Vasodilatory Effect of Basic Fibroblast Growth Factor in Isolated Rat Cerebral Arterioles: Mechanisms Involving Nitric Oxide and Membrane Hyperpolarization

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Abstract

Basic fibroblast growth factor (bFGF), a potent mitogen, acutely dilates cerebral blood vessels and may be effective in reducing cerebral infarction. However, the vasodilatory mechanism, which may involve nitric oxide (NO), is not completely understood. This study investigated whether membrane hyperpolarization is also involved in this mechanism. Membrane potential (MP) of smooth muscle cells and vessel diameter of isolated intracerebral arterioles were simultaneously measured following extraluminal application of bFGF in rats. The involvement of NO and adenosine triphosphate-sensitive potassium (K_{ATP}) channels in bFGF-induced vasodilation and membrane hyperpolarization was evaluated using specific inhibitors, N^G -monomethyl-L-arginine (L-NMMA, 10^{-4} M) and glibenclamide (GB, 10^{-5} M), respectively. The resting MP was recorded at a mean value of -31.9 ± 4.5 mV. bFGF (1 to 1000 ng/ml) produced significant vasodilation and hyperpolarization. Treatment with L-NMMA caused vasoconstriction and significantly attenuated bFGF-induced vasodilation without affecting membrane hyperpolarization. In the presence of GB, the membrane potential was significantly depolarized but the vessel diameter was only marginally reduced, so bFGF-induced membrane hyperpolarization was inhibited while arteriolar dilation was attenuated. These results suggest that bFGF-induced vasodilation is mediated by a mechanism involving both NO and membrane hyperpolarization, and that membrane hyperpolarization is caused by the activation of K_{ATP} channels.

Key words: cerebral arterioles, basic fibroblast growth factor, membrane potential, vasodilation, N^G -monomethyl-L-arginine, glibenclamide

Introduction

Basic fibroblast growth factor (bFGF) was originally described as a potent mitogen for many types of cells such as fibroblasts, endothelial cells, and smooth muscle cells (SMCs).²⁵⁾ An acute hypotensive effect was observed in rats and rabbits, establishing a non-mitogenic function of bFGF as an affector of vascular tone.⁵⁾ Subsequent in vivo studies have shown that bFGF increased cortical blood flow¹⁹⁾ or dilated pial arterioles²⁰⁾ and rat basilar arteries,¹⁵⁾ suggesting that bFGF may contribute to the regulation of pial microcirculation^{19,20)} and the cerebral posterior circulation.¹⁵⁾ bFGF is found constitutively in neurons and astroglia⁸⁻¹⁰⁾ with receptors found in cerebral blood vessels,²⁶⁾ suggesting that bFGF may contrib-

ute to cerebrovascular regulation.²⁰⁾ bFGF is of clinical interest because of its ability to reduce cerebral infarction.^{1,12,22)} However, the mechanism of bFGF-induced vasodilation is not completely understood.

Previous studies have suggested that endothelium-derived nitric oxide (EDNO) is central in the bFGF-induced vasodilatory process,^{5,15,20)} but may not be solely responsible.⁵⁾ Electrophysiological studies have revealed that membrane hyperpolarization of SMCs, which is likely to be mediated by endothelium-derived hyperpolarizing factor (EDHF), is associated with EDNO-dependent vasodilation.¹⁷⁾ A possible explanation for this phenomenon is that an increase in the endothelial cytosolic concentration of Ca^{2+} ions in response to agonists triggers both EDHF and EDNO release.¹⁷⁾ bFGF increases intracellular Ca^{2+} ,¹⁸⁾ possibly by binding to a tyrosine kinase receptor.²⁾

This study investigated whether bFGF simultane-

ously causes vasodilation and membrane hyperpolarization of rat intracerebral arterioles, and, if so, whether nitric oxide (NO) contributes to these responses. In addition, the possible involvement of hyperpolarizing adenosine triphosphate-sensitive K^+ channels (K_{ATP} channels), as suggested by Cuevas et al.,⁵⁾ was tested using a selective K_{ATP} -channel inhibitor.

