ATP-Sensitive Potassium Channels Mediate Dilatation of Basilar Artery in Response to Intracellular Acidification In Vivo

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Background and Purpose—During cerebral ischemia, both hypoxia and hypercapnia appear to produce marked dilatation of the cerebral arteries. Hypcapnia and hyperoxia may be accompanied by extracellular and intracellular acidosis, which is another potent dilator of cerebral arteries. However, the precise mechanism by which acidosis produces dilatation of the cerebral arteries is not fully understood. The objective of the present study was to examine the mechanisms by which intracellular acidosis produces dilatation of the basilar artery in vivo.

Methods—Using a cranial window in anesthetized rats, we examined responses of the basilar artery to sodium propionate, which was used to cause intracellular acidosis specifically. Expression of subunits of potassium channels was determined by reverse transcription and polymerase chain reaction (RT-PCR).

Results—Topical application of propionate increased diameter of the basilar artery in a concentration-related manner. Propionate-induced dilatation of the artery was attenuated by glibenclamide, an inhibitor of ATP-sensitive potassium channels. However, inhibitors of nitric oxide synthase (N\textsuperscript{G}-nitro-L-arginine), large-conductance calcium-activated potassium channels (iberiotoxin), and cyclooxygenase (indomethacin) did not affect the vasodilatation. Expression of mRNA for SUR2B and Kir6.1 was detected, with the use of RT-PCR, in the cultured basilar arterial muscle cells.

Conclusions—The findings suggest that intracellular acidification may produce dilatation of the basilar artery through activation of ATP-sensitive potassium channels in vivo. Kir6.1/SUR2B may be the major potassium channels that mediate propionate-induced dilatation of the artery. (Stroke. 2003;34:1276-1280.)

Key Words: muscle, smooth ■ propionic acids ■ vasodilation

During cerebral ischemia, both hypoxia and hypercapnia appear to produce marked dilatation of the cerebral arteries.\textsuperscript{1} The vasodilator responses may increase cerebral perfusion for maintenance of oxygen delivery to brain tissue. Both hypoxia and hypercapnia may be accompanied by extracellular and/or intracellular acidosis. Recent evidence has suggested that acidosis itself produces marked relaxation of the cerebral arteries in vitro.\textsuperscript{2,3} Thus, extracellular and intracellular acidosis may play a major role in dilatation of the cerebral arteries during hypoxia and hypercapnia.\textsuperscript{4}

Hypoxic dilatation of the cerebral arteries appears to be mediated by activation of ATP-sensitive potassium (K\textsubscript{ATP}) channels.\textsuperscript{5,6} On the other hand, vasodilatation induced by hypercapnia is reported to be mediated mainly by nitric oxide (NO).\textsuperscript{7,8} Thus, the mechanisms by which acidosis produces dilatation of the cerebral arteries may be quite different between hypoxia and hypercapnia. It is reported that extracellular pH (pH\textsubscript{e}) rather than intracellular pH (pH\textsubscript{i}) may be the major determinant of hypercapnia-induced, NO-dependent relaxation of the cerebral arteries in vitro.\textsuperscript{9} Hypoxia appears to produce dilatation of the cerebral arteioles through activation of K\textsubscript{ATP} channels that is independent of pH\textsubscript{i} changes,\textsuperscript{4} suggesting that pH\textsubscript{i} may not be important in hypoxia-induced activation of K\textsubscript{ATP} channels in cerebrovascular muscle.

Recently, Xu et al\textsuperscript{9} showed that K\textsubscript{ATP} channels expressed in \textit{Xenopus} oocytes are activated directly by intracellular but not extracellular acidosis. The findings suggest that K\textsubscript{ATP} channels contain their pH-sensitive sites in the inside of the cell membrane. However, it is not known whether selective intracellular acidosis activates K\textsubscript{ATP} channels and thereby produces dilatation of the cerebral arteries in vivo. Thus, the first objective of the present study was to test the hypothesis that intracellular acidosis of the basilar arterial muscle cells produces dilatation of the artery through activation of K\textsubscript{ATP} channels in vivo.

