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. N⁵-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

Hypercapnia 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-
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 administration of CO2 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 during administration of 5% and 7% CO2. 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 Nω-nitro-L-arginine (L-NNA, 300 μmol/L), an inhibitor of NO synthase. This concentration of L-NNA produces selective inhibition of dilation 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 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 Nω-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 muscule. Synthetic activators of ATP-sensitive potassium channels produce relaxation of cerebral arteries in vitro and dilatation of the basilar artery and cerebral arterioles in vivo. The 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 in cerebral vessels of the rabbit in vitro and in vivo are in contrast to our previous findings in the rat basilar artery in vivo, in which glibenclamide had no effect on dilatation in response to acetylcholine. The reason for this difference is not clear, but the difference may reflect species or regional differences in the contribution of glibenclamide-sensitive potassium channels to acetylcholine-induced relaxation of cerebral blood vessels.

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...
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 rats5 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. 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.20,24 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.

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References


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 (Paco2, 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.

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