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Mild Hypercapnia Induces Vasodilation via Adenosine Triphosphate–sensitive K^+ Channels in Parenchymal Microvessels of the Rat Cerebral Cortex

Katsutoshi Nakahata, M.D., Hiroyuki Kinoshita, M.D., Ph.D.,† Yusei Hirano, M.D.,‡ Yoshiki Kimoto, M.D.,§ Hiroshi Iranami, M.D.,‖ Yoshio Hatano, M.D., Ph.D.#*

Background: **Carbon dioxide is an important vasodilator of cerebral blood vessels. Cerebral vasodilation mediated by adenosine triphosphate (ATP)–sensitive K^+ channels has not been demonstrated in precapillary microvessel levels. Therefore, the current study was designed to examine whether ATP-sensitive K^+ channels play a role in vasodilation induced by mild hypercapnia in precapillary arterioles of the rat cerebral cortex.**

Methods: **Brain slices from rat cerebral cortex were prepared and superfused with artificial cerebrospinal fluid, including normal (P_{CO_2} = 40 mmHg; pH = 7.4), hypercapnic (P_{CO_2} = 50 mmHg; pH = 7.3), and hypercapnic normal pH (P_{CO_2} = 50 mmHg; pH = 7.4) solutions. The ID of a cerebral parenchymal arteriole (5–9.5 μm) was monitored using computerized videomicroscopy.**

Results: **During contraction to prostaglandin $F_{2\alpha}$ (5×10^{-7} M), hypercapnia, but not hypercapnia under normal pH, induced marked vasodilation, which was completely abolished by the selective ATP-sensitive K^+ channel antagonist glibenclamide (5×10^{-6} M). However, the selective Ca^{2+}-dependent K^+ channel antagonist iberiotoxin (10^{-7} M) as well as the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (10^{-4} M) did not alter vasodilation. A selective ATP-sensitive K^+ channel opener, levcromakalim (3×10^{-8} to 3×10^{-7} M), induced vasodilation, whereas this vasodilation was abolished by glibenclamide.**

Conclusion: **These results suggest that in parenchymal microvessels of the rat cerebral cortex, decreased pH corresponding with hypercapnia, but not hypercapnia itself, contributes to cerebral vasodilation produced by carbon dioxide and that ATP-sensitive K^+ channels play a major role in vasodilator responses produced by mild hypercapnia.**

ADENOSINE triphosphate (ATP)–sensitive K^+ channels, which regulate vascular contractility concurrently with the changes in membrane potential of vascular smooth muscle cells, play an important role in vasodilator responses in cerebral circulation.[1–3] ATP-sensitive K^+ channels are activated by decreased intracellular levels of ATP as well as increased levels of adenosine diphosphate, indicating that these channels are involved in metabolic regulation in the pathophysiologic condition ischemia, including hypoxia, hypercapnia, and acidosis.[1–3] A previous study demonstrated that intraparenchymal injection of cromakalim produced a glibenclamide-sensitive increase in local cerebral blood flow, suggesting that even in peripheral circulation of the brain, ATP-sensitive K^+ channels seem to be present.[4] Vascular smooth muscle tone produced by cerebral arterioles, including those existing in the brain parenchyma, appears to be a crucial determinant of cerebral vascular resistance.[5] However, cerebral vasodilation mediated by ATP-sensitive K^+ channels has not been demonstrated in precapillary microvessel levels.

Carbon dioxide is one of the well-known vasodilator substances acting on cerebral blood vessels.[6] A previous study of rabbits demonstrated that glibenclamide is partly capable of reducing dilation of pial arterioles induced by hypercapnia, suggesting that ATP-sensitive K^+ channels may marginally contribute to cerebral vasodilation during hypercapnia.[7] In addition, our previous study of the canine basilar artery demonstrated that extracellular acidosis, which can coexist with hypercapnia, causes vasorelaxation partly *via* ATP-sensitive K^+ channels.[8] These previous studies of cerebral arteries as well as relatively larger cerebral arterioles indicate the involvement of ATP-sensitive K^+ channels in cerebral vasodilation induced by pathophysiologic stimuli. However, the role of ATP-sensitive K^+ channels in hypercapnia-induced vasodilation has not been studied in cerebral precapillary arterioles.

Therefore, the current study was designed to determine whether ATP-sensitive K^+ channels contribute to cerebral vasodilation mediated by a mild increase in concentrations of carbon dioxide in parenchymal arterioles of the rat cerebral cortex.

Materials and Methods

The institutional animal care and use committee approved this study. Male Wistar rats (300–400 g) were anesthetized with inhalation of halothane, 3%, in 100% oxygen (3 l/min). A midline thoracotomy was performed; 50 ml saline was infused intracardially into the left ventricle; simultaneously, a right atrial incision was made for blood drainage. The animals were then decapitated, and the brains were rapidly removed and rinsed with artificial cerebrospinal fluid (control solution) with

* Staff Anesthesiologist, ‖ Chief Anesthesiologist, Department of Anesthesia, Japanese Red Cross Society, Wakayama Medical Center. † Assistant Professor, ‡ Staff Anesthesiologist, § Instructor, Professor and Chairman, Department of Anesthesiology, Wakayama Medical University.

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Address reprint requests to Dr. Kinoshita: Department of Anesthesiology, Wakayama Medical University, 811–1 Kimiidera, Wakayama, Wakayama 641–0012, Japan. Address electronic mail to: hkinoshi@pd5.so-net.ne.jp.