Materials and Methods

I. Preparation of arterioles

Animal experimentation was conducted in conformity with the regulations of the Institutional Animal Care and Use Committee. Methods for the isolation techniques of rat intracerebral arterioles were the same as those previously described in detail.⁶⁾ Briefly, 24 male Sprague-Dawley rats, weighing 300 to 400 g, were anesthetized with pentobarbital sodium (65 mg/kg, intraperitoneal administration) and 43 penetrating intracerebral arterioles, 39–76 μ m in diameter, were isolated surgically at 4°C from the first (M_1) portion of the middle cerebral arteries. Vessels were transferred to an organ bath (2.5 ml volume) on the stage of a Nikon-inverted microscope (Nikon, Tokyo) and were cannulated at 22°C with glass pipettes. The diameter of the vessel was monitored with a sensitive video camera (CCD 72 with GenIIsis intensifier; Dage-MTI, Michigan City, Ind., U.S.A.) and displayed on a monochrome monitor. After cannulation, arteriolar transmural pressure (TMP) was set to 60 mmHg via the cannulating pipette. Bath temperature was brought to 37.5°C, and the vessels were allowed to equilibrate for 45 minutes in an extraluminal bath of pH 7.3. The organ bath was continuously perfused at 0.5 ml/min with albumin-free physiological salt solution (PSS) using a peristaltic pump (model 203; Scientific Industries, Inc., Bohemia, N.Y., U.S.A.). During the equilibration period of 45 minutes, the vessels developed spontaneous tone, contracting to approximately 70% of their maximum passive diameters. Vessel responsiveness was then assessed by changing the extraluminal pH from 7.3 to 6.8 and 7.65. Vessels with poor spontaneous tone (<20% decrease in diameter) or poor pH response (<12% change of diameter after pH changes) were discarded at this stage.

II. Measurement of vessel diameter

A calibrated video-dimensional analyzer (Model 321; Colorado Video, Boulder, Colo., U.S.A.) was used to measure the luminal vessel diameter. To determine whether continuous changes in diameter occurred simultaneously with changes in membrane

potential (MP), a microcomputer-based imaging system (video resolution 320 $\times$ 200 pixel with 256 grayscale levels; Diamtrak; Montech Pty Ltd., Ringwood, Vic., Australia) was additionally used, which tracked the distance between either the two darkest vertical lines of the vessel wall (Diameter Tracking Image Vertical), or the two vertical lines dividing the areas of greatest dark/light contrast (Diameter Tracking Blood Vessel). We chose the function which offered greater tracking stability for each vessel with a resolution of 0.5 μ m/pixel. Diameter measurements were recorded on a strip chart recorder (Model 7WC8PA; Grass Instrument, Quincy, Mass., U.S.A.).

III. Electrophysiological studies

The methods used for measuring MP were the same as those described by Dietrich and Dacey.⁷⁾ Briefly, to obtain intracellular measurements, electrodes were pulled from filament glass capillaries (aluminosilicate or borosilicate, 1.0 mm outer diameter; Sutter Instruments, Novato, Calif., U.S.A.) and backfilled with 4 M potassium acetate (Sigma Chemical, St. Louis, Mo., U.S.A.) dissolved in distilled water. To allow lateral flexibility, the electrodes were connected to a short piece of potassium acetate-filled glass capillary containing elastic silicone tubing. This assembly was then inserted into a Teflon electrode holder with an Ag-AgCl wire for electric connection and attached to the preamplifier of a direct current (DC) amplifier (input impedance of 300 M Ω ; World Precision Instruments, Sarasota, Fla., U.S.A.). An Ag-AgCl wire in the organ bath was used as a reference electrode.