The activity of K\textsubscript{ATP} channels has been shown in cerebrovascular muscle and appears to play an important role in dilator responses of the cerebral arteries.\textsuperscript{5,6,10,11} It has been shown that K\textsubscript{ATP} channels are hetero-octamers consisting of 4 sulfonylurea receptors (SUR) interacting with 4 channel subunits (Kir channels).\textsuperscript{12,13} SUR2B may be the major SUR in the smooth muscle cells of systemic arteries.\textsuperscript{14} Both Kir6.1 and Kir6.2 are reported to be present in vascular muscle.
cells. However, it is not known which Kir is expressed in the cerebral arterial muscle. Thus, the second objective was to determine SUR and Kir subunits expressed in the basilar arterial muscle cells.

Materials and Methods

This experiment was reviewed by the Committee on the Ethics of Animal Experiments in the Faculty of Medicine, Kyushu University, and performed under the control of the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Japanese government.

Cranial Window

Experiments were performed on male Sprague-Dawley rats (weight, 400±8 g; age, 3.4±0.2 months [mean±SEM]) anesthetized with amobarbital (100 mg/kg IP). Anesthesia was supplemented intravenously at 20 to 25 mg/kg per hour. The trachea was cannulated, and the animals were mechanically ventilated with room air and supplemental oxygen. Skeletal muscle paralysis was produced with d-tubocurarine chloride (2 mg/kg). Depth of anesthesia was evaluated by applying pressure to a paw or the tail and observing changes in heart rate or blood pressure. When such changes occurred, additional anesthetic was administered. Catheters were placed in both femoral arteries to measure systemic arterial pressure and to obtain arterial blood samples. A femoral vein was cannulated for infusion of drugs.

A craniotomy was prepared over the ventral brain stem as previously described in detail. After a part of the dura was opened, the cranial window was suffused with artificial cerebrospinal fluid (CSF) (temperature=37°C; ionic composition [in mmol/L]: 132 NaCl, 2.95 KCl, 1.71 CaCl2, 0.65 MgCl2, 24.6 NaHCO3, 3.69 D-glucose) that was bubbled continuously with appropriate gases. The diameter of the blood vessel was measured with the use of a microscope equipped with a television camera coupled to an auto-width analyzer (C3161, Hamamatsu Photonics KK). After cranietomy was performed, pH, PCO2, and PO2 of arterial blood were adjusted by changing rate and volume of the respirator and the oxygen content of inspiratory air. We also monitored arterial blood gas during the experiments and kept the values within normal limits (pH=7.45±0.01, PCO2=39±1 mm Hg, and PO2=93±4 mm Hg).

We examined responses of the basilar artery to topical application of sodium propionate (10-6 to 10-3 mol/L). Sodium propionate permeates the cells in its protonated form and releases protons, which produces intracellular acidification. We also examined responses to sodium nitroprusside (10-9 to 10-6 mol/L), an NO donor. Each drug was mixed in artificial CSF and suffused over the cranietomy for 5 minutes. Diameters of the basilar artery were measured immediately before and during the last minute of application of each agonist. After application of a specific drug, the vessel diameter returned to baseline level within a few minutes before application of another one. The application sequence was alternative. We also examined pH, PCO2, and PO2 of artificial CSF in the absence and presence of 1 mmol/L sodium propionate. Application of the concentration of propionate did not affect these parameters of the CSF (control: pH=7.43±0.02, PCO2=35±1 mm Hg, and PO2=119±3 mm Hg; propionate: pH=7.42±0.02, PCO2=35±1 mm Hg, and PO2=119±3 mm Hg; n=10).

We used glibenclamide (10-6 mol/L), an inhibitor of KirATP channels, and N,N'-nitro-L-arginine (L-NNA) (10-5 mol/L), an inhibitor of NO synthase; ibritenzin (10-4 mol/L), an inhibitor of large conductance calcium-activated potassium channels; and indomethacin (10 mg/kg IV), an inhibitor of cyclooxygenase. Each inhibitor appears to produce maximum inhibition of its target molecule at the concentration described above. Glibenclamide was dissolved in dimethyl sulfoxide (DMSO). The maximum final concentration of DMSO was 0.1%. We found that 0.1% DMSO did not cause any significant changes in diameter of the basilar artery (1±2%; n=5).

