ATP-Sensitive K⁺ Channels Mediate Dilatation of Cerebral Arterioles During Hypoxia

Hisao Taguchi, Donald D. Heistad, Takanari Kitazono, Frank M. Faraci

Abstract We tested the hypothesis that dilatation of cerebral arterioles during hypoxia is mediated by activation of ATP-sensitive K⁺ channels. The diameter of pial arterioles was measured through a closed cranial window in anesthetized rabbits. Topical application of aprikalim (10⁻⁷ mol/L), a direct activator of ATP-sensitive K⁺ channels, dilated pial arterioles by 18±3% (mean±SEM). Glibenclamide (10⁻⁶ mol/L), an inhibitor of ATP-sensitive K⁺ channels, virtually abolished aprikalim-induced vasodilatation. When arterial Po2 was reduced from 129±3 to 25±1 mm Hg, the diameter of cerebral arterioles increased by 66±9% (P<.05). Glibenclamide inhibited dilatation of pial arterioles during hypoxia by 46±5% (P<.05). In contrast, vasodilatation in response to sodium nitroprusside was not altered by glibenclamide. Topical application of adenosine (10⁻⁴ mol/L) increased arteriolar diameter by 21±4%. Glibenclamide did not affect adenosine-induced vasodilatation. These findings suggest that dilatation of cerebral arterioles in response to hypoxia is mediated, in part, by activation of ATP-sensitive K⁺ channels. (Circ Res. 1994; 74:1005-1008.)

Key Words • cerebral microcirculation • aprikalim • glibenclamide • adenosine • sodium nitroprusside

Hypoxia produces relaxation of cerebral blood vessels and marked increases in cerebral blood flow.1-4 The mechanism that mediates hypoxia-induced vasodilatation in the cerebral circulation is poorly defined, although several studies suggest that adenosine may play an important role.5-7 Activation of ATP-sensitive K⁺ channels appears to be a major mechanism that mediates vasodilatation.8-11 In the coronary circulation, dilatation in response to hypoxia appears to be mediated by activation of ATP-sensitive K⁺ channels.12 The first goal of the present study was to test the hypothesis that activation of ATP-sensitive K⁺ channels mediates dilatation of cerebral arterioles during hypoxia in vivo. We attempted to determine whether dilatation of cerebral arterioles in response to hypoxia is attenuated by glibenclamide, a selective inhibitor of ATP-sensitive K⁺ channels.9,10 Several lines of evidence suggest that adenosine may contribute to cerebral vasodilatation during hypoxia. In some blood vessels, adenosine may activate ATP-sensitive K⁺ channels.12-14 Thus, the second goal of the present study was to determine whether vasodilatation in response to adenosine is attenuated by glibenclamide.

Materials and Methods

Animal Preparation

Experiments were performed on 31 male New Zealand White rabbits (2.3 to 2.7 kg) anesthetized with pentobarbital (30 mg/kg IV). Pentobarbital was supplemented intravenously at a rate of 20 to 40 mg/kg per hour. Pressure was applied to a paw approximately every 30 minutes to evaluate the depth of anesthesia. When changes in blood pressure were observed, additional pentobarbital was administered intravenously. A tracheotomy was performed, and the animals were mechanically ventilated with room air and supplemental oxygen. Skeletal muscle paralysis was produced with gallamine triethiodide (4 mg/kg IV).

A catheter was placed in a femoral artery to measure blood pressure and obtain arterial blood samples. A femoral vein was cannulated for infusion of drugs. Arterial blood gases were monitored and maintained at normal levels, except during the experimental induction of hypoxia. Core body temperature was measured continuously and maintained at 38±0.5°C with a heating pad.

A closed cranial window was placed over the left parietal cortex as described in detail previously.15 The cranial window was filled with artificial cerebrospinal fluid (temperature, 37.5°C; ionic composition [mmol/L]: NaCl 132, KCl 2.95, CaCl₂ 1.71, MgCl₂ 0.65, NaHCO₃ 24.6, and d-glucose 3.69). Cerebrospinal fluid had a pH of 7.36±0.04, a PaCO₂ of 35±2 mm Hg, and a PaO₂ of 62±3 mm Hg.