The amplifier output was monitored with an oscilloscope, which was connected to a strip-chart recorder for simultaneous recording of the potential traces. The electrode resistance and tip potentials were measured using the built-in test circuitry of the DC amplifier. Only electrodes with a resistance greater than 60 M Ω and a tip potential smaller than 100 mV were used. Tip potentials were compensated to 0 mV using the amplifier's DC offset. For the intracellular measurements, the preamplifier was mounted on a precision micromanipulator. Under microscopic control, the preamplifier with an attached electrode was advanced at an oblique angle of approximately 45° toward the cannulated arteriole until the electrode just touched the vessel wall. An ideal site had little or no visible adventitial tissue. SMCs were identified according to their location (the cells next to the clearly visible endothelium). The electrode was gently advanced and allowed to penetrate the vessel wall with a Piezo Stepper (Model 5172; Eppendorf, Westbury, N.Y., U.S.A.).

After the removal of the electrode, the observed potential had to return to the baseline for the MP measurement to be considered acceptable.

IV. Protocols

The experiments were divided into two parts. In the first protocol, after a stable MP was obtained under control conditions (TMP of 60 mmHg and temperature of 37°C in PSS of pH 7.3), both the MP and the vessel diameter were recorded. Then the bath PSS was exchanged with PSS containing bFGF (concentration ranging from 0.1 to 1000 ng/ml) using a peristaltic pump and continuous perfusion, and the vessel diameter and MP were simultaneously measured after equilibration. One to two bFGF concentrations were used per vessel and a dose/response curve for the vessel diameter and MP was constructed. In the second protocol, MP and vessel diameter were first measured under control conditions. Then, to determine the role of NO and K_{ATP} channels in bFGF-induced vasodilation and hyperpolarization, the vessels were pretreated with 10^{-4} M N^G-monomethyl-L-arginine (L-NMMA) or 10^{-5} M glibenclamide (GB). bFGF (0.1 to 100 ng/ml) was then applied to the arterioles as in the first protocol.

V. Drugs and solutions

All drugs used in this study were obtained from Sigma Chemical. bFGF (recombinant human, 147 amino acids) was dissolved in a 0.2 μ m-filtered solution containing 1% bovine serum albumin in buffered saline. GB was dissolved in a 5% dextrose solution containing 0.2 M NaOH and stored at a concentration of 10^{-2} M. L-NMMA was dissolved in distilled water. All stock solutions were stored at -20°C. All drugs were diluted in PSS and adjusted to a pH of 7.3 before use.

The PSS used in this preparation was a modified Ringer's solution, composed of 144 mM NaCl, 3.0 mM KCl, 2.5 mM $CaCl_2$, 1.5 mM $MgSO_4$, 2.0 mM 3-(N-morpholino)-propanesulfonic acid, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM ethylenediaminetetra-acetic acid, 1.21 mM NaH_2PO_4 , and 0.9–1.0 g/100 ml bovine serum albumin. The extraluminal solutions contained no albumin. One percent albumin was added to the intraluminal buffer.⁽⁶⁾ The pH of the solutions was maintained at 7.3 except when vessel responsiveness to pH change was assessed. The pH was adjusted by adding either HCl or NaOH.

VI. Statistical analysis

Values were given as mean $\pm$ SD. The control vessel diameter and MP were defined as the values recorded before each treatment. Diameter and MP

changes were then expressed as the percentage change from the control. Each group's mean diameter and MP within a treatment series were first compared by analysis of variance (ANOVA) and subsequently by the Fisher Protected Least Significant Difference multiple range test. The significance of the difference in vessel diameter and MP at a given dose of bFGF between non-treated control and L-NMMA- or GB-treated arterioles was evaluated by repeated measures ANOVA with the Student-Newmann-Keuls post-test. The ANOVA test was also applied to evaluate the difference in baseline vessel characteristics, such as vessel diameter and pH response to a pH of 6.8 and 7.65, between the arterioles used in each treatment series. Linear regression was used to evaluate the relationship between relative diameter change and MP. Regression slopes were compared using Student's t-test. bFGF-induced relative diameter and MP changes in L-NMMA- and GB-treated vessels were combined after normalization and compared against the untreated control vessels. The level of significance was $p < 0.05$.

