Cerebral Vasodilation During Hypercapnia
Role of Glibenclamide-Sensitive Potassium Channels and Nitric Oxide

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Background and Purpose The purpose of these experiments was to examine mechanisms by which hypercapnia produces vasodilatation in brain. We examined the hypothesis that dilatation of cerebral arterioles during hypercapnia is dependent on activation of ATP-sensitive potassium channels and formation of nitric oxide.

Methods Diameters of cerebral arterioles were measured using a closed cranial window in anesthetized rabbits. Changes in diameter of arterioles were measured in response to topical application of acetylcholine and sodium nitroprusside and during two levels of systemic hypercapnia.

Results Increasing arterial PCO₂ from 32±1 mm Hg (mean±SE) to 54±1 and 66±1 mm Hg dilated cerebral arterioles by 25±3% and 38±5%, respectively, from a control diameter of 93±3 μm. The response to the low level of hypercapnia was attenuated (25±3% versus 16±4%, P<.05) by glibenclamide (1 μmol/L), an inhibitor of ATP-sensitive potassium channels. Vasodilatation in response to the high level of hypercapnia was not affected by glibenclamide. Increases in arteriolar diameter in response to sodium nitroprusside were not inhibited by glibenclamide. NOS-nitro-L-arginine (300 μmol/L), an inhibitor of nitric oxide synthase, completely inhibited dilatation of cerebral arterioles in response to the low level of hypercapnia and inhibited vasodilatation during the high level of hypercapnia by 66%.

Conclusions Thus, activation of glibenclamide-sensitive potassium channels may contribute to dilatation of cerebral arterioles during hypercapnia. Cerebral vasodilatation during hypercapnia is dependent in large part on production of nitric oxide. (Stroke. 1994;25:1679-1683.)

Key Words • acetylcholine • hypercapnia • nitric oxide • vasodilation • rabbits

Hypocapnia is a potent dilator of cerebral blood vessels through a mechanism that requires development of extracellular acidosis. The overall goal of the present study was to further examine mechanisms that mediate cerebral vasodilatation during hypercapnia.

Hyperpolarization of vascular muscle in response to activation of potassium channels is a major mechanism of relaxation of blood vessels. Activity of ATP-sensitive potassium channels may reflect changes in the cellular metabolic state and may be activated by reductions in PO₂ and pH. We have provided evidence that cerebral vasodilatation during hypoxia is mediated by activation of ATP-sensitive potassium channels. The first goal of the present study was to examine the hypothesis that dilatation of cerebral arterioles during hypercapnia, which produces acidosis, is mediated by ATP-sensitive potassium channels. We determined whether glibenclamide, which is considered to be a selective inhibitor of ATP-sensitive potassium channels, attenuates cerebral vasodilatation during hypercapnia.

Several studies performed in rats suggest that increases in cerebral blood flow during hypercapnia are dependent on production of nitric oxide (NO). How-ever, it is unclear whether NO mediates increases in cerebral blood flow during hypercapnia in other species. Species differences in the mechanism that mediates cerebral vasodilatation during hypercapnia would not necessarily be surprising. For example, indomethacin has been reported to inhibit increases in cerebral blood flow during hypercapnia in rats but not cerebral vasodilatation during hypercapnia in rabbits or cats. Thus, the second goal of the present study was to examine the hypothesis that dilatation of cerebral arterioles during hypercapnia in rabbits is dependent on formation of NO.

Methods

Animal Preparation

Experiments were performed on New Zealand White rabbits (2.5 to 3.5 kg) that were anesthetized with pentobarbital sodium (35 to 40 mg · kg⁻¹ · IV). Pentobarbital was supplemented regularly at approximately 10 mg · kg⁻¹ · hr⁻¹ · IV. The trachea was cannulated, and the animals were ventilated mechanically with air and supplemental oxygen. Arterial blood gases were monitored and maintained within normal limits throughout the experiment. A femoral artery was cannulated for measurement of systemic pressure and to sample arterial blood. A femoral vein was cannulated for infusion of drugs. To control ventilation effectively during hypercapnia, it was necessary to produce paralysis of skeletal muscle using gallamine triethiodide (5 mg · kg⁻¹ · IV).

Rabbits were placed in a head holder, and a closed cranial window was placed over the parietal cortex as described previously. The cranial window was filled with artificial cerebrospinal fluid (CSF) warmed to 37°C. Diameters of pial arterioles were measured using a microscope equipped with a video camera coupled to a video monitor. Images were re-

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corded on videotape, and vessel diameters were measured later with an image analyzer.

