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 vasodilation 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. Vasodilation 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. N'N'-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 vasodilation 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 vasodilation during hypercapnia is dependent in large part on production of nitric oxide.

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

Hypocapnia is a potent dilator of cerebral blood vessels1 through a mechanism that requires development of extracellular acidosis.2 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.3 Activity of ATP-sensitive potassium channels may reflect changes in the cellular metabolic state3 and may be activated by reductions in PO2 and pH.4,5 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,6,7 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).7,14 However, 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 rats9 but not cerebral vasodilatation during hypercapnia in rabbits10 or cats.16,17 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.18 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-
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 \(\mu\)mol/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 CO\(_2\). For each level of CO\(_2\), vessel diameter was measured at 9 to 10 minutes, at which time a steady state had been obtained. Application of acetylcholine and administration of CO\(_2\) 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 \(\mu\)mol/L), and during administration of 5% and 7% CO\(_2\). After a 60-minute recovery period, application of acetylcholine and administration of CO\(_2\) were repeated in the presence of glibenclamide (1 \(\mu\)mol/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 vasodilatation in response to aprikalim (a direct activator of ATP-sensitive potassium channels), calcitonin gene-related peptide, and hypoxia.

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 \(\mu\)mol/L) and sodium nitroprusside (1 and 10 \(\mu\)mol/L). After a 60-minute recovery period, application of acetylcholine and nitroprusside was repeated in the presence of glibenclamide (1 \(\mu\)mol/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 \(\mu\)mol/L) and sodium nitroprusside (1 and 10 \(\mu\)mol/L), and during administration of CO\(_2\). After a 60-minute recovery period, applications of acetylcholine, nitroprusside, and CO\(_2\) were repeated in the presence of N\(^5\)–nitro-L-arginine (L-NNA, 300 \(\mu\)mol/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. 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. 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 P\(_{CO_2}\) 32±1 mm Hg; arterial P\(_{O_2}\) 118±2 mm Hg; arterial pH, 7.47±0.01), diameter of cerebral arterioles averaged 93±3 \(\mu\)mol/L. Arterial P\(_{CO_2}\) during hypercapnia was similar in the different groups and averaged 54±1 mm Hg (arterial P\(_{O_2}\) 116±2 mm Hg; arterial pH, 7.31±0.01) and 65±1 mm Hg (arterial P\(_{O_2}\) 115±2 mm Hg; arterial pH, 7.23±0.01) during inspiration of 5% and 7% CO\(_2\), 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 \(\mu\)mol/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 N\(^5\)-Nitro-L-Arginine
Treatment with L-NNA had no significant effect on baseline diameter of cerebral arterioles (101±6 versus 98±4 \(\mu\)mol/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 mus-

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**Figure 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.

**Figure 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 N⁵-nitro-L-arginine (L-NNA, 300 μmol/L). Values are means±SE. *P<.05 versus control response.

**Figure 4.** Bar graphs showing change in diameter of cerebral arterioles during two levels of hypercapnia (n=7) in the absence and presence of N⁵-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.

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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 rats3 but not cerebral vasodilation 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. It is possible that normal basal levels of NO (or cyclic GMP) are required for vasodilatation during hypercapnia to occur.31 Although vasodilatation during hypercapnia appears to be partially dependent on production of NO, the cellular source of NO is not known.

It is somewhat surprising that both L-NNA and glibenclamide inhibited vasodilatation during hypercapnia, although the effect of L-NNA (and thus the role for NO) was much greater than the effect of glibenclamide. Although an interaction between NO and ATP-sensitive potassium channels cannot be excluded, it is unlikely that NO activates potassium channels in cerebral vessels. NO does not hyperpolarize cerebral 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 (2015170). P.M. Faraci is an Established Investigator of the American Heart Association. The authors thank Dr J.E. Brian for critical evaluation of the manuscript.

References


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**Editorial Comment**

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 vasodilation during hypercapnia. This latter finding suggests that cerebral vasodilation during hypercapnia is dependent on the production of nitric oxide. This latter conclusion is somewhat controversial; in fact, in the literature one can find studies indicating that nitric oxide synthase inhibition both is and is not involved in cerebral vasodilation with hypercapnia. Much depends on the dose of hypercapnia; at moderate levels (Paco\textsubscript{2} around 50 mm Hg), attenuation of cerebral vasodilation is apparent, but at higher levels (70 mm Hg), there is no effect. The data in the present article indicate attenuation at 50 mm Hg and at 90 mm Hg. It also may be difficult to explain the lack of effect of nitric oxide synthase inhibition in the hypercapnic response when one considers the time after the administration of the drug in which the hypercapnia is tested. In some investigations, the hypercapnic response may have been tested too early after the drug was administered, and thus nitric oxide synthase inhibition was not complete at that time. Finally, as the authors point out in this article, there may be a species difference. It has never been to my liking to express the physiological differences that occur in animals to a species difference. If a response, for example, to hypercapnia is so well described in all species of animals one would think that the mechanism of action of this universal dilation is similar. Yet, here the authors make a point that these mechanisms may be species related. While one has no idea why these differences would exist, species differences always seem bothersome, particularly for this universal hypercapnic response.

**Richard J. Traystman, PhD, Guest Editor**

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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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/25/8/1679

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