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. (Stroke. 2002;33:844-849.)

Key Words: acid-base equilibrium ■ cerebral circulation ■ hydrogen-ion concentration ■ microcirculation ■ rats
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 vaso dilatation 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, 450-28 g; 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% dialized 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 micropipettes. The internal diameter of arteriole was continuously monitored under an inverted microscope (Dolphin, 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 drugs were purchased: tetraethylammonium ion (TEA), glibenclamide, 4-aminopyridine (4-AP), KCl, BaCl2, N-monomethyl-L-arginine (LNMA), N-nitro-L-arginine (L-NNA), ouabain, and indomethacin (Sigma). N-Methylsulfonyl-6-(2-propargyloxyphenyl) hexanamide (MS-PPOH) was a gift from Dr John R. Falck (Department of Biochemistry and Pharmacology, University of Texas Southwestern Medical Center).

Experimental Protocols

Acidosis-induced dilation was studied by adding HCl (0.1N) to MOPS-buffered saline to reduce extravascular pH. The internal diameter was measured at pH 7.4 (as control), 7.2, 7.0, and 6.8 before and after endothelial impairment and/or administration of several inhibitors. Previously, we confirmed that acidosis-induced dilations were reproducible.

The high-K+ saline (30 mmol/L) was used to negate any effects of K+ channels. Isotonic high-K+ MOPS-buffered saline was prepared by substituting NaCl with an equimolar amount of KCl.

To confirm what type of K+ channels is involved in acidosis-induced dilation, we used 4 K+ channel inhibitors: 3 μmol/L glibenclamide specific for KATP channels, 1 mmol/L TEA specific for calcium-activated K+(KCa) 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 arterial 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 smooth muscle cell damage with extraluminal propidium iodide (5 μmol/L) after air embolism. After air embolization, we previously reported that L-NMMA did not cause constriction of our preparation. This result indicated that 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-NAGA (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−Dbase)/(Dmax−Dacidosis)×100, where Dmax, Dbase, and Dacidosis 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. Dmax 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 100μmol/L 4-AP (n=4; Table 2) altered the
dilation.

Effect of L-NMMA and Indomethacin
Indomethacin (10μmol/L) did not affect the control diameter and the dilation in response to acidosis (n=4; Table 2). Extraluminally applied L-NMMA (10μmol/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 (3μmol/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 (10μmol/L) constricted the vessels (Table 1) and inhibited the dilation in response to acidosis (n=5; Table 3).
acidity is regulated by endothelial NO and smooth muscle K\(_{\text{ATP}}\) 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 acidity-induced dilation via both potassium and calcium channels in vascular smooth muscle.\(^2\)

### Role of Potassium Channels in Dilation in Response to Acidity

Potassium ion (30 mmol/L) can inhibit the activity of all potassium channels.\(^9,15\) In this study the acidity-induced dilation is regulated by endothelial NO and smooth muscle K\(_{\text{ATP}}\) channel.

### Discussion

The results of the present study indicate the following: (1) The dilation in response to acidity is attenuated by glibenclamide, BaCl\(_2\), 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 acidity before and after endothelial impairment. We\(^\text{17}\) and others\(^\text{18}\) confirmed that up to 3 \(\mu\)mol/L glibenclamide was sufficient and specific for K\(_{\text{ATP}}\) channels because this concentration abolished the K\(_{\text{ATP}}\) channel opener–induced dilation. In addition, we previously reported that acidity caused hyperpolarization of smooth muscle cells in rat cerebral arterioles.\(^4\) Thus, acidity can hyperpolarize the smooth muscle cell, resulting in dila-

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

<table>
<thead>
<tr>
<th>Arteriolar Diameter, (\mu)m</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>BaCl(_2) (30 (\mu)mol/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 (\mu)mol/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 (\mu)mol/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 (\mu)mol/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 (\mu)mol/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 (\mu)mol/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.
tion via activation of smooth muscle $K_{\text{ATP}}$ channels in rat cerebral arterioles. However, we cannot exclude the possibility that endothelial $K_{\text{ATP}}$ 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 $K_{\text{IR}}$ channels$^{26}$ that does not inhibit $K_{\text{ATP}}$ 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 $K_{\text{IR}}$ as well as $K_{\text{ATP}}$ channels in rat cerebral arterioles. However, we cannot exclude the contribution of $K_{\text{IR}}$ channels to acidosis-induced dilation. Nguyen et al$^{18}$ recently have shown that both $K_{\text{ATP}}$ and $K_{\text{IR}}$ 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 along the rat arterial tree. Since the action of glibenclamide as an inhibitor of $K_{\text{ATP}}$ channels was consistent for both macrovessels and microvessels,$^{18,27}$ we speculate that activation of $K_{\text{ATP}}$ channels may be essential to acidosis-induced dilation rather than $K_{\text{IR}}$ channels.

$K_{\text{Ca}}$ and $K_{\text{V}}$ 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 extra-cellular pH in cat cerebral arterial smooth muscle cells.$^{29}$ Wang et al$^{30}$ have demonstrated that, in rat pial arterioles in vivo, $K_{\text{Ca}}$ channel inhibitor did not block the dilatory response to acidosis under basal conditions, whereas $K_{\text{Ca}}$ 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-

<table>
<thead>
<tr>
<th>TABLE 3. Effect of L-NNA With or Without Glibenclamide on Dilation in Response to Acidosis (pH 6.8) in Rat Cerebral Arteriole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>(10 $\mu$mol/L)</td>
</tr>
<tr>
<td>% Maximum diameter</td>
</tr>
<tr>
<td>81.8±6.2</td>
</tr>
</tbody>
</table>

*Significant differences (P<0.05) from control.†Significant differences (P<0.05) from L-NNA.

sis. Irikura et al$^{32}$ have suspected that neuronal but not endothelial NOS may be activated by acidosis. In addition, neuronal NO does 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 acido-
sis.$^{36,37}$ (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 is 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 prostanooids and electrogenic Na$^+$ pump in acidosis-induced cerebral dilation has been postulated in newborn pigs,$^{40}$ monkeys,$^{34}$ 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 prostanooids 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$^4$ are known to be 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 not 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 hyperperfusion seen after subarachnoid hemorrhage or traumatic injury despite the acidosis caused by these injuries.
In conclusion, the present study shows that acidosis stimulates smooth muscle $K_{ATP}$ channels, resulting in dilation 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 dilation caused by acidosis, which differentiates cerebral arteriolar responses from responses of large cerebral arteries.

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**References**


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