**Experimental Protocol**

Four groups of animals were studied. In group 1 (n=5), arteriolar diameter was measured under control conditions and 1 to 2 minutes after the window was filled with CSF containing acetylcholine (1 and 10 μmol/L). Acetylcholine was used to examine reactivity of the preparation. After administration of acetylcholine, the cranial window was flushed with artificial CSF, and the diameter of cerebral arterioles returned to baseline in 15 to 30 minutes. Flushing the window with fresh CSF maintained at 37°C did not alter diameter of cerebral arterioles. Diameter of cerebral arterioles was also measured under control conditions and during hypercapnia produced by administering 5% and 7% inspired CO₂. For each level of CO₂, vessel diameter was measured at 9 to 10 minutes, at which time a steady state had been obtained. Application of acetylcholine and nitroprusside were repeated after a 60-minute recovery period. This group of animals served as a time control to establish the reproducibility of responses to acetylcholine and the two levels of hypercapnia.

In group 2 (n=13), arteriolar diameter was measured under control conditions and after the window was filled with CSF containing acetylcholine (1 and 10 μmol/L), and during administration of 5% and 7% CO₂. After a 60-minute recovery period, application of acetylcholine and administration of CO₂ were repeated in the presence of glibenclamide (1 μmol/L). The cranial window was treated with glibenclamide for 15 minutes before responses to acetylcholine and hypercapnia were tested. We have shown previously that this concentration of glibenclamide produces marked, but selective, inhibition of cerebral vasodilation in response to aprikalim (a direct activator of ATP-sensitive potassium channels), calcitonin gene-related peptide, and hypoxia.5-19

In group 3 (n=5), arteriolar diameter was measured under control conditions and after the window was filled with CSF containing acetylcholine (1 and 10 μmol/L) and sodium nitroprusside (1 and 10 μmol/L). After a 60-minute recovery period, application of acetylcholine and nitroprusside was repeated in the presence of glibenclamide (1 μmol/L). The cranial window was treated with glibenclamide for 15 minutes before responses to acetylcholine and nitroprusside were tested. The purpose of these experiments was to examine the specificity of glibenclamide.

In group 4 (n=8), arteriolar diameter was measured under control conditions, after the window was filled with CSF containing acetylcholine (1 and 10 μmol/L) and sodium nitroprusside (1 and 10 μmol/L), and during administration of CO₂. After a 60-minute recovery period, applications of acetylcholine, nitroprusside, and CO₂ were repeated in the presence of 5N'-nitro-L-arginine (L-NNA, 300 μmol/L), an inhibitor of NO synthase. This concentration of L-NNA produces selective inhibition of dilatation of cerebral arterioles in response to acetylcholine and seizures.22 The cranial window was treated with L-NNA for 15 minutes before responses to acetylcholine and nitroprusside were tested and for the duration of the experiment. After responses to acetylcholine and nitroprusside were measured, the effects of hypercapnia on arteriolar diameter in the presence of L-NNA were tested. Because responses to hypercapnia were tested after examining responses to acetylcholine and nitroprusside, the cranial window had been treated with L-NNA for approximately 1 hour before producing hypercapnia.

**Statistics**

To examine the effects of interventions on baseline vessel diameter, paired t tests were used on absolute values (not percent change). For comparison of percent change data in the absence and presence of inhibitors, statistical analysis was performed using Wilcoxon's test. All values are expressed as means±SE. A value of P<.05 was considered significant.

**Results**

**Control Responses**

Under control conditions (arterial PCO₂, 32±1 mm Hg; arterial PO₂, 118±2 mm Hg; arterial pH, 7.47±0.01), diameter of cerebral arterioles averaged 93±3 μmol/L. Arterial PCO₂ during hypercapnia was similar in the different groups and averaged 54±1 mm Hg (arterial PO₂, 116±2 mm Hg; arterial pH, 7.31±0.01) and 65±1 mm Hg (arterial PO₂, 115±2 mm Hg; arterial pH, 7.23±0.01) during inspiration of 5% and 7% CO₂, respectively. There were no differences (P>.05) in responses of cerebral arterioles during the first and second treatment of either acetylcholine or hypercapnia (data not shown). Arterial pressure averaged 80±1 mm Hg and was not altered significantly by hypercapnia (data not shown).