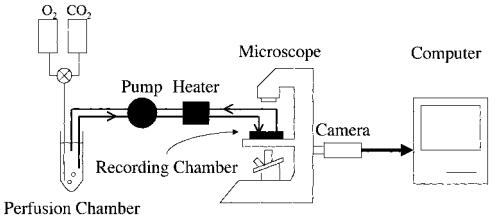

Fig. 1. The schema of our system for evaluation of cerebral microvessel function in the rat bran slice preparations. The brain slice was placed in a recording chamber and superfused with artificial cerebrospinal fluid bubbled with a mixture of oxygen and carbon dioxide at 37°C. The image of a parenchymal arteriole was transmitted to a video camera and displayed on a computer. The change in intraluminal diameter of the cerebral arteriole was recorded as the computer image file and, thereafter, was analyzed using the image analysis software.

the following composition (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 25; and glucose, 11. In general, brain slice preparation was done according to previously reported methods.[9,10] Brains were cut freehand into blocks containing the neocortex, followed by immediate sectioning into slices (200 μm thick) with a mechanical tissue slicer (Vibratomes 1000; Ted Pella, Inc., Redding, CA). Throughout the slicing procedure, brain blocks were continuously bathed in the control solution bubbled with 93% O_2 + 7% CO_2 at 4°C. An individual slice was then transferred to a recording chamber filled with control solution, which was mounted on an inverted microscope (IX70; Olympus, Tokyo, Japan).

The recording system consisted of a recording chamber (3 ml) and a tubing compartment (7 ml) including the perfusion chamber (fig. 1). The slices were continuously superfused with control solution at a flow rate of 1.5 ml/min bubbled with 93% O_2 + 7% CO_2 (P_{CO_2} = 40 mmHg; pH = 7.4; 37°C) and 90% O_2 + 10% CO_2 (P_{CO_2} =50 mmHg; pH = 7.3; 37°C) for normal and hypercapnic conditions, respectively. In some experiments, artificialcerebrospinal fluid was changed from the normocapnic control solution to the hypercapnic normal pH solution (P_{CO_2} = 50 mmHg; pH = 7.4; 37°C), which was prepared by modifying the composition of NaCl (109 mM) and $NaHCO_3$ (35 mM) bubbled with 90% O_2 + 10% CO_2. An intraparenchymal arteriole (ID, 5–9.5 μm) was located within the neuronal tissue, and its ID was continuously monitored with live computerized videomicroscopy.

The videomicroscopy equipment consisted of an inverted microscope, a ×40 objective (Olympus), and a ×2.25 video projection lens (Olympus). Arterioles were microscopically identified and differentiated from venules by the presence of the layer of vascular smooth muscle cells, and these were confirmed by hematoxylin–eosin staining of the slice after each experiment (unpublished data, Hiroyuki Kinoshita, M.D., Ph.D., Wakayama, Japan [October 2001]). The image of a parenchymal arteriole was transmitted to a video camera (C6790–81; Olympus) and displayed on a computer *via* a media converter (Physio-Tech, Tokyo, Japan). We defined the intraluminal diameter as the length between the internal margins of the arteriolar walls. Changes in the intraluminal diameter in cerebral microvessels were recorded on computer image files and then analyzed using image analysis software with a sensitivity to 0.01 μm (Physio-Tech).

Each slice was equilibrated for at least 30 min before the start of the experimental protocols. All experiments were performed during submaximal constriction in response to prostaglandin $F_{2\alpha}$ (5×10^{-7} M). Glibenclamide (5×10^{-6} M), iberiotoxin (10^{-7} M), or N^G-nitro-L-arginine methyl ester (10^{-4} M) was applied 10 min before addition of prostaglandin $F_{2\alpha}$ (5×10^{-7} M). In some experiments, the condition of perfusion was changed from normal to hypercapnic once during constriction in response to prostaglandin $F_{2\alpha}$. Concentration responses to acetylcholine (10^{-6} to 10^{-4} M), levcromakalim (3×10^{-8} to 3×10^{-7} M), and sodium nitroprusside (3×10^{-8} to 3×10^{-6} M) were obtained in the absence or the presence of glibenclamide, iberiotoxin, or N^G-nitro-L-arginine methyl ester (10^{-4} M). Concentration responses were obtained in a cumulative fashion by adding vasoconstrictor or vasodilator substances into the bubbling chamber connected to, but separated from, the recording chamber. Only one concentration response was made for each slice. The experiment duration for each slice was within 3 h. In the preliminary experiments, we found that prostaglandin $F_{2\alpha}$ (5×10^{-7} M) can produce submaximal vasoconstrictor effects (≈70% constriction compared with maximal contraction induced by prostaglandin $F_{2\alpha}$ [10^{-5} M]) on the cerebral arterioles under our experimental conditions (unpublished data, Katsutoshi Nakahata, M.D., Wakayama Japan [October 2002]). The amount of dilation of the cerebral arteriole induced by a mild increase in levels of carbon dioxide (50 mmHg), acetylcholine, levcromakalim, or sodium nitroprusside was normalized by using the constriction produced by prostaglandin $F_{2\alpha}$ (5×10^{-7} M) in each arteriole. Therefore, the percent dilation was calculated by the following equation: . The percent constriction was calculated by the following equation:

$$\% \text{ dilation} = 100 \times (D_{dilator} - D_{PGF})/(D_{control} - D_{PGF})$$

$$\% \text{ constriction to prostaglandin } F_{2\alpha} = 100 \times (D_{PGF} - D_{control})/D_{control}$$

$D_{control}$, D_{PGF}, and $D_{dilator}$ were the arteriolar diameter during the control condition, the arteriolar diameter after administration of prostaglandin $F_{2\alpha}$ (5×10^{-7} M), and that after addition of the vasodilator including carbon dioxide, respectively.