Statistical Analysis

All values were expressed as mean±SEM. One-way repeated-measures ANOVA was used to compare concentration-dependent responses to vasodilators. Two-way repeated-measures ANOVA was used to compare responses under control conditions and during interventions. When a significant F value was found, post hoc analysis was performed with the Wilcoxon test. A value of P<0.05 was considered significant.

| TABLE 1. Nucleotide Sequences of Primers Used for PCR and Expected Size of PCR Products |
|-----------------------------|------------------|------------------|
| mRNA Expected Size Primer Sequence (5′→3′) |
| rKir6.1 1275 Forward-ATGCTGGCAGGAAGAGCAT Reverse-TCATGATTGCTAGTGGAGCAT |
| rKir6.2 1173 Forward-ATGCTGGCTGGGAAGACCAT Reverse-TCAGGACAGAATGTCGGAG |
| rSUR2B 858 Forward-GTGTAAGGAACCTGGCGGA Reverse-TCACATGTCCGACAGAA |

from 5 minutes before application of sodium propionate or sodium nitroprusside. Topical application of these agents did not cause any changes in systemic arterial pressure.

To confirm the importance of pH changes in propionate-induced vasodilatation, we tested the effects of 5-N,N-hexamethylenemalonamide, an inhibitor of Na-H exchanger, on the vasodilatation. 5-N,N-Hexamethylenemalonamide (3 μmol/L) was dissolved in DMSO and suffused 5 minutes before and during application of sodium propionate or sodium nitroprusside. The final concentration of DMSO in the CSF was 0.1%.

Reverse Transcription and Polymerase Chain Reaction

Basilar arterial muscle cells were collected from the basilar artery of male Sprague-Dawley rats (aged 4 to 6 weeks). After they were anesthetized with diethyl ether, the animals were decapitated, and the basilar artery was quickly removed under sterile conditions. The arterial segments were carefully cleaned of connective tissue, cut into small fragments, and placed in culture dishes coated with collagen type 1. The growth medium (Dulbecco’s modified Eagle’s medium) was supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution. The dishes were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO2. After a few days, colonies of vascular muscle cells proliferated from the basilar arterial segments. After the arterial segment was removed, the primary vascular muscle cell colonies were gently scraped off and subcultured in other dishes. Final characterization of the cells was performed by demonstrating the absence of di-I LDL uptake and the expression of smooth muscle–specific α-actin.

Total RNA was prepared from cultured basilar arterial muscle cells with TRIzol reagent (Life technologies, Inc). With the use of 1 μg total RNA, first strand cDNA synthesis was performed by AMV transcriptase in 20 μL (Roche Diagnostics). With the use of aliquots (1 μL) of reverse transcriptase (RT) products, polymerase chain reaction (PCR) reactions were performed in 50 μL final volumes with the gene-specific primers listed in Table 1. Amplification was performed for 30 cycles of denaturation at 94°C (30 seconds), annealing at 60°C (30 seconds), and extension at 72°C (60 seconds), followed by 7 minutes of extension reaction at 72°C. PCR products were separated on 1% agarose gel.

We also examined the expression of KATP channels in pancreatic cells. After they were anesthetized with diethyl ether, male Sprague-Dawley rats (aged 6 weeks) were decapitated, and the pancreas was quickly removed. The organ was dispersed with a dispersing generator (POLYTRON PT2100, Kinematica AG), and total RNA was prepared with TRIzol reagent. RT-PCR was performed as described above.
Results

Propionate-Induced Vasodilation

Under control conditions, diameter of the basilar artery was 224±7 μm (n=20). Topical application of sodium propionate (10⁻⁶ to 10⁻³ mol/L) produced dilatation of the basilar artery in a concentration-related manner (Figure 1). Vasodilation induced by propionate (10⁻⁶ to 10⁻³ mol/L) was reproducible since there was no significant attenuation of the response during repeated application of propionate (first: 11±2%, 19±3%, 28±5%, 36±6%, respectively; second: 10±2%, 19±4%, 29±6%, 34±5%, respectively; n=5). Glibenclamide, a selective inhibitor of K<sub>ATP</sub> channels, had no effects on the baseline diameter of the basilar artery but inhibited dilatation of the basilar artery in response to propionate (Figure 1). We found that 10⁻⁶ mol/L glibenclamide inhibited vasodilation in response to 10⁻³ mol/L sodium propionate by 46±11%. Sodium nitroprusside (10⁻⁸ to 10⁻⁶ mol/L) also had no effect on the dilatation of the basilar artery (Figure 2). Glibenclamide (10⁻⁶ mol/L) did not affect vasodilation produced by sodium nitroprusside (Figure 2). We also tested the effects of L-NNA (10⁻⁵ mol/L), iberiotoxin (10⁻⁵ mol/L), and indomethacin (10 mg/kg IV) on propionate-induced dilatation of the basilar artery. None of the inhibitors affected propionate-induced vasodilation (Table 2).

