 mmol/L to 10 μmol/L) before and after air emboli. Extraluminal application of acetylcholine does not produce dilation of this preparation; we therefore confirmed endothelial but not neuronal NO played an important role in regulation of our preparations.

We also examined the effect of MS-PPOH (1 μmol/L) and ouabain (10 μmol/L). MS-PPOH and ouabain were used as inhibitors of cytochrome P450 epoxygenase and Na-K ATPase, respectively.

In a separate series of experiments, we studied the effect of 10 μmol/L L-NNA (NOS inhibitor) with or without 3 μmol/L glibenclamide on acidosis-induced dilation. Each antagonist was incubated for at least 20 minutes.

**Statistical Analysis**

Only 1 vessel was studied from 1 rat brain. All data are presented as mean±SEM. The n denotes the number of the vessels used in this study. For concentration-response curves, the results are presented as percentage of the maximum diameter of the arteriole and calculated by the following equation: % maximum diameter = [(Dmax−Dmin)/(Dmax−Dbaseline)]×100, where Dmax, Dbaseline, and Dmin are the maximum diameter of the vessel at 60 mm Hg, the control diameter of the vessel before introducing acidosis, and the diameter of the vessel after dilation, respectively. Dbase is the diameter after application of transmural pressure of 60 mm Hg at room temperature and before development of the spontaneous tone, which is comparable to maximal dilation induced by papaverine. Significant differences (P<0.05) were determined by repeated-measures ANOVA with a post hoc Student-Newman-Keuls test and paired Student’s t test, as appropriate.

**Results**

The passive maximum arteriolar diameter (the maximum diameter) was 64.7±1.6 μm (n=48). All vessels developed spontaneous tone, constricting to an average diameter of 45.7±1.4 μm (−29.4±1.2% of the maximum diameter).

**Effect of High Potassium and Potassium Channel Inhibitors**

Extraluminal application of 30 mmol/L KCl significantly decreased the arteriolar diameter (Table 1). High-K+ solution significantly attenuated but did not abolish the dilation in response to acidosis (n=5; Figure 1). BaCl2 (30 μmol/L) and 4-AP (100 μmol/L) constricted the vessel (Table 1), while neither TEA (1 mmol/L) nor glibenclamide (3 μmol/L) affected the control diameter (Table 1). The acidosis-induced dilation was inhibited by either 3 μmol/L glibenclamide (n=5; Figure 2) or 30 μmol/L BaCl2 (n=4; Figure 3). Treatment with 3 μmol/L glibenclamide in addition to 30 μmol/L BaCl2 did not produce further reduction of dilation (n=4; Figure 3). In contrast, neither 1 mmol/L TEA (n=4;
Table 2) nor 100 μmol/L 4-AP (n=4; Table 2) altered the dilation.

Effect of L-NMMA and Indomethacin
Indomethacin (10 μmol/L) did not affect the control diameter and the dilation in response to acidosis (n=4; Table 2). Extraluminally applied L-NMMA (10 μmol/L) caused a significant constriction (Table 1) and attenuated the acidosis-evoked dilation (n=5; Figure 4).

Effect of Endothelial Impairment
The air emboli significantly constricted the vessel (Table 1). Functional endothelial impairment blunted the dilation in response to acidosis (n=5; Figure 5). Administration of glibenclamide (3 μmol/L) after air emboli induced further inhibition of the dilation (n=5; Figure 5). SNP-induced dilations of cerebral arterioles were not different before and after air embolus (n=5, Figure 6). This result shows that smooth muscle viability did not change before and after air emboli.

Effect of MS-PPOH and Ouabain
MS-PPOH (n=4) and ouabain (n=3) contributed to neither the control diameter nor acidosis-induced dilation (Table 2). Ouabain itself constricted the vessel transiently, but the vessel diameter returned to control within 15 minutes.

Effect of L-NNA With Glibenclamide
L-NNA (10 μmol/L) constricted the vessels (Table 1) and inhibited the dilation in response to acidosis (n=5; Table 3).

Figure 1. Effects of 30 mmol/L KCl (closed circles) on acidosis-induced dilation (n=5). *Significant differences (P<0.05) from control (open circles).

Figure 2. Effects of 3 μmol/L glibenclamide (closed circles) on acidosis-induced dilation (n=5). *Significant differences (P<0.05) from control (open circles).