Drugs

The following pharmacologic agents were used: acetylcholine, dimethyl sulfoxide, glibenclamide, N^G-nitro-

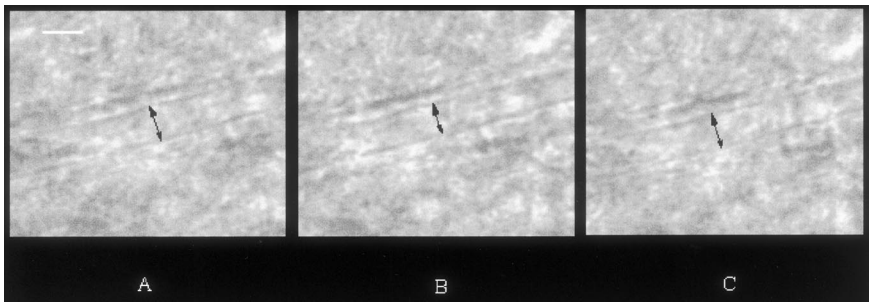

Fig. 2. The representative vasodilator response of rat cerebral parenchymal arterioles induced by the addition of carbon dioxide. In the control arteriole (ID, 7.3 μm) (*A*), which was contracted with prostaglandin $F_{2\alpha}$ (5×10^{-7} M) (ID, 5.9 μm) (*B*), an increase in levels of carbon dioxide (P_{CO_2} = 50 mmHg) produced almost maximal vasodilation (ID, 7.2 μm) within 20 min (*C*). *Black arrows* = the intraluminal diameter (defined as the length between the internal margin of the arteriolar wall); *bar* = 10 μm.

L-arginine methyl ester, prostaglandin $F_{2\alpha}$, sodium nitroprusside (Sigma, St. Louis, MO), and levcromakalim (GlaxoSmithKline plc, Greenford, United Kingdom). Drugs were dissolved in distilled water such that volumes of less than 60 μl were added to the perfusion system. Stock solutions of levcromakalim (10^{-5} M) and glibenclamide (10^{-5} M) were prepared in dimethyl sulfoxide (3×10^{-4} M). The drug concentrations are expressed as final molar concentrations.

Statistical Analysis

The data are expressed as mean ± SD; n refers to the number of rats from whom brain slices were obtained. Statistical analysis was performed using repeated-measures ANOVA followed by the Sheffé F test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Figure 2 shows a representative example of vasodilator responses induced by a mild increase in levels of carbon dioxide (50 mmHg; pH = 7.3). Prostaglandin $F_{2\alpha}$ (5×10^{-7} M) produced vasoconstriction of parenchymal arterioles, and addition of carbon dioxide (P_{CO_2} = 50 mmHg) induced marked vasodilation that reached the maximum within 20 min. The cumulative data demonstrate significant vasodilation induced by hypercapnia in the cerebral parenchymal arterioles, whereas the selective ATP-sensitive K^+ channel antagonist glibenclamide (5×10^{-6} M) completely abolished this vasodilation (fig. 3). In contrast, the selective Ca^{2+}-dependent K^+ channel antagonist iberiotoxin (10^{-7} M) did not alter the vasodilation induced by hypercapnia in arterioles contracted with prostaglandin $F_{2\alpha}$ (fig. 4).

Hypercapnia under normal pH (P_{CO_2} = 50 mmHg; pH = 7.4) produced no vasodilation in cerebral parenchymal arterioles (n = 5; fig. 5).

The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (10^{-4} M) did not affect the vasodilation induced by a mild increase in levels of carbon dioxide (50 mmHg) (fig. 6A), whereas this compound completely abolished the vasodilation induced by acetylcholine (10^{-6} to 10^{-4} M) (fig. 6B).

In arterioles contracted with prostaglandin $F_{2\alpha}$ (5×10^{-7} M), the selective ATP-sensitive K^+ channel opener levcromakalim (3×10^{-8} to 3×10^{-7} M) induced vasodilation in a concentration-dependent fashion (fig. 7). The highest concentration of levcromakalim used in the

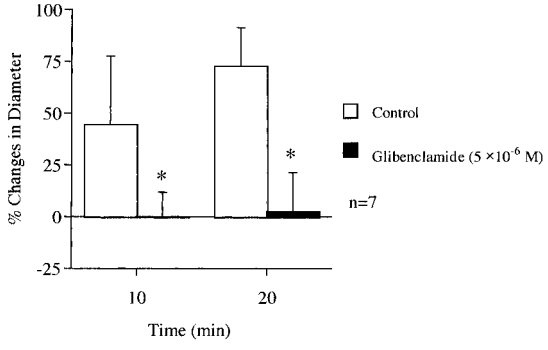

Fig. 3. Vasodilator responses to an increase in levels of carbon dioxide (P_{CO_2} = 50 mmHg) in the absence or presence of glibenclamide (5×10^{-6} M) in the rat cerebral parenchymal arteriole. Baseline diameters were 6.5 ± 1.5 and 6.3 ± 1.8 μm for control arterioles and arterioles treated with glibenclamide, respectively. Vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (5×10^{-7} M) were 18.1 ± 5.4 and 23.9 ± 8.0% for control arterioles and arterioles treated with glibenclamide, respectively. Data are expressed as mean ± SD. * The difference between control arterioles and arterioles treated with glibenclamide was statistically significant.