We studied one vessel in each animal. Diameters of pial arterioles were measured with a microscope equipped with a television camera coupled to a monitor and an image-shearing device (model 907, Instrumentation for Physiology and Medicine, San Diego, Calif). The images were recorded on videotape for later analysis.

Experimental Protocol

Diameters of cerebral arterioles were measured under control conditions and during hypoxia. Hypoxia was induced by administering 8% O₂ to the animals. Diameters of cerebral arterioles were measured immediately before and during 5 minutes of hypoxia. After discontinuing the hypoxic exposure, the animals were ventilated with room air and supplemental O₂, and arteriolar diameter returned to baseline within a few minutes. We waited a minimum of 30 minutes before repeating the hypoxic exposure. Arterial blood was sampled for analysis of blood gases before and during hypoxia.

We also examined changes in diameters of pial arterioles in response to the topical application of 10⁻⁷ and 10⁻⁸ mol/L aprikalim (a direct activator of ATP-sensitive K⁺ channels), 10⁻⁴ and 10⁻³ mol/L sodium nitroprusside, and 10⁻³ and 10⁻⁴ mol/L adenosine. The drugs were mixed in artificial cerebrospinal fluid and applied within the cranial window for 5

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minutes. The diameters reached a maximum =3 minutes after application of these vasodilators and were maintained for at least 3 minutes. Thus, diameters of cerebral arterioles were measured immediately before and during the last minute of application of each agonist.

We examined the effects of glibenclamide, an inhibitor of ATP-sensitive K+ channels,9-10 on vasodilatation during hypoxia or in response to specific agonists. Glibenclamide (10^-6 mol/L) was dissolved in dimethyl sulfoxide (DMSO) and applied 5 minutes before and during exposure to hypoxia. Because the concentration of DMSO in the glibenclamide solution was 0.1%, we used the same concentration of DMSO in the control experiments. This concentration of DMSO did not affect the diameters of the vessels. After application of a specific agonist was stopped, vessel diameter returned to baseline within a few minutes. The order of application of agonists was randomized.

**Statistical Analysis**

All values are expressed as mean±SEM. A paired t test was used to compare absolute values under control conditions and during interventions, and Wilcoxon's test was used to compare percentage changes. A value of P<.05 was considered significant.

**Results**

**Responses of Pial Arterioles to Aprikalim**

Under control conditions, diameters of the pial arterioles were 56±5 μm. Topical application of aprikalim produced concentration-related increases in the diameters of cerebral arterioles (Fig 1). Glibenclamide (10^-6 mol/L), which had no significant effect on baseline diameter, almost abolished dilator responses of pial arterioles to aprikalim (Fig 1).

Sodium nitroprusside also produced dilatation of pial arterioles (Fig 2). In contrast to the effects on responses to aprikalim, glibenclamide did not attenuate vasodilatation in response to sodium nitroprusside (Fig 2). These findings suggest that ATP-sensitive K+ channels are functional in cerebral arterioles of the rabbit in vivo.

**Effects of Glibenclamide on Hypoxia-Induced Vasodilatation**

Exposure of rabbits to 8% O2 reduced PaO2 from 129±3 to 25±1 mm Hg without affecting pH (control, 7.40±0.01; hypoxia, 7.43±0.01) or PacO2 (control, 35±1 mm Hg; hypoxia, 36±1 mm Hg). Hypoxia did not produce a significant change in arterial blood pressure (control, 89±3 mm Hg; hypoxia, 91±4 mm Hg) (P>.05).

Hypoxia produced marked dilatation of cerebral arterioles (Fig 3). Diameters of cerebral arterioles began to increase within 30 seconds after the beginning of hypoxia and reached a maximum increase in arteriolar diameter of 66±9% at 5 minutes. In animals that served as time controls (n=6), there was no significant difference in the increase in vessel diameter between the first and second hypoxic exposures (P>.05; data not shown).

Glibenclamide (10^-6 mol/L) significantly inhibited dilator responses of pial arterioles during hypoxia (P<.05, Fig 3). The increase in diameter of cerebral arterioles was inhibited by 46±5% at 5 minutes. Severity of hypoxia did not differ between control (PaO2, 25±1 mm Hg) and glibenclamide-treated (PaO2, 25±1 mm Hg) animals. These results suggest that dilatation of pial arterioles during hypoxia is mediated, in part, by activation of ATP-sensitive K+ channels.