Because intracellular acidification activates the Na⁺/H⁺ exchanger, which causes efflux of protons to the extracellular space and thereby normalizes intracellular pH, inhibition of the Na⁺/H⁺ exchanger enhances intracellular acidification. Thus, we anticipated that inhibition of the exchanger would enhance propionate-induced vasodilation. In the present study 5-N,N-hexamethyleneamiloride (3 μmol/L), an inhibitor of Na⁺/H⁺ exchanger, enhanced propionate-induced dilatation of the basilar artery (Figure 3); 3 μmol/L 5-N,N-hexamethyleneamiloride did not affect vasodilator responses to sodium nitroprusside (10⁻⁷ to 10⁻⁶ mol/L). The findings suggest that propionate-induced vasodilation is dependent on intracellular acidification.

Presence of Kir6.1 and SUR2B

RT-PCR reactions yielded an expected-size 1275-bp PCR product for Kir6.1 but not an expected-size 1173-bp product for Kir6.2 (Figure 4), indicating that the vascular smooth muscle cells mainly expressed Kir6.1. Identical results were obtained with several independent vascular muscle isolates. On the other hand, pancreatic cells expressed Kir6.2 but not Kir6.1 (Figure 4). We also tested whether the vascular cells expressed SUR2B, which is reported to be present in rat coronary arterial muscle cells. RT-PCR reactions yielded an expected-size 858-bp PCR product for SUR2B (Figure 4).

![Figure 1](https://stroke.ahajournals.org/)

Figure 1. Effects of glibenclamide on propionate-induced vasodilation. Changes in diameter of the basilar artery were measured in response to sodium propionate (10⁻⁶ to 10⁻³ mol/L) under control conditions and in the presence of glibenclamide (10⁻⁶ mol/L). Values are mean±SEM; n=6. *P<0.05 vs control response.

![Figure 2](https://stroke.ahajournals.org/)

Figure 2. Effects of glibenclamide on nitroprusside-induced vasodilation. Changes in diameter of the basilar artery were measured in response to sodium nitroprusside (10⁻⁸ to 10⁻⁶ mol/L) under control conditions and in the presence of glibenclamide (10⁻⁶ mol/L). Values are mean±SEM; n=6.

![Figure 3](https://stroke.ahajournals.org/)

Figure 3. Effects of 5-N,N-hexamethyleneamiloride (HMA) on propionate-induced vasodilation. Changes in diameter of the basilar artery were measured in response to sodium propionate (10⁻⁶ to 10⁻³ mol/L) under control conditions and in the presence of 5-N,N-hexamethyleneamiloride (3×10⁻⁶ mol/L). Values are mean±SEM; n=6. *P<0.05 vs control response.

Table 2. Effects of Inhibitors on Propionate-Induced Vasodilatation

<table>
<thead>
<tr>
<th>Propionate, mol/L</th>
<th>Control (n=7)</th>
<th>Iberiotoxin (n=5)</th>
<th>Indomethacin (n=5)</th>
<th>L-NNA (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁶</td>
<td>12±3</td>
<td>11±2</td>
<td>12±4</td>
<td>11±3</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>18±3</td>
<td>20±4</td>
<td>20±4</td>
<td>17±4</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>31±6</td>
<td>27±4</td>
<td>28±4</td>
<td>23±3</td>
</tr>
<tr>
<td>10⁻³</td>
<td>38±6</td>
<td>35±5</td>
<td>36±4</td>
<td>38±4</td>
</tr>
</tbody>
</table>
appears to decrease pHi without affecting pH o . We also showed that propionate did not affect pH o , P CO 2 , or P O 2 in the cerebral arteries,2,3 decrease of pH o and pH i may play an important role in dilatation of the cerebral arteries during hypoxia and hypercapnia. In the present study we focused on intracellular acidosis because that may be the first phenomenon that occurs after ischemic insult. Sodium propionate appears to decrease pH without affecting pH i .18 We also showed that propionate did not affect pH i , P CO 2 , or P O 2 in the artificial CSF. Because propionate caused marked dilatation of the basilar artery, intracellular acidosis of vascular muscle itself may produce dilatation of the artery in vivo. Moreover, 5-N,N-hexamethylenamiloride enhanced dilatation of the basilar artery in response to sodium propionate. The findings may also support the interpretation that propionate-induced dilatation of the basilar artery may be mediated by intracellular acidification in vivo.