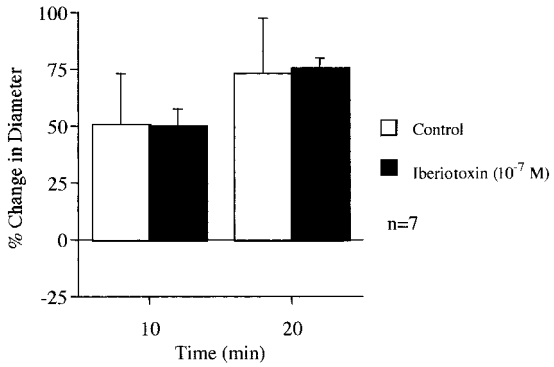

Fig. 4. Vasodilator responses to increase in levels of carbon dioxide (P_{CO_2} = 50 mmHg) in the absence or presence of the Ca^{2+}-dependent K^+ channel antagonist iberiotoxin (10^{-7} M) in rat cerebral parenchymal arterioles. Baseline diameters were 6.5 ± 2.3 and 6.4 ± 2.7 μm for control arterioles and arterioles treated with iberiotoxin, respectively. Vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (5×10^{-7} M) were 18.4 ± 6.2 and 21.3 ± 5.3% for control arterioles and arterioles treated with iberiotoxin, respectively. Data are expressed as mean ± SD.

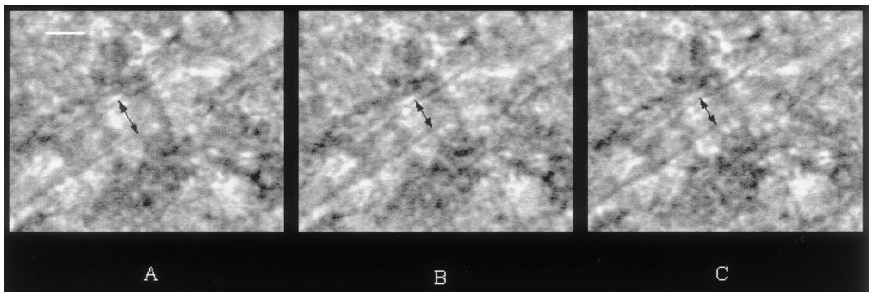

Fig. 5. The representative vasodilator response of rat cerebral parenchymal arterioles induced by the addition of carbon dioxide under the condition of normal pH. In the control arteriole (ID, 6.4 μm) (*A*), which was contracted with prostaglandin $F_{2\alpha}$ (5×10^{-7} M) (ID, 5.2 μm) (*B*), an increase in levels of carbon dioxide (P_{CO_2} = 50 mmHg) produced no vasodilation of the parenchymal arterioles under the condition of normal pH (pH = 7.4; ID, 5.2 μm) (*C*). *Black arrows* = the intraluminal diameter (defined as the length between the internal margin of arteriolar wall); *bar* = 10 μm.

current study produced almost full vasodilation. This vasodilator effect of levcromakalim was completely abolished by glibenclamide (5×10^{-6} M) (fig. 7). Glibenclamide did not alter the vasodilator effect of the nitric oxide donor sodium nitroprusside (3×10^{-8} to 3×10^{-6} M) in cerebral parenchymal arterioles (fig. 8).

Discussion

To our knowledge, this is the first study demonstrating that in rat cerebral parenchymal arterioles, a mild increase in levels of carbon dioxide (50 mmHg) induces vasodilation, which is completely abolished by glibenclamide but not by N^G-nitro-L-arginine methyl ester. These results suggest that in parenchymal microvessels of the rat cerebral cortex, ATP-sensitive K^+ channels, but not nitric oxide derived from endothelial as well as neuronal enzymes, play a major role in vasodilator responses produced by mild hypercapnia.

The specific effects of carbon dioxide on parenchymal cerebral arterioles have not been well studied, because penetrating microvessels are quite small and difficult to access when embedded within the neuronal tissues. However, Lee *et al.*[10] introduced a new system, which is capable of evaluating microvessels in the brain parenchyma using live videomicroscopy. The advantage of this method is the capacity to directly evaluate vasodilation or vasoconstriction in smaller vessels, approximately greater than 20 μm in diameter. In addition to these beneficial points, we developed this microscopic system with computer image analysis. Indeed, the image analysis software used in the current study has a higher sensitivity of 0.01 μm (compared with previously reported systems), showing that much smaller arterioles with IDs of less than 10 μm can be examined.[10,11] Although a previous study of rat brain slices demonstrated that cerebral microvessels are capable of responding to the alteration in carbon dioxide tension,[11] the role of ATP-sensitive K^+ channels in intraparenchymal cerebral arterioles has not been studied.