Results

Under control conditions, 39 intraparenchymal arterioles (passive diameter of $75.2 \pm 14.8 \mu$ m) constricted to a spontaneous tone of $55.4 \pm 13.2 \mu$ m ($73.7 \pm 5.9\%$ of the passive diameter) with a resting MP of -31.9 ± 4.5 mV. Vessels dilated to $115.3 \pm 2.6\%$ of the control diameter when the pH of the extraluminal solution was lowered to 6.8, and constricted to $84.9 \pm 2.4\%$ of the control diameter when the pH was raised to 7.65. Baseline characteristics of the arterioles, such as control vessel diameter, percent spontaneous vasomotor tone, and pH response to 6.8 or 7.65, were not significantly different between the experimental groups (ANOVA, $p = 0.269$ – 0.444). Additionally, in four vessels the vehicles of bFGF (PSS containing 0.1% bovine serum albumin) and GB (PSS containing 0.005% dextrose) were applied to arterioles as a control and caused no change in vessel diameter ($100.3 \pm 1.4\%$ and $99.7 \pm 0.9\%$, respectively).

I. Effect of bFGF on vessel diameter and MP

Seventeen arterioles developed spontaneous tone ($71.6 \pm 5.0\%$ of maximum passive diameter) under control conditions with a resting MP of -32.3 ± 4.4 mV and a vessel diameter of $50.7 \pm 17.2 \mu$ m. One to 1000 ng/ml bFGF produced significant vasodilation and membrane hyperpolarization (Fig. 1). A concentration of 100 ng/ml bFGF ($n = 6$) caused maximum vasodilation from 51.5 ± 18.5 to $59.4 \pm 20.4 \mu$ m ($115.3 \pm 4.9\%$) accompanied by maximum

Fig. 1 Effect of basic fibroblast growth factor (bFGF) on membrane potential (MP) and vessel diameter. Bar graph and line graph indicate relative MP and vessel diameter (%), respectively. bFGF (1 to 1000 ng/ml) caused significant vasodilation and membrane hyperpolarization. Data are means $\pm$ SD. * $p < 0.05$ vs. corresponding 100% control. (n) indicates the number of observations.

Table 1 Comparison of arteriolar diameter and membrane potential (MP) with different treatments

	n	Diameter (μ m)	MP (mV)
Control	6	51.5 $\pm$ 18.5	-31.9 $\pm$ 2.8
+ bFGF (100 ng/ml)	6	59.4 $\pm$ 20.4*	-35.7 $\pm$ 3.1*
Control	12	56.4 $\pm$ 8.9	-30.1 $\pm$ 4.4
+ L-NMMA (10^{-4} M)	12	47.5 $\pm$ 8.3*	-28.7 $\pm$ 4.6
Control	10	59.0 $\pm$ 13.5	-33.3 $\pm$ 4.2
+ GB (10^{-5} M)	10	56.0 $\pm$ 13.1	-29.7 $\pm$ 3.6*

Values are mean $\pm$ SD. *Significant difference from that control ($p < 0.05$). bFGF: basic fibroblast growth factor, GB: glibenclamide, L-NMMA: N^G-monomethyl-L-arginine.

hyperpolarization from -31.9 ± 2.8 to -35.7 ± 3.1 mV ($111.9 \pm 8.5\%$) (Table 1, Fig. 1; example in Fig. 2A) and was thus used for the subsequent experiments.