**Effect of Glibenclamide**

Glibenclamide (1 μmol/L) had no effect on the diameter of cerebral arterioles under control conditions (change in diameter of 1±2%). Vasodilatation in response to the low concentration of acetylcholine was inhibited by glibenclamide by approximately 38% (Fig 1). Increases in diameter of cerebral arterioles during the low level of hypercapnia were also inhibited by 36% by glibenclamide (Fig 2). Cerebral vasodilatation in response to more severe hypercapnia was not affected significantly by glibenclamide (Fig 2). In contrast to the results with acetylcholine and moderate hypercapnia, glibenclamide did not inhibit vasodilatation in response to sodium nitroprusside (Fig 1). Thus, inhibitory effects of glibenclamide on the responses of cerebral arterioles to acetylcholine and moderate hypercapnia were modest but specific. These findings suggest that responses of cerebral arterioles to a low concentration of acetylcholine and moderate hypercapnia are dependent in part on activation of glibenclamide-sensitive potassium channels.

**Effect of N⁵-Nitro-L-Arginine**

Treatment with L-NNA had no significant effect on baseline diameter of cerebral arterioles (101±6 versus 98±4 μmol/L). L-NNA produced marked inhibition of
vasodilatation in response to acetylcholine (Fig 3) and hypercapnia (Fig 4). The increase in diameter of cerebral arterioles in response to the low level of hypercapnia was abolished completely, and vasodilatation during the higher level of hypercapnia was inhibited by 66% (Fig 4). In contrast to responses to acetylcholine and hypercapnia, vasodilatation in response to sodium nitroprusside was not inhibited by L-NNA (Fig 3). These findings suggest that increases in diameter of cerebral arterioles in response to acetylcholine and hypercapnia are dependent in large part on production of NO.

Discussion

There are two major findings in the present study. First, glibenclamide attenuated dilatation of cerebral arterioles in response to a low concentration of acetylcholine and moderate hypercapnia. These findings suggest that cerebral vasodilatation in response to acetylcholine and hypercapnia is dependent in part on activation of ATP-sensitive potassium channels. Second, L-NNA produced marked inhibition of dilatation of cerebral arterioles during hypercapnia. These findings suggest that cerebral vasodilatation during hypercapnia is dependent in large part on production of NO.

Role of Potassium Channels

Activation of ATP-sensitive potassium channels produces hyperpolarization and relaxation of vascular muscle. Synthetic activators of ATP-sensitive potassium channels produce relaxation of cerebral arteries in vitro and dilatation of the basilar artery in vivo. These findings suggest that ATP-sensitive potassium channels are present in cerebral blood vessels. ATP-sensitive potassium channels may also be activated by endogenous compounds such as endothelium-derived hyperpolarizing factor and calcitonin gene-related peptide.

Endothelium-dependent hyperpolarization and relaxation of the rabbit middle cerebral artery in response to acetylcholine appear to be mediated in part by an endothelium-derived hyperpolarizing factor. Actions of this hyperpolarizing factor are inhibited by glibenclamide. In the present study, dilatation of cerebral arterioles in response to a submaximal concentration of acetylcholine was also dependent in part on activation of a glibenclamide-sensitive potassium channel. These findings suggest that glibenclamide-sensitive potassium channels are present in cerebral vessels. ATP-sensitive potassium channels may also be activated by endogenous compounds such as endothelium-derived hyperpolarizing factor and calcitonin gene-related peptide.

Previous studies have suggested that acidosis may activate ATP-sensitive potassium channels in blood vessels. Hypercapnia produces cerebral vasodilatation through a mechanism that requires development of acidosis. Our findings with glibenclamide suggest that potassium channels in cerebral arterioles may also be activated during hypercapnia, although the overall influence of these channels on vascular responses during hypercapnia appears to be modest.

Although several studies suggest glibenclamide is a specific inhibitor of ATP-sensitive potassium channels, some data suggest that glibenclamide also has an effect on other potassium channels. For example, 10 μmol/L glibenclamide (10-fold greater than the concentration used in this study) has been reported to produce some inhibition of calcium-activated potassium channels in the aorta. Based on the majority of evidence, the most likely mechanism of action of glibenclamide in

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**Fig 2.** Bar graphs showing change in diameter of cerebral arterioles during two levels of hypercapnia (n=12) in the absence and presence of glibenclamide (1 μmol/L). Values are means±SE. Arterial Paco2 was 54±1 and 66±1 mm Hg in the absence and 53±1 and 64±1 mm Hg in the presence of glibenclamide, respectively. *P<.05 versus control response.

**Fig 3.** Bar graphs showing change in diameter of cerebral arterioles in response to acetylcholine (n=8) and sodium nitroprusside (n=8) in the absence and presence of glibenclamide, respectively. *P<.05 versus control response.