Figure 3. Effects of 30 μmol/L BaCl2 (n=4; closed circles) and 30 μmol/L BaCl2 + 3 μmol/L glibenclamide (n=4; open triangles) on acidosis-induced dilation. *Significant differences (P<0.05) from control (open circles).

Figure 4. Effects of 10 μmol/L L-NMMA (n=5; closed circles) on acidosis-induced dilation. *Significant differences (P<0.05) from control (open circles).
Conclude that in cerebral arterioles, dilation in response to acidosis, which was further decreased by glibenclamide. We indomethacin, MS-PPOH, and ouabain. (2) Endothelial impairment (open circles); *Significant differences (P<0.05) from control (open circles); †significant differences (P<0.05) from endothelial impairment.

Additional treatment with glibenclamide (3 μmol/L) produced further impairment of dilation without change of basal diameter (n=5; Table 3).

Discussion
The results of the present study indicate the following: (1) The dilation in response to acidosis is attenuated by glibenclamide, BaCl2, and L-NMMA but not by TEA, 4-AP, indomethacin, MS-PPOH, and ouabain. (2) Endothelial impairment and L-NNA attenuated the dilation in response to acidosis, which was further decreased by glibenclamide. We conclude that in cerebral arterioles, dilation in response to acidosis is regulated by endothelial NO and smooth muscle KATP channel.

Hypercapnic and Normocapnic Acidosis
Previous studies indicate that there are differences in the mechanisms of hypercapnic and normocapnic acidosis between vascular beds.1,2 However, both lead to an increase in hydrogen ions, and the direct and/or indirect effects of them are related to acidosis-induced dilation via both potassium and calcium channels in vascular smooth muscle.3

Role of Potassium Channels in Dilation in Response to Acidosis
Potassium ion (30 mmol/L) can inhibit the activity of all potassium channels.4,5 In this study the acidosis-induced dilation is significantly attenuated but not abolished by extracellular K+ ion. This result suggests that there are at least 2 mechanisms for the dilation in response to acidosis: K+ channel–dependent and –independent mechanisms.

It is known that smooth muscle KATP channels are important mediators of the dilation in response to acidosis in cerebral arteries9 as well as pial arterioles.7 These findings are consistent with our study because glibenclamide (3 μmol/L) diminished the dilation in response to acidosis before and after endothelial impairment. We and others confirmed that up to 3 μmol/L glibenclamide was sufficient and specific for KATP channels because this concentration abolished the KATP channel opener–induced dilation. In addition, we previously reported that acidosis can hyperpolarize the smooth muscle cell, resulting in dilation.

Table 1. Arteriolar Diameter Before and After Administration of Inhibitors or Air Embolization

<table>
<thead>
<tr>
<th>Arteriolar Diameter, μm</th>
<th>n</th>
<th>Before</th>
<th>After</th>
<th>% of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl (30 mmol/L)</td>
<td>5</td>
<td>44.2±4.8</td>
<td>33.3±5.4</td>
<td>-25.1±3.7*</td>
</tr>
<tr>
<td>BaCl2 (30 μmol/L)</td>
<td>4</td>
<td>50.9±3.6</td>
<td>46.9±4.0</td>
<td>-8.1±1.9*</td>
</tr>
<tr>
<td>4-AP (100 μmol/L)</td>
<td>4</td>
<td>52.3±4.8</td>
<td>47.4±5.8</td>
<td>-10.0±2.9*</td>
</tr>
<tr>
<td>TEA (1 mmol/L)</td>
<td>4</td>
<td>52.4±3.4</td>
<td>50.1±4.0</td>
<td>-4.2±1.7</td>
</tr>
<tr>
<td>Glibenclamide (3 μmol/L)</td>
<td>5</td>
<td>47.2±4.6</td>
<td>48.2±4.2</td>
<td>2.5±1.6</td>
</tr>
<tr>
<td>L-NMMA (10 μmol/L)</td>
<td>5</td>
<td>44.5±2.8</td>
<td>33.6±2.3</td>
<td>-24.6±2.0*</td>
</tr>
<tr>
<td>L-NNA (10 μmol/L)</td>
<td>5</td>
<td>40.3±6.7</td>
<td>30.8±5.6</td>
<td>-23.4±4.4*</td>
</tr>
<tr>
<td>Air emboli</td>
<td>5</td>
<td>54.9±3.7</td>
<td>51.9±4.0</td>
<td>-5.6±1.7*</td>
</tr>
</tbody>
</table>

Values are mean±SE; n is number of observations. *Significant difference from control diameter.