II. Effect of L-NMMA on bFGF-induced vasodilation and hyperpolarization

After the development of spontaneous tone ($75.2 \pm 6.5\%$ of maximum passive vessel diameter), the mean vessel diameter and resting MP were $56.4 \pm 8.9 \mu\text{m}$ and -30.1 ± 4.4 mV, respectively, in 12 vessels (Table 1). Treatment with 10^{-4} M L-NMMA caused arterioles to constrict to $47.5 \pm 8.3 \mu\text{m}$ ($84.2 \pm 4.8\%$ of control) within 20 minutes (Table 1). MP slightly depolarized to -28.7 ± 4.6 mV ($95.3 \pm 3.2\%$), but was not significantly affected by this treatment (Table 1). In L-NMMA-treated arterioles, additional application of 100 ng/ml of bFGF increased the vessel diameter to 88.5% of control ($49.3 \pm 5.9 \mu\text{m}$) (Fig. 3). Addition of 10 and 100 ng/ml of bFGF caused a significant membrane hyperpolarization (maximum value of -32.4 ± 4.5 mV or $106.0 \pm 4.6\%$ of control at 100 ng/ml) (Fig. 3). An example recording is shown in Fig. 2B.

III. Effect of GB on bFGF-induced vasodilation and hyperpolarization

Under control conditions, 10 arterioles developed spontaneous tone to $75.5 \pm 6.2\%$ of their passive diameter. The control vessel diameter and resting MP were $59.0 \pm 13.5 \mu\text{m}$ and -33.3 ± 4.2 mV, respectively (Table 1). GB (10^{-5} M) had no significant effect on the vessel diameter ($56.0 \pm 13.1 \mu\text{m}$ or $94.9 \pm 4.4\%$ of control), but the MP depolarized to $89.1 \pm 3.4\%$ (-29.7 ± 3.6 mV) in 30 minutes (Table 1). Ten and 100 ng/ml of bFGF significantly dilated GB-treated arterioles (maximum dilation of $103.3 \pm 4.8\%$ of control at 10 ng/ml) but did not change the MP (Fig. 3). An example recording is shown in Fig. 2C.

IV. Relationship between bFGF-induced dilation and MP

Figure 4 shows the bFGF-induced relative diameter change versus the corresponding MP using data from Fig. 3. Linear regression confirms a strong correlation between the diameter change and the MP under control conditions ($r^2 = 0.998$). Addition of L-NMMA causes a left-shift of the data, indicating vessel constriction but no MP change. However, a strong correlation between bFGF-induced vessel diameter increase and membrane hyperpolarization remains ($r^2 = 0.886$). GB data are down-shifted, indicating depolarization, with a small but statistically not significant vasoconstriction. Addition of bFGF caused a significant vasodilation independent of MP change ($r^2 = 0.055$). Normalizing the L-NMMA- and GB-treated vessel data to 100% control and then combining the normalized bFGF-induced diameter and MP changes of the L-NMMA- and GB-treated

Fig. 2 (A) Recording showing 100 ng/ml basic fibroblast growth factor (bFGF)-induced changes in vessel diameter and membrane potential (MP). bFGF caused vasodilation accompanied by membrane hyperpolarization. (B) Recording showing the effect of N^G-monomethyl-L-arginine (L-NMMA) (10^{-4} M) on 100 ng/ml bFGF-induced changes in vessel diameter and MP. Treatment with L-NMMA constricted arterioles and blunted bFGF-induced vasodilation without affecting membrane hyperpolarization. (C) Recording showing the effect of glibenclamide (GB) (10^{-5} M) on 100 ng/ml bFGF-induced changes in vessel diameter and MP. Treatment with GB slightly depolarized MP, and inhibited the bFGF-induced vasodilation and hyperpolarization.

vessels resulted in a data set that was not different from untreated vessels. These data indicate that after blocking NO with L-NMMA, bFGF-induced vasodilation has a MP-dependent component, whereas after blocking K_{ATP} channels with GB, the vasodilation was MP-independent. Furthermore, combining the bFGF-induced diameter and MP changes of the L-NMMA- and GB-treated vessels resulted in a data set which was indistinguishable from the untreated controls. Thus, only two components, a MP-dependent component unmasked by L-NMMA and a MP-independent component unmasked by GB, are sufficient to describe the bFGF-induced vasodilation.