In parenchymal arterioles of the rat cerebral cortex, a mild increase in levels of carbon dioxide produced marked vasodilation, which was completely abolished by glibenclamide but not by iberiotoxin. Glibenclamide and iberiotoxin are selective ATP-sensitive and Ca^{2+}-dependent K^+ channel antagonists, respectively, suggesting that vasodilation induced by carbon dioxide in this preparation is mediated by ATP-sensitive K^+ channels.[8,12,13] The selectivity of glibenclamide as an ATP-sensitive K^+ channel antagonist is supported by the following results. In the current study, glibenclamide abolished vasodilation in response to an ATP-sensitive K^+ channel opener, levcromakalim. In addition, the concentration of glibenclamide used in the current study did

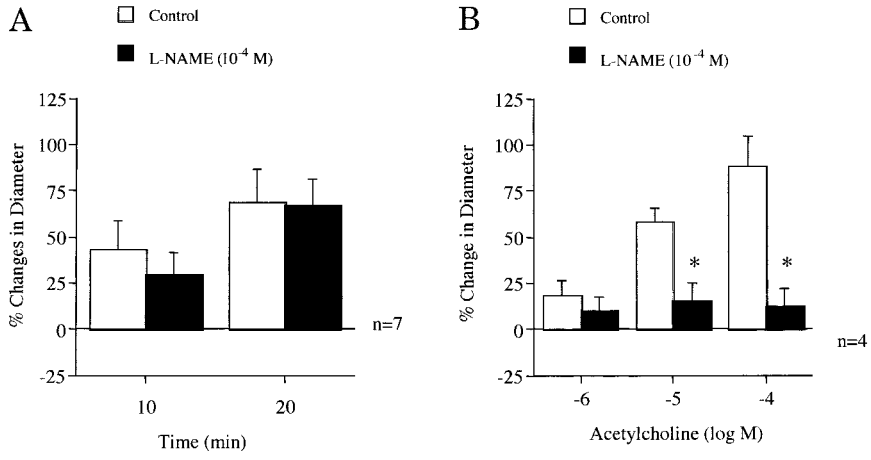

Fig. 6. Vasodilator responses to an increase in levels of carbon dioxide (P_{CO_2} = 50 mmHg) (*A*) or acetylcholine (10^{-6}, 10^{-5}, and 10^{-4} M) (*B*) in the absence or presence of N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M) in rat cerebral parenchymal arterioles. Baseline diameters were 7.3 ± 1.7 and 8.2 ± 1.9 μm for control arterioles and arterioles treated with L-NAME for carbon dioxide–induced vasodilation and 6.5 ± 1.6 and 6.5 ± 1.3 μm for control arterioles and arterioles treated with L-NAME for acetylcholine-induced vasodilation, respectively. Vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (5×10^{-7} M) were 15.2 ± 4.8 and 14.1 ± 3.2% for control arterioles and arterioles treated with L-NAME for carbon dioxide–induced vasodilation and 10.9 ± 3.6 and 18.1 ± 11.0% for control arterioles and arterioles treated with L-NAME for acetylcholine-induced vasodilation, respectively. Data are expressed as mean ± SD. * The difference between control arterioles and arterioles treated with L-NAME for acetylcholine-induced vasodilation was statistically significant.

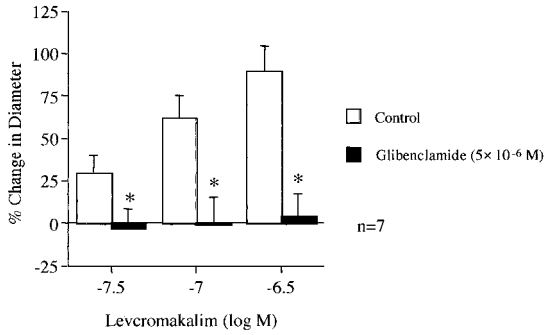

Fig. 7. Vasodilator responses to levcromakalim (3×10^{-8}, 10^{-7}, and 3×10^{-7} M) in the absence or presence of glibenclamide (5×10^{-6} M) in rat cerebral parenchymal arterioles. Baseline diameters were 6.4 ± 2.1 and 6.4 ± 1.9 μm for control arterioles and arterioles treated with glibenclamide, respectively. Vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (5×10^{-7} M) were 17.3 ± 2.9 and 17.7 ± 6.2% for control arterioles and arterioles treated with glibenclamide, respectively. Data are expressed as mean ± SD. * The difference between control arterioles and arterioles treated with glibenclamide was statistically significant.

not affect vasodilation of cerebral parenchymal arterioles induced by the nitric oxide donor sodium nitroprusside. Therefore, it appears that, at least in the rat cerebral parenchymal arterioles, mild hypercapnia–induced vasodilation is mediated by the relatively selective activation of ATP-sensitive K^+ channels.

The current study was designed to evaluate the role of ATP-sensitive K^+ channels in cerebral microvessels in mild hypercapnia rather than severe hypercapnia, because mild hypercapnia appears to be more clinically relevant than the severe type. A previous study of larger cerebral arterioles demonstrated that mild hypercapnia, similar to that studied in the current study, induced vasodilation only partly *via* ATP-sensitive K^+ channels.[7] In humans, administration of glibenclamide did not alter the blood flow of the middle cerebral artery in response to mild hypercapnia, suggesting that the activity of ATP-sensitive K^+ channels may not contribute to hypercapnia-induced vasodilation in the large cerebral artery.[14] In contrast, in cerebral parenchymal arterioles, mild hypercapnia produced marked vasodilation, which was completely abolished by a selective antagonist of ATP-sensitive K^+ channels, suggesting that these channels expressed in microvessels of cerebral cortex are very sensitive to the mild increase in levels of carbon dioxide. Therefore, ATP-sensitive K^+ channels may play a major role in cerebral vasodilation in mild hypercapnia only in cerebral microvessels. However, we cannot completely rule out the possibility that methodologic as well as species differences may contribute to these differential results.