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**Fig 1.** Bar graph showing the effects of glibenclamide on aprikalim-induced dilatation of pial arterioles. Changes in diameters of pial arterioles were measured in response to aprikalim under control conditions and in the presence of glibenclamide (10^-6 mol/L). Values are mean±SEM in five rabbits. *P<.05 compared with control.

**Fig 2.** Bar graph showing the effects of glibenclamide on sodium nitroprusside-induced dilatation of pial arterioles. Changes in diameters of pial arterioles were measured in response to sodium nitroprusside under control conditions and in the presence of glibenclamide (10^-6 mol/L). Values are mean±SEM in six rabbits.

**Fig 3.** Bar graph showing the effects of glibenclamide on hypoxia-induced dilatation of pial arterioles. Changes in diameters of pial arterioles were measured in response to hypoxia (8% oxygen) under control conditions and in the presence of glibenclamide (10^-6 mol/L). Values are mean±SEM in eight rabbits. *P<.05 compared with control.
Response of Pial Arterioles to Adenosine

Topical application of adenosine produced increases in diameters of pial arterioles (Fig 4). Glibenclamide (10^{-6} mol/L) did not attenuate dilatation of pial arterioles in response to adenosine (P > .05, Fig 4). These results suggest that adenosine may not contribute to the activation of ATP-sensitive K^+ channels during hypoxia.

Discussion

The major new finding in the present study is that hypoxia produces marked dilatation of cerebral arterioles in vivo that appears to be mediated, in part, by activation of ATP-sensitive K^+ channels. In addition, we found that dilatation of cerebral arterioles in response to adenosine is not mediated by activation of ATP-sensitive K^+ channels. Adenosine may not contribute to activation of ATP-sensitive K^+ channels in cerebral arterioles during hypoxia.

ATP-Sensitive K^+ Channels in Cerebral Arterioles

Activation of ATP-sensitive K^+ channels is an important mechanism of vasodilatation.\(^8\)-\(^11\) Activation of ATP-sensitive K^+ channels produces relaxation of cerebral arteries in vitro.\(^16\),\(^17\) Aprikalim, a direct activator of ATP-sensitive K^+ channels,\(^8\) dilates the basilar artery and pial arterioles of the rat in vivo.\(^18\),\(^19\) In the present study, we observed dilatation of pial arterioles in the rabbit in response to aprikalim in vivo. Glibenclamide, which is considered to be a selective inhibitor of ATP-sensitive K^+ channels at the concentrations used in the present study,\(^9\),\(^10\) almost abolished aprikalim-induced vasodilatation. These findings suggest that ATP-sensitive K^+ channels are functional in cerebral arterioles in vivo.

Aprikalim-induced dilatation of cerebral arterioles appears to be mediated exclusively by activation of ATP-sensitive K^+ channels. Responses to aprikalim are inhibited by glibenclamide, a selective inhibitor of ATP-sensitive K^+ channels.\(^8\),\(^10\) Dilatation of cerebral arterioles in response to aprikalim is not attenuated by an inhibitor of nicotinic oxidation synthase or inhibitors of other K^+ channels (apamin and charybdotoxin).\(^19\)

We considered the possibility that glibenclamide or aprikalim might affect neuronal activity or cerebral metabolism. The lack of effect of glibenclamide on vessel diameter under control conditions suggests that glibenclamide does not affect local neuronal activity or metabolism. It also seems unlikely that aprikalim alters local neuronal activity. If aprikalim activated K^+ channels in neurons, the response probably would be hyperpolarization. Such an effect would decrease neuronal activity or neurotransmitter release, which would result in constriction of the arterioles. Thus, dilatation of cerebral arterioles in response to aprikalim probably is not secondary to an effect on neurons.

Role of ATP-Sensitive K^+ Channels in Hypoxia-Induced Vasodilatation

Hypoxia relaxes cerebral blood vessels and produces marked increases in cerebral blood flow. The level of hypoxia in the present study dilated pial arterioles by \(\approx 65\%\). Because arterial Pco_2, pH, and blood pressure did not change significantly during hypoxia, we can exclude the possibility that factors in addition to hypoxia accounted for dilator responses of pial arterioles in these experiments.