Discussion

This study has shown that bFGF caused vasodilation as well as membrane hyperpolarization dose-dependently at concentrations of 1 to 1000 ng/ml in rat isolated intracerebral arterioles. Treatment with L-NMMA, which inhibits the NO pathway, constricted arterioles without significantly changing MP. Furthermore, L-NMMA attenuated the bFGF-induced vasodilation, but did not affect the bFGF-induced hyperpolarization. Lastly, blocking K_{ATP} channels caused membrane depolarization, reduced bFGF-induced vasodilation, and inhibited hyperpolarization.

The observation of vasodilation in response to bFGF is consistent with previous *in vivo* data of pial arterioles obtained in cranial window experiments. The topical application of bFGF (5 to 200 ng/ml) produced dose-dependent arteriolar dilation to a maximum of 120% of control in rats.²⁰⁾ Superfusion of 25 g bFGF in rabbits, which is a higher concentration than that used in rats, increased cortical blood flow by 33%.¹⁹⁾ Together, these findings suggest that bFGF, which is widely distributed in the neonatal and adult central nervous system, is important in the regulation of both cortical and intracerebral microcirculation.

bFGF is synthesized in endothelial cells and vascular SMCs as well as in the neurons and astroglia.^{8-10,21,23)} bFGF production increases under certain pathological conditions⁹⁾ and may prevent neuronal injury. A number of studies have shown the protective effect of bFGF in cerebral ischemia. For example, in a focal ischemia model in the rat, intravenous administration of bFGF reduced the infarct size.^{1,12,22)} In models of middle cerebral artery occlusion in rat, treatment with bFGF demonstrated a decrease in infarct size.²²⁾ However, the mechanism of infarct reduction by bFGF remains poorly understood. One possible explanation is that bFGF in-

Fig. 3 (left) Effect of N^G-monomethyl-L-arginine (L-NMMA) (10^{-4} M) and glibenclamide (GB) (10^{-5} M) on vasodilation induced by basic fibroblast growth factor (bFGF). Pretreatment with L-NMMA (closed columns) and GB (shaded columns) both decreased bFGF-induced vasodilation. (right) Effect of L-NMMA and GB on hyperpolarization induced by bFGF. Pretreatment with GB (shaded columns) significantly inhibited this hyperpolarization, whereas L-NMMA (closed columns) did not affect membrane hyperpolarization. Data are means $\pm$ SD. * $p < 0.05$ vs. untreated (bFGF 0 ng/ml, 100% control) (open columns), † $p < 0.05$ vs. pretreated with L-NMMA (bFGF 0 ng/ml) or GB (bFGF 0 ng/ml). (n) indicates the number of observations.

Fig. 4 Correlation of basic fibroblast growth factor (bFGF)-induced relative diameter and membrane potential (MP) change in untreated vessels (control, closed circles) and vessels treated with N^G-monomethyl-L-arginine (L-NMMA) (open circles) and glibenclamide (GB) (closed triangles). bFGF concentration increases from left to right (bFGF 0 to 100 ng/ml, see Fig. 3) for each series. There is a strong correlation between bFGF-induced diameter and MP change. L-NMMA treatment caused vasoconstriction with no effect on MP, but the bFGF-induced vasodilation remained strongly MP dependent. GB hyperpolarized the vessels with small vasoconstriction, but the bFGF-induced vasodilation is MP independent. Normalizing and combining the L-NMMA + GB diameter data versus the corresponding MP data resulted in a data set (open triangles) indistinguishable from the control (Student's t-test). Thus, the vasodilatory effect of bFGF can be described by only two mechanisms: one involving nitric oxide which is MP independent and one involving adenosine triphosphate-sensitive potassium channels which is MP dependent. straight lines: Linear regression, curved lines: 95% confidence interval.

creases regional cerebral blood flow (rCBF), causing the neuroprotective effect encountered after stroke. Indeed, reduction in infarct size was accompanied by an increased rCBF in the peri-infarct area.²²⁾ Furthermore, bFGF has a potent neurotrophic function²⁵⁾ and this may cause the therapeutic effect on cerebral infarction. This protective effect may be independent of rCBF increase.¹⁾ Further studies will be required to clarify these issues. Recently, the level of bFGF in bovine aortic SMCs is proportional to the amount of shear stress applied to endothelial cells, suggesting that a hemodynamic factor is a possible regulator of bFGF synthesis.²¹⁾ If this relationship was to exist in cerebral arterioles, vascular bFGF production may regulate rCBF in conjunction with hemodynamic conditions.