To examine the role of K ATP channels in propionate-induced dilatation of the basilar artery, we tested effects of glibenclamide, an inhibitor of K ATP channels,6 on the vasodilation. Glibenclamide inhibited propionate-induced dilatation of the artery by approximately 50%. Thus, dilatation of the artery in response to intracellular acidosis may be mediated, in large part, by activation of K ATP channels in vivo. The findings are similar to those of Ishizaka and Kuo27 that acidosis-induced relaxation of porcine coronary artery is mediated primarily by activation of K ATP channels in vitro. The mechanisms of acidosis-induced activation of K ATP channels are still unclear. Ishizaka and Kuo27 have suggested that acidosis produces activation of pertussis toxin–sensitive GTP-binding protein and thereby causes vasorelaxation. Recently, Xu et al,9 using a patch-clamp technique, showed that protons activate the intracellular domain of K ATP channels and increase the open probability of the channels. Thus, it may be possible that selective intracellular acidification activates K ATP channels of basilar arterial muscle cells and thereby causes dilatation of the artery.

We also tested the effects of 3 other inhibitors, ie, L-NNA, iberiotoxin, and indomethacin. However, none of these inhibitors affected propionate-induced dilatation of the basilar artery. Thus, NO, BKCa channels, and prostanooids may not be involved in propionate-induced dilatation of the basilar artery in vivo. Horiiuchi et al28 examined responses of the cerebral arteries in response to extracellular acidification by HCl and showed that both NO and K ATP channels are involved in acidosis-induced relaxation of the arteries in vitro, pH i instead of pH i appears to be the major determinant of hypercapnia-induced, NO-dependent relaxation of the cerebral arteries in vitro.3 Thus, it may be possible that application of HCl to the vascular muscle reduces pH i as well as pH i and thereby activated both NO production and K ATP channels of the cerebral arterioles.

Glibenclamide at the concentration of 10−5 mol/L reduced but did not abolish propionate-induced dilatation of the basilar artery. Several mechanisms may be involved in the residual vasodilatation. It is reported that normocapnic acidosis inhibited calcium influx without affecting membrane potential of vascular muscle cells.29 Thus, acidosis may also inhibit calcium channels in the vascular muscle independent of the activity of potassium channels, and the residual vasodilatation after inhibition of K ATP channels is mediated by such direct inhibitory actions of acidosis on calcium channels. The activity of inward rectifier potassium channels is reported to be modulated by acidosis.30,31 Thus, we cannot exclude the possibility that inward rectifier potassium channels may be involved in the residual dilatation of the basilar artery. We also cannot exclude the possibility that other unknown mechanisms are involved in propionate-induced dilatation of the basilar artery in vivo.

K ATP channels are hetero-octamers consisting of 4 sulfonylurea receptors (SUR) interacting with 4 channel subunits (Kir channels). SUR2B appears to represent the SUR in the vascular muscle type K ATP channels. We also found that SUR2B was expressed in the basilar arterial muscle cells. On the other hand, both Kir6.1 and Kir6.2 are reported to be present in vascular muscle cells. Isomoto et al15 showed that coexpression of Kir6.2 and SUR2B reconstitutes the pharmacological and electrophysiological properties of K ATP channels described in smooth muscle cells. However, Yamada et
suggested that K+ channels composed of Kir6.1 and SUR2B closely resemble K_ATP channels observed in vascular muscle cells. In the present study we found that Kir6.1 is expressed in the basilar arterial muscle cells; however, significant expression of Kir6.2 was not observed in the muscle cells. Thus, Kir6.1/SUR2B may be the major K_ATP channels in the basilar arterial muscle cells. Xu et al32 showed that protons act directly on the Kir6 subunits and thereby increase channel activity. Thus, dilatation of the basilar artery in response to intracellular acidosis may be produced by activation of Kir6.1/SUR2B channels in the basilar arterial muscle cells by protons. However, we cannot exclude the possibility that the pattern of expression of the channels in cultured muscle cells is different from that in intact cells.

In conclusion, sodium propionate produced dilatation of the basilar artery in vivo. Propionate-induced vasodilatation may be due to intracellular acidification of the basilar arterial muscle cells and may be mediated in part by activation of K_ATP channels. Kir6.1/SUR2B may be the major K_ATP channels in the basilar arterial muscle cells.

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References

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