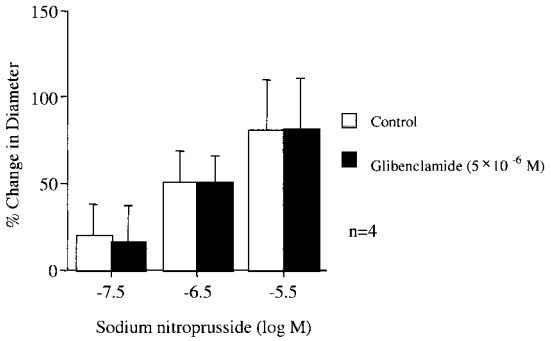

Fig. 8. Vasodilator responses to the nitric oxide donor sodium nitroprusside (3×10^{-8}, 3×10^{-7}, and 3×10^{-6} M) in the absence or presence of glibenclamide (5×10^{-6} M) in rat cerebral parenchymal arterioles. Baseline diameters were 7.4 ± 2.3 and 7.1 ± 1.2 μm for control arterioles and arterioles treated with glibenclamide, respectively. Vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (5×10^{-7} M) were 20.0 ± 11.7 and 22.7 ± 6.0% for control arterioles and arterioles treated with glibenclamide, respectively. Data are expressed as mean ± SD.

In the cerebral microvessels, N^G-nitro-L-arginine methyl ester did not affect the vasodilation induced by a mild increase in levels of carbon dioxide. Because N^G-nitro-L-arginine methyl ester is a well-known nitric oxide synthase inhibitor, these results suggest that nitric oxide derived from endothelial as well as neuronal enzymes may not mediate vasodilation induced by mild hypercapnia in the cerebral parenchymal arteriole.[15] In larger cerebral arteries as well as arterioles, nitric oxide, in the diverse degree, appears to play a role in cerebral vasodilation in response to hypercapnia.[7,16–19] In the human cerebral circulation, it is contradictory whether the activity of nitric oxide synthase contributes to this vasodilation.[19,20] Therefore, differential species as well as methodologic differences among these studies are the most likely explanation for these differential findings obtained for variable species including humans.

In our preparation, vasodilation in response to acetylcholine was completely abolished by treatment with N^G-nitro-L-arginine methyl ester, indicating the intact function of nitric oxide synthases in our experimental model. Previous studies of the isolated pressurized artery demonstrated that extraluminally applied acetylcholine can induce the activation of the endothelial enzyme concurrently with increased levels of endothelial Ca^{2+}, resulting in production of nitric oxide, which suggests the possible role of endothelial nitric oxide synthase in vasodilation produced by acetylcholine in our preparation.[21] However, we cannot rule out the possibility that in our preparation vasodilator substances induced the activation of neuronal nitric oxide synthase, because parenchymal arterioles in the brain slice are embedded in the neuronal tissues.

Previous studies demonstrated that ATP-sensitive K^+ channels can be activated by increased intracellular levels of adenosine diphosphate as well as decreased ATP levels, suggesting that these K^+ channels are closely related to intracellular energy status.[22] In addition to these nucleotides, intracellular levels of protons reportedly regulate ATP-sensitive K^+ channels.[23] Indeed, a recent study of cloned mouse ATP-sensitive K^+ channels expressed in smooth muscle cells demonstrated that

hypercapnia as well as intracellular acidosis is capable of augmenting these currents, suggesting that the cloned ATP-sensitive K^+ channels are regulated by intracellular protons.[24] In contrast, studies on isolated rat cerebral arteries indicated that carbon dioxide acts on ATP-sensitive K^+ channels from the outer side of the vascular smooth muscle cell membrane.[25] Therefore, it has been unclear whether the activation of these channels induced by hypercapnia is mediated by the changes of extracellular carbon dioxide levels themselves or by those of intracellular proton levels indirectly produced by extracellular carbon dioxide. Under the condition of normal pH, applied carbon dioxide produced no vasodilation of the parenchymal arterioles in our preparation, indicating that in the cerebral arteriole the extracellular change of proton levels, but not carbon dioxide molecule levels, is responsible for vasodilation produced by hypercapnia. This conclusion is supported by many previous studies demonstrating that decreased extracellular pH plays an important role in cerebral vasodilation in response to hypercapnic acidosis.[8,18,25] However, we cannot completely rule out the possibility that species and methodologic differences between these previous studies may contribute to the differential results regarding the mechanisms of hypercapnia action on ATP-sensitive K^+ channels expressed in vascular smooth muscle cells.