Extraluminal application of bFGF caused vasodilation accompanied by membrane hyperpolarization. Mean hyperpolarization of -2.5 mV at 100 ng/ml of bFGF was accompanied by a 10% increase in vessel diameter. In our previous study,⁷⁾ the relationship between relative change in arteriolar diameter (%) and MP (mV) was evaluated as a linear correlation, resulting in changes of -6.6 mV/ 10% for extraluminal pH change and -4.2 mV/ 10% for potassium chloride. However, this ratio calculated from the present data obtained in L-NMMA-treated vessels (with the MP-dependent dilatory mechanism unmasked) increases to -7.7 mV/ 10% diameter change, which correlates with our previous data.⁷⁾

Treatment with L-NMMA constricted the arterioles,¹⁶⁾ but had little effect on the resting MP. Furthermore, L-NMMA inhibited bFGF-induced vasodilation without affecting membrane hyperpolarization. These findings indicate that arteriolar NO is involved in basal vascular tone¹⁶⁾ and mediates vasodilation due to bFGF, but is not involved in membrane hyperpolarization. Recent studies have shown that EDNO-dependent vasodilators, such as bradykinin and acetylcholine, hyperpolarize the membrane of SMCs in rabbit cerebral arteries and guinea-pig arteries.^{3,17)} However, the nature of the mediating factor causing the hyperpolarization is controversial.¹⁷⁾ A number of previous studies^{4,17)} have suggested the possible involvement of EDHF (yet to be identified), which is distinct from EDNO since NO synthase blockers and NO do not change MP. More recently, EDHF was suggested to be a cytochrome P450-derived arachidonic acid metabolite.¹¹⁾ Our electrophysiological study also suggests that another EDNO-independent pathway involved with hyperpolarization exists in the bFGF-induced vasodilatory process.

The K_{ATP} channel is known to be essential for

maintaining systemic arteriolar tone.^{13,14)} Previous *in vivo* studies of dogs¹³⁾ found that K_{ATP} channels were 55% responsible for basal coronary blood flow. Superfusion with 2 M GB constricted hamster muscle arterioles to approximately 72% of control.¹⁴⁾ Compared with these data in systemic arteries, treatment with GB in our study slightly constricted arterioles to 94.9% and depolarized the membrane of SMCs by 3.6 mV. Although the possibility exists that a non- K_{ATP} channel effect of GB¹⁴⁾ contributed to our observations, this finding may suggest that K_{ATP} channels contribute to basal cerebral arteriolar tone and resting MP. Furthermore, our results are in accordance with other studies in cerebral arterioles²⁴⁾ which indicate that the contribution of the K_{ATP} channel to basal cerebral microcirculatory tone is small, because the K_{ATP} channels are mostly inactive at rest.

Combining the bFGF-induced diameter and MP changes of the L-NMMA- and GB-treated vessels after normalization resulted in a similar data set to that for the untreated controls, suggesting that bFGF dilated arterioles by a MP-independent mechanism and membrane hyperpolarization. The underlying mechanisms behind membrane hyperpolarization by endothelium-dependent vasodilators are incompletely understood. In this study, treatment with GB inhibited hyperpolarization by bFGF, which suggests that hyperpolarization is mediated through the activation of K_{ATP} channels. Several recent studies^{4,17)} have demonstrated that K^+ channels are involved in endothelium-dependent hyperpolarization, with EDHF being one possible channel activator. The responsible K^+ -channel subtypes seem to differ among artery types, species, and agonists.¹⁷⁾ For example, Ca^{2+} -activated K^+ channels may be involved in bradykinin-induced dilation of bovine and porcine coronary arteries.¹¹⁾ Other studies of rabbit cerebral arteries³⁾ have reported that GB inhibited hyperpolarization in response to acetylcholine. This study did not examine the effect of other K^+ -channel subtype inhibitors in bFGF-induced hyperpolarization, and therefore we could not exclude the possibility of their involvement in this response. Also, we did not test arterioles in the posterior circulation or in different rat species. Further study is required to determine the mechanism behind the observed hyperpolarization.