**Fig 4.** Bar graphs showing change in diameter of cerebral arterioles during two levels of hypercapnia (n=7) in the absence and presence of Nω-nitro-L-arginine (L-NNA, 300 μmol/L). Values are means±SE. Arterial Paco2 was 53±2 and 63±2 mm Hg in the absence and 51±2 and 62±2 mm Hg in the presence of L-NNA, respectively. *P<.05 versus control response.
the present study is inhibition of ATP-sensitive potassium channels, although we cannot exclude some role for calcium-activated potassium channels. Effects of glibenclamide in the present study were specific for responses to acetylcholine and hypercapnia because vasodilatation in response to nitroprusside was not inhibited by glibenclamide.

Role of Nitric Oxide

Recent studies suggest that cerebral vasodilatation during hypercapnia is dependent on formation of NO. This conclusion was based on studies in which increases in cerebral blood flow during hypercapnia were attenuated by inhibitors of NO synthesis.7,14,28 These previous findings all were obtained in rats, and it was not clear whether cerebral vascular responses to hypercapnia are dependent on production of NO in other species. Species differences in mechanisms that mediate cerebral vasodilatation during hypercapnia would not necessarily be surprising. For example, indomethacin inhibits increases in cerebral blood flow during hypercapnia in rats8 but not cerebral vasodilatation during hypercapnia in rabbits15 or cats.16,17

The present study indicates that dilatation of cerebral arterioles during hypercapnia is inhibited profoundly by L-NNA (an inhibitor of NO synthase), suggesting that vascular responses during hypercapnia are dependent on production of NO in rabbits. L-NNA also inhibited dilatation of cerebral arterioles in response to acetylcholine, as we have reported previously.18,22,29 These inhibitory effects were selective, however, because vasodilatation in response to nitroprusside was not inhibited by L-NNA. We have shown in this model that inhibitory effects of L-NNA are reversed by L-arginine.18,29

The mechanism by which hypercapnia increases activity of NO synthase is not clear. Acidosis has been reported to increase activity of brain NO synthase.30 If increased NO synthase activity in response to acidosis contributes to vasodilatation during hypercapnia, other mechanisms may also be involved because we observed only partial inhibition of the vasodilator response during the higher level of hypercapnia. Similar to our finding, increases in cerebral blood flow during very high levels of hypercapnia have been reported to be unaltered by an inhibitor of NO synthase and thus mediated by an NO-independent mechanism.11 Although it is clear that L-NNA is an inhibitor of NO synthase, we cannot exclude the possibility that inhibitory effects of L-NNA on the response to hypercapnia are through some action unrelated to inhibition of NO synthase.

It is presently not known whether NO is the mediator of relaxation of vascular muscle during hypercapnia. If NO is a mediator of relaxation of vascular muscle,25,32 and glibenclamide does not inhibit cerebral vasodilatation in response to NO donors such as nitroprusside (present study)19,20 or nitroglycerin.21 Similar to our findings with hypercapnia, both glibenclamide and L-NNA inhibit relaxation of the middle cerebral artery in response to acetylcholine.33 It is possible that hypercapnia causes production of both NO and a hyperpolarizing factor that activates ATP-sensitive potassium channels.

A recent study suggests that some nitrovasodilators (nitroprusside and nitroglycerin) produce vasodilatation in part by release of calcitonin gene-related peptide from trigeminal sensory fibers that innervate cerebral vessels.34 We therefore considered the possibility that release of NO during hypercapnia might cause release of calcitonin gene-related peptide, activating glibenclamide-sensitive potassium channels in cerebral vessels.30,34 Because, however, trigeminal ganglionectomy does not inhibit cerebral vasodilatation during hypercapnia,35 it is very unlikely that release of calcitonin gene-related peptide accounts for activation of ATP-sensitive potassium channels during hypercapnia.

Acknowledgments

This work was supported by National Institutes of Health grants HL-38901, HL-16066, HL-14388, AG-10269, and NS-24621, research funds from the Veterans Administration, and a Grant-In-Aid from the American Heart Association (2901570). P.F. Faraci is an Established Investigator of the American Heart Association. The authors thank Dr J.E. Brian for critical evaluation of the manuscript.

References

This is an interesting and very well-written article in which the authors tested the hypothesis that dilation of cerebral arterioles during hypercapnia is dependent on activation of ATP-sensitive potassium channels and the formation of nitric oxide. There are two major findings of this study. First, glibenclamide attenuated cerebral arteriolar dilation in response to low concentrations of acetylcholine and moderate hypercapnia. This finding suggests that cerebral vasodilation in response to acetylcholine and hypercapnia is at least in part dependent on the activation of ATP-sensitive potassium channels. Second, L-NNA produced a marked inhibition of cerebral arterioles during hypercapnia.

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Cerebral vasodilation during hypercapnia. Role of glibenclamide-sensitive potassium channels and nitric oxide.

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Stroke. 1994;25:1679-1683
doi: 10.1161/01.STR.25.8.1679

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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