Table 2. Acidosis-Induced Dilation (pH 6.8) of Rat Cerebral Arteriole Before and After Administration of Antagonists

<table>
<thead>
<tr>
<th></th>
<th>TEA</th>
<th>4-AP</th>
<th>Indomethacin</th>
<th>Ouabain</th>
<th>MS-PPOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>75.4±5.6</td>
<td>88.7±3.9</td>
<td>76.7±8.3</td>
<td>82.8±10.9</td>
<td>85.8±4.0</td>
</tr>
<tr>
<td>After</td>
<td>66.6±11.7*</td>
<td>84.4±7.3*</td>
<td>71.0±5.6</td>
<td>76.8±11.9*</td>
<td>90.3±1.0*</td>
</tr>
</tbody>
</table>

Values are mean±SE; n is number of observations. The dilation induced by acidosis (pH 6.8) is expressed as percent maximum response.

*P=NS.
tion via activation of smooth muscle $K_{ATP}$ channels in rat cerebral arterioles. However, we cannot exclude the possibility that endothelial $K_{ATP}$ channels also contribute to acidosis-induced dilation.

Recently, Suzuki and colleagues$^{25}$ demonstrated that a pH-sensitive $K^+$ channel is present in the rabbit cerebral arterial smooth muscle. It is characterized by an inward rectifier current and blocked by Ba$^{2+}$. BaCl$_2$ is a concentration-dependent $K^+$ channel inhibitor,$^{9,18}$ and a low concentration of BaCl$_2$ ($<50 \mu$mol/L) has been shown to be a selective antagonist of $K_{IR}$ channels$^{26}$ that does not inhibit $K_{ATP}$ channels in rat basilar artery.$^{27,28}$ In the present study BaCl$_2$ (30 $\mu$mol/L) also attenuated the dilation in response to acidosis, which was similar in degree to the reduction induced by glibenclamide. However, additional treatment with glibenclamide did not produce a further reduction of dilation. These results indicate that 30 $\mu$mol/L BaCl$_2$ may inhibit $K_{IR}$ as well as $K_{ATP}$ channels in rat cerebral arterioles. However, we cannot exclude the contribution of $K_{IR}$ channels to acidosis-induced dilation. Nguyen et al$^{18}$ recently have shown that both $K_{ATP}$ and $K_{IR}$ channels are blocked by 30 $\mu$mol/L BaCl$_2$ in rat cerebral arterioles, similar to our preparation. Thus, there may be a difference in the action of BaCl$_2$ as an inhibitor of potassium channels among the rat arterial tree. Since the action of glibenclamide as an inhibitor of $K_{ATP}$ channels was consistent for both macrovessels and microvessels,$^{18,27}$ we speculate that activation of $K_{ATP}$ channels may be essential to acidosis-induced dilation rather than $K_{IR}$ channels.

$K_{IR}$ and $K_{ATP}$ channels in rat cerebral arterioles appear to be ruled out as mediators of dilation because TEA and 4-AP did not affect the dilation in response to acidosis. On the other hand, the $K_v$ channel is involved in reduction of the extracellular pH in cat cerebral arterial smooth muscle cells.$^{29}$ Wang et al$^{30}$ have demonstrated that, in rat pial arterioles in vivo, $K_{Ca}$ channel inhibitor did not block the dilatory response to acidosis under basal conditions, whereas $K_{Ca}$ channels can be activated by acidosis in the presence of neuronal NOS inhibition. These studies strongly suggest that several types of potassium channels may participate in acidosis-mediated cerebral dilatory responses depending on a variety of factors, such as species or organ studied.