Several vasoactive substances including endothelium-derived relaxing factor (EDRF or nitric oxide) or prostaglandins could potentially mediate hypoxia-induced vasodilatation. However, inhibition of nitric oxide synthase does not have a significant effect on increases in cerebral blood flow during hypoxia.\(^20\),\(^22\) Inhibition of prostaglandin synthesis with indomethacin also does not affect dilator responses of cat pial arterioles during hypoxia in vivo.\(^23\)

Recently, it was suggested that activation of ATP-sensitive K^+ channels is involved in hypoxia-induced vasodilatation in the heart.\(^12\) This conclusion was based on the finding that glibenclamide inhibits coronary vasodilatation during hypoxia.\(^12\) In the present study, glibenclamide inhibited hypoxia-induced vasodilatation without affecting vasodilatation in response to nitroprusside. These results suggest that hypoxia-induced dilatation of the pial arterioles is mediated, in part, by activation of ATP-sensitive K^+ channels. A recent study using isolated cerebral vascular muscle has suggested that severe hypoxia activates glibenclamide-sensitive K^+ channels in vitro.\(^24\)

Glibenclamide inhibits activation of both Ca^{2+}-activated and ATP-sensitive K^+ channels in response to cromakalim or pinacidil in the rabbit aorta in vitro.\(^25\) Using a patch-clamp technique, the authors demonstrated that 10 \(\mu\)mol/L glibenclamide reversed the pinacidil or cromakalim-induced increase in the open probability of Ca^{2+}-activated K^+ channels from aortic smooth muscle. The concentration of glibenclamide used in the present study (1 \(\mu\)mol/L) was lower than the concentration (10 \(\mu\)mol/L) used previously.\(^25\) It is important to note, in addition, that several other studies suggest that glibenclamide does not inhibit Ca^{2+}-dependent K^+ channels.\(^26\) Nevertheless, we cannot completely exclude the possibility that activation of Ca^{2+}-activated K^+ channels may contribute to hypoxia-induced dilatation of cerebral arterioles.
Role of Adenosine in Activation of ATP-Sensitive K⁺ Channels

Extracellular levels of adenosine increase substantially during hypoxia in the brain. Vasodilatation in response to adenosine appears to be mediated by activation of ATP-sensitive K⁺ channels in some noncerebral arteries. Thus, we anticipated that adenosine may produce dilatation in cerebral vessels via activation of ATP-sensitive K⁺ channels. Adenosine produced dilatation of pial arterioles, but the response was not attenuated by glibenclamide. These results suggest that dilatation of pial arterioles in response to adenosine is not mediated by activation of ATP-sensitive K⁺ channels. Although adenosine may contribute to vasodilatation in brain during hypoxia, our data suggest that adenosine-induced dilatation of cerebral arterioles is not mediated by the activation of ATP-sensitive K⁺ channels.

The opening of ATP-sensitive K⁺ channels produces hyperpolarization of vascular muscle and relaxation. ATP-sensitive K⁺ channels are inhibited by the binding of ATP at intracellular sites. Intracellular ADP blocks this inhibition, possibly by competitive binding in place of ATP. Thus, either decreases in [ATP], or increases in the [ADP]-to-[ATP] ratio may increase the opening of ATP-sensitive K⁺ channels. Alterations in the levels of adenosine nucleotides during hypoxia may result in activation of ATP-sensitive K⁺ channels in vascular muscle. However, we cannot exclude the possibility that factors other than adenosine that are released from tissues may contribute to the activation of ATP-sensitive K⁺ channels during hypoxia.

Because glibenclamide attenuated but did not abolish vasodilatation during hypoxia in the present study, mechanisms in addition to activation of ATP-sensitive K⁺ channels appear to contribute to vasodilator responses to hypoxia. As discussed above, EDRF or prostanoioids may not contribute to hypoxia-induced vasodilatation. Adenosine that is released from tissues during hypoxia may produce vasodilatation via a mechanism that is independent of ATP-sensitive K⁺ channels. We also cannot exclude the possibility that activation of other K⁺ channels may contribute to vasodilatation in response to hypoxia that is not inhibited by glibenclamide.

In summary, dilator responses of cerebral arterioles during hypoxia are mediated, in part, by activation of glibenclamide-sensitive K⁺ channels in vivo. Adenosine-induced dilatation of the pial arterioles does not appear to be mediated by activation of these K⁺ channels.

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