bFGF caused vasodilation accompanied by membrane hyperpolarization. L-NMMA produced vasoconstriction and attenuated bFGF-induced vasodilation without affecting membrane hyperpolarization. Treatment with GB slightly constricted arterioles and caused membrane depolarization. In GB-treated arterioles, bFGF-induced vasodilation

was attenuated and hyperpolarization was inhibited. Our findings suggest that bFGF-induced vasodilation is mediated by a mechanism involving NO which is independent of MP. The simultaneous membrane hyperpolarization may be due to an activation of K_{ATP} channels. Thus, only two mechanisms, a NO-dependent mechanism and a K_{ATP} -dependent mechanism, are sufficient to describe the bFGF-induced vasodilation in rat cerebral arterioles.

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Commentary

In this interesting experimental study, Kajita et al. have described the mechanism involving both nitric oxide (NO) and membrane hyperpolarization in the vasodilatory effect of basic fibroblast growth factor (bFGF). They also presented results suggesting that membrane hyperpolarization is caused by the activation of adenosine triphosphate-sensitive K⁺ channels (K_{ATP} channels). bFGF is of clinico-physiological interest because of its ability to acutely dilate cerebral vessels and reduce cerebral infarction. The mechanisms of vasodilatation and regulation of cerebral vessels induced by bFGF have been investigated in some previous reports. However, the true mechanisms of these processes are not completely understood. The authors organized a theoretical study and presented the results suggesting the vasodilatory effect involves NO and membrane hyperpolarization. bFGF was originally described as a potent mitogen for many types of cells such as fibroblast, endothelial cells and smooth muscles, and its significance has been also noticed in other clinical categories including blood supply and enlargement of brain tumor. This study may be extremely important for understanding the dilatory mechanism of cerebral vessels, and additionally contribute to develop studies related to bFGF in other fields.

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The authors have demonstrated that basic FGF (bFGF) dilates cerebral arterioles through the nitric oxide pathway and membrane hyperpolarization, and furthermore that these mechanisms are independent. The mechanism of bFGF-induced vascular dilation was previously postulated but not confirmed for the cerebral arterioles. This study may help to elucidate the mechanisms of bFGF-induced brain protection against ischemic insults. In addition, it is very interesting that both of the mechanisms they have shown consist of two different endothelial derived factors, so the function of bFGF is closely related to the vascular endothelium where a certain amount of bFGF is produced.

The method they used was isolated arterial preparation and this is well adjusted to test the hypothesis of this study, because the techniques originated from the co-author, Dr. Dacey. While the results they have shown are quite acceptable and compatible with the previous literature, the results of this study should not be overestimated. As the authors mentioned in the paper, bFGF causes hypotension due to its vasodilation effect and the cerebral arteries in the living animal are affected by various factors such as systemic blood pressure, pH, and other vasoactive substances which may affect endothelial functions.

The results of this study are very important for the exploration of the cerebral blood flow regulation. However, further study will be important to investigate the mechanism by which dose bFGF acts on the cerebral arteries in vivo, which is a very complex environment.

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The authors have demonstrated two mechanisms of basic FGF-induced vasodilation in rat cerebral arterioles, a NO-dependent mechanism and a K_{ATP}-dependent mechanism. Although many previous studies have showed the basic FGF-induced vasodilation, the underlying mechanism was poorly understood. To elucidate the mechanism of this contribution of basic FGF to cerebrovascular regulation, the authors conducted electrophysiological studies and demonstrated the subcellular mechanism. The experimental concept is acceptable. The results shown are acceptable and compatible with the previous study. In this paper, the

authors have shown very important data, which may contribute to the management of cerebral ischemia.

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