Role of NO in Dilation in Response to Acidosis

The finding that L-NMMA, L-NNA, and endothelial impairment reduced the acidosis-induced dilation, but indomethacin did not, indicates that endothelial NO is involved. We previously demonstrated that L-NMMA (10 $\mu$mol/L) and L-NNA (10 $\mu$mol/L) were sufficient as the NOS inhibitor.$^{19,20}$ On the basis of in vivo studies, $^{7,8,31}$ NO is an important mediator of cerebral dilation in response to acido-

s. Irikura et al$^{32}$ have suspected that neuronal but not endothelial NOS may be activated by acidosis. In addition, neuronal NO dose not appear to be from the perivascular nerves of the vessels.$^{31}$ On the other hand, in vitro studies showed acidosis induced endothelium-independent dilation in large cerebral arteries such as dog or monkey middle cerebral or rat basilar arteries,$^{33–35}$ suggesting that endothelial NO did not alter this response. Recently, Lindauer et al$^{11}$ confirmed in isolated rat middle cerebral artery that denudation did not alter the response to acidic dilation. However, the present study in rat cerebral arterioles clearly demonstrated that endothelial NO contributes to acidosis-induced dilation. Thus, there appears to be a regional difference between large cerebral arteries and cerebral arterioles in response to acidosis-mediated dilation.

There are 2 possible explanations for the contribution of endothelial NO to acidic dilation: (1) Acidosis increases the production of NO because NOS may be activated by acidosis.$^{36,37}$ (2) Acidosis protects spontaneously released NO from its degradation because NO is stable in acidosis.$^{38,39}$ In the cerebral circulation, it would seem that NO acts as an amplifier but not a mediator for the response to dilation caused by acidosis.$^{39}$ We previously reported that endothelial NO was basally released and regulated arteriolar tone in rat cerebral arterioles.$^{19}$ Thus, it is possible that acidosis increases the net action of basal NO, resulting in dilation of arterioles.

Role of Other Mediators

Several substances may be considered to be other mediators of dilation in response to acidosis. Involvement of prostanoids and electrogenic Na$^+$ pump in acidosis-induced cerebral dilation has been postulated in newborn pigs,$^{40}$ monkeys,$^{41}$ and dogs.$^{33}$ However, treatment with indomethacin or ouabain failed to attenuate the acidosis-mediated dilation in isolated rat cerebral arterioles. These observations indicate that prostanoids and electrogenic Na$^+$ pump play little role in the response to acidosis. It is possible that newly synthesized products of cytochrome P450 epoxygenase contribute to the dilation during acidosis because acidosis causes smooth muscle hyperpolarization$^4$ and endothelial impairment attenuates the dilation in response to acidosis in isolated rat cerebral arterioles. Cytochrome P450 metabolites$^{41}$ are known to be one of the endothelium-derived hyperpolarizing factors. However, MS-PPOH, the cytochrome P450 epoxygenase inhibitor,$^{23,24}$ had no effect on acidosis-induced dilation in our study.

Physiological and Pathophysiological Consequences

Our data indicate that in cerebral arterioles the endothelium contributes to acidic vasodilation, which differentiates these vessel from large cerebral arteries where the endothelium seems to contribute to acidic dilation.$^{11}$ Pathological conditions such as subarachnoid hemorrhage or traumatic brain injury can cause endothelial dysfunction$^{42,43}$ and cerebral acidosis.$^{44,45}$ Decreased microvascular dilation due to lack of endothelial pH response may therefore contribute to the cerebral hypoperfusion seen after subarachnoid hemorrhage or traumatic injury despite the acidosis caused by these injuries.

### TABLE 3. Effect of L-NNA With or Without Glibenclamide on Dilation in Response to Acidosis (pH 6.8) in Rat Cerebral Arteriole

<table>
<thead>
<tr>
<th>Control L-NNA and Glibenclamide</th>
<th>L-NNA (10 $\mu$mol/L)</th>
<th>L-NNA and Glibenclamide (3 $\mu$mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Maximum diameter</td>
<td>81.8±6.2</td>
<td>53.7±10.8*</td>
</tr>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td></td>
<td>35.5±9.5†</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

*Significant differences (P<0.05) from control. †Significant differences (P<0.05) from L-NNA.
In conclusion, the present study shows that acidosis stimulates smooth muscle \( K_{\text{ATP}} \) channels, resulting in dilatation of isolated rat cerebral arterioles, but depends neither on cyclooxygenase or cytochrome P450 products nor on activation of Na-K ATPase. In addition, endothelial NO modulates the dilatation caused by acidosis, which differentiates cerebral arteriolar responses from responses of large cerebral arteries.

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References
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