In the rat cerebral parenchymal microvessels, 3×10^{-7} M levcromakalim produced almost maximal vasodilation, whereas this concentration of the compound can induce only 40% dilation of isolated aorta and carotid artery in the rat.[26,27] A previous study of rats also demonstrated that in middle cerebral arteries with diameters of 200 to 300 μm, this concentration of levcromakalim induced up to 70% vasodilation.[28] These results indicate that ATP-sensitive K^+ channel openers can produce more potent vasodilator responses in cerebral arteries than conduit arteries and that, even in the cerebral arteries, the arterioles that peripherally exist in brain parenchyma are likely to respond better to these openers than are relatively proximal arteries. In the current study of cerebral parenchymal arterioles, both the endothelium-dependent agonist acetylcholine and the nitric oxide donor sodium nitroprusside had somewhat less potent effects than the effects seen in larger conduit arteries like the aorta.[29,30] These results suggest that the vasodilator response *via* nitric oxide may not be a major regulatory mechanism in peripheral cerebral circulation, compared with that in conduit arteries.

In the current study, we used prostaglandin $F_{2\alpha}$ as a vasoconstrictor substance to produce resting vascular tone to mimic the *in vivo* condition, because previous studies already demonstrated that this compound is capable of producing strong constriction of cerebral parenchymal as well as pial arterioles in the rat, indicating that these prostanoids play an important role in the regulation of cerebral parenchymal circulation.[31,32] Previous studies on rat brain parenchymal arterioles also documented that vasoconstrictor substances other than prostaglandin $F_{2\alpha}$, including potassium chloride or endothelin-1, can induce strong vasoconstriction.[10] These results suggest that the above-mentioned substances are similarly effective as vasoconstrictors in cerebral parenchymal arterioles.

One may argue that the parenchymal arterioles in the current study were not perfused *in vitro* and thus their reactivity to the vasodilator as well as vasoconstrictor substances may be altered as compared with the vasomotor reactivity *in vivo*. It is important to note that endothelial as well as nonendothelial control of vascular tone produced by shear stress is one of the determinants of vascular tone, especially in the resistance blood vessels including cerebral arterioles.[33,34] Therefore, it may be difficult to extrapolate directly our results to *in vivo* situations because we cannot rule out the possibility that the absence of intraluminal flow in cerebral arterioles may alter the behavior of our preparation. However, parenchymal arterioles in our preparation, similar to pressurized cerebral arterioles, responded well to vasoactive substances. Because the parenchymal arterioles of the brain slice are embedded in the intact neuronal tissues composed of both neuronal and glial cells attached to the arterioles, the resting vascular tone of these arterioles may be close to that seen under *in vivo* conditions. In addition, even under *in vivo* conditions, the intraluminal blood flow as well as the pressure of arterioles that exist in precapillary levels should be fairly low. These results appear to support the conclusion that the nonpressurized situation itself may not be a major problem during the study of arterioles embedded in the cerebral parenchyma.

A hypercapnia-induced increase in cerebral blood flow appears to be one of the mechanisms that modulates the acid–base balance in neuronal cells, resulting in maintenance of normal neuronal activity. Indeed, ATP-sensitive K^+ channels can be activated in ischemic situations including hypoxia, hypercapnia, and acidosis, indicating that these channels are involved in the regulation of cerebral blood flow during critical situations for neuronal cells.[7,8,35] Our study using cerebral parenchymal arterioles may provide a hint to improve cerebral microcirculation during anesthesia.

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References

1. Kitazono T, Faraci FM, Taguchi H, Heistad DD: Role of potassium channels in cerebral blood vessels. Stroke 1995; 26:1713–23
2. Nelson MT, Quayle JM: Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995; 268:C799–822
3. Faraci FM, Sobey CG: Role of potassium channels in regulation of cerebral vascular tone. J Cereb Blood Flow Metab 1998; 18:1047–63

4. Reid JM, Davies AG, Ashcroft FM, Paterson DJ: Effect of L-NMMA, cromakalim, and glibenclamide on cerebral blood flow in hypercapnia and hypoxia. Am J Physiol 1995; 269:H916–22

5. Faraci FM, Heistad DD, Mayhan WG: Role of large arteries in regulation of blood flow to brain stem in cats. J Physiol 1987; 387:115–23

6. Kontos AH, Wei EP, Jarrell Raper A, Patterson JL: Local mechanism of CO_2 action on cat pial arterioles. Stroke 1977; 8:226–9

7. Faraci FM, Breese KR, Heistad DD: Cerebral vasodilation during hypercapnia: Role of glibenclamide-sensitive potassium channels and nitric oxide. Stroke 1994; 25:1679–83

8. Kinoshita H, Katusic ZS: Role of potassium channels in relaxations of isolated canine basilar arteries to acidosis. Stroke 1997; 28:433–8

9. Staunton M, Drexler C, Schmid PG III, Havlik HS, Hudetz AG, Farber NE: Neuronal nitric oxide synthase mediates halothane-induced cerebral microvascular dilation. ANESTHESIOLOGY 2000; 92:125–32

10. Sagher O, Zhang X, Szeto W, Thai QA, Jin Y, Kasssell NF, Lee KS: Live computerized videomicroscopy of cerebral microvessels in brain slices. J Cereb Blood Flow Metab 1993; 13:676–82

11. Harkin CP, Schmeling WT, Kampine JP, Farber NE: The effects of hyper- and hypocarbia on intraparenchymal arterioles in rat brain slices. Neuroreport 1997; 8:1841–4

12. Meisheri KD, Khan SA, Martin JL: Vascular pharmacology of ATP-sensitive K^+ channels: Interactions between glyburide and K^+ channel openers. J Vasc Res 1993; 30:2–12

13. Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ, Garcia ML: Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion Buthus tamulus. J Biol Chem 1990; 265:11083–90

14. Bayerle-Eder M, Wolzt M, Polska E, Langenberger H, Pleiner J, Teherani D, Rainer G, Polak K, Eichler H-G, Schmetterer L: Hypercapnia-induced cerebral and ocular vasodilation is not altered by glibenclamide in humans. Am J Physiol 2000; 278:R1667–73

15. Kinoshita H, Katusic ZS: Nitric oxide and effects of cationic polypeptides in canine cerebral arteries. J Cereb Blood Flow Metab 1997; 17:470–80

16. Wang Q, Bryan RM Jr, Pelligrino DA: Calcium-dependent and ATP-sensitive potassium channels and the 'permissive' function of cyclic GMP in hypercapnia-induced pial arteriolar relaxation. Brain Res 1998; 793:187–96

17. Najarian T, Marrache AM, Dumont I, Hardy P, Beauchamp MH, Hou X, Peri K, Gobeil F Jr, Varma DR, Chemtob S: Prolonged hypercapnia-evoked cerebral hyperemia via K^+ channel- and prostaglandin E_2-dependent endothelial nitric oxide synthase induction. Circ Res 2000; 87:1149–56

18. Toda N, Hatano Y, Mori K: Mechanisms underlying response to hypercapnia and bicarbonate of isolated dog cerebral arteries. Am J Physiol 1989; 257: H141–6

19. Schmetterer L, Findl O, Strenn K, Graselli U, Kastner J, Eichler H-G, Wolzt M: Role of NO in the O_2 and CO_2 responsiveness of cerebral and ocular circulation in humans. Am J Physiol 1997; 273:R2005–12

20. White RP, Deane C, Vallance P, Markus HS: Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. Stroke 1998; 29:467–72

21. Ungvari Z, Sun D, Huang A, Kaley G, Koller A: Role of endothelial $[Ca^{2+}]_i$ in activation of eNOS in pressurized arterioles by agonists and wall shear stress. Am J Physiol 2001; 281:H606–12

22. Fujita A, Kurachi Y: Molecular aspects of ATP-sensitive K^+ channels in the cardiovascular system and K^+ channel openers. Pharmacol Ther 2000; 85:39–53

23. Quayle JM, Nelson MT, Standen NB: ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev 1997; 77:1165–232

24. Piao H, Cui N, Xu H, Mao J, Rojas A, Wang R, Abdulkadir L, Li L, Wu J, Jiang C: Requirement of multiple protein domains and residues for gating KATP channels by intracellular pH. J Biol Chem 2001; 276:36673–80

25. Tian R, Vogel P, Lassen NA, Mulvany MJ, Andreasen F, Aalkjaer C: Role of extracellular and intracellular acidosis for hypercapnia-induced inhibition of tension of isolated rat cerebral arteries. Circ Res 1995; 76:269–75

26. Kinoshita H, Iranami H, Kimoto Y, Dojo M, Hatano Y: Mild alkalinization and acidification differentially modify the effects of lidocaine or mexiletine on vasorelaxation mediated by ATP-sensitive K^+ channels. ANESTHESIOLOGY 2001; 95:200–6

27. Kinoshita H, Iranami H, Kimoto Y, Dojo M, Hatano Y: Cibenzoline has an inhibitory effect on vasorelaxation mediated by adenosine triphosphate-sensitive K^+ channels in the rat carotid artery. Anesth Analg 2001; 93:282–6

28. Zimmermann PA, Knot HJ, Stevenson AS, Nelson MT: Increased myogenic tone and diminished responsiveness to ATP-sensitive K^+ channel openers in cerebral arteries from diabetic rats. Circ Res 1997; 81:996–1004

29. Ishida K, Kinoshita H, Kobayashi S, Sakabe T: Thiopentone inhibits endothelium-dependent relaxations of rat aortas regulated by endothelial Ca^{2+}-dependent K^+ channels. Eur J Pharmacol 1999; 371:179–85

30. Kinoshita H, Ishikawa T, Hatano Y: Role of K^+ channels in augmented relaxations to sodium nitroprusside induced by mexiletine in rat aortas. ANESTHESIOLOGY 2000; 92:813–20

31. Harkin CP, Hudetz AG, Schmeling WT, Kampine JP, Farber NE: Halothane-induced dilatation of intraparenchymal arterioles in rat brain slices: A comparison to sodium nitroprusside. ANESTHESIOLOGY 1997; 86:885–94

32. Hoffman SW, Moore S, Ellis EF: Isoprostanes: Free radical-generated prostaglandins with constrictor effects on cerebral arterioles. Stroke 1997; 28:844–9

33. Ngai AC, Winn HR: Modulation of cerebral arteriolar diameter by intraluminal flow and pressure. Circ Res 1995; 77:832–40

34. Bryan RM Jr, Marrelli SP, Steenberg ML, Schildmeyer LA, Johnson TD: Effects of luminal shear stress on cerebral arteries and arterioles. Am J Physiol 2001; 280:H2011–22

35. Taguchi H, Heistad DD, Kitazono T, Faraci FM: ATP-sensitive K^+ channels mediate dilatation of cerebral arterioles during hypoxia. Circ Res 1993; 74: 1005–8