Blockade of ATP-Sensitive Potassium Channels in Cerebral Arterioles Inhibits Vasoconstriction From Hypocapnic Alkalosis in Cats

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Background and Purpose—Recent studies have shown that the cerebral arteriolar dilation from hypercapnic acidosis is blocked by agents which inhibit K<sub>ATP</sub> channels. These findings suggested that this response is due to opening of K<sub>ATP</sub> channels. Because the repose to CO<sub>2</sub> is a continuum, with hypercapnic acidosis causing vasodilation and hypocapnic alkalosis causing vasoconstriction, it would be expected that the response to hypocapnic alkalosis would be due to closing of K<sub>ATP</sub> channels. There are no studies of the effect of inhibition of K<sub>ATP</sub> channels on the response to hypocapnic alkalosis.

Methods—We investigated the effect of 3 agents that in earlier studies were found to inhibit K<sub>ATP</sub> channels—N<sup>G</sup>-nitro-L-arginine, hydroxylysine, and glyburide—on the cerebral arteriolar constriction caused by graded hypocapnia induced by hyperventilation in anesthetized cats equipped with cranial windows.

Results—Hypocapnic alkalosis caused dose-dependent vasoconstriction that was inhibited completely by each of the 3 inhibitors of K<sub>ATP</sub> channels. The blockade induced by these agents was eliminated in the presence of topical L-lysine (5 μmol/L).

Conclusions—The findings show that agents which inhibit ATP-sensitive potassium channels in cerebral arterioles inhibit the vasoconstriction from hypocapnic alkalosis. These and earlier results showing that inhibition of K<sub>ATP</sub> channels inhibited dilation from hypercapnic acidosis demonstrate that the response to CO<sub>2</sub> in cerebral arterioles is mediated by the opening and closing of K<sub>ATP</sub> channels. (Stroke. 1999;30:851-854.)

Key Words: carbon dioxide ■ glyburide ■ hydroxylysine ■ microcirculation ■ nitroarginine ■ vasoconstriction ■ cats

Several investigators have found that the cerebral vasodilation in response to hypercapnic acidosis is blocked by arginine analogs, such as N<sup>G</sup>-nitro-L-arginine (L-NNA) or N<sup>ω</sup>-monomethyl-L-arginine (L-NMMA).1 Because the main action of these agents is the blockade of the synthesis of nitric oxide, these findings led to the hypothesis that the vasodilation from hypercapnic acidosis is mediated by increased synthesis and release of nitric oxide.1

Recent findings,2 however, have shown that the arginine analogs also block ATP-sensitive potassium (K<sub>ATP</sub>) channels and that the vasodilation from hypercapnic acidosis is also blocked by known inhibitors of K<sub>ATP</sub> channels, such as glyburide, which do not affect nitric oxide synthesis. It is therefore likely that the vasodilation from hypercapnic acidosis is due to opening of K<sub>ATP</sub> channels.

Although many studies tested the effect of agents that block K<sub>ATP</sub> channels or nitric oxide synthase on the response to hypercapnic acidosis, we can find no studies in which the effect of these agents on the response to hypocapnic alkalosis was tested. Because the response to CO<sub>2</sub> is a continuum, with hypercapnic acidosis causing vasodilation and hypocapnic alkalosis causing vasoconstriction, it would be expected that the response to hypocapnic alkalosis would also be mediated by the same mechanism as that due to hypercapnic acidosis.

In the present experiments we tested the effect of 3 agents that block K<sub>ATP</sub> channels in cerebral arterioles on the vasoconstrictor response to hypocapnic alkalosis in anesthetized cats.

Materials and Methods
Experiments were performed in cats anesthetized with sodium pentobarbital (30 mg/kg IV). Additional doses of anesthetic were given as required to maintain surgical anesthesia, based on testing of corneal reflexes and on responses to tail pinch. The animals were subjected to tracheostomy and ventilated with a positive-pressure respirator. The end-expiratory CO<sub>2</sub> of the animals was continuously monitored with a CO<sub>2</sub> analyzer and was maintained at a constant level of approximately 30 mm Hg during the control period. Arterial blood pressure was measured with a pressure transducer connected to a cannula introduced into the aorta via the femoral artery. Arterial blood samples were collected for determination of arterial blood oxygen, CO<sub>2</sub> partial pressures, and pH at appropriate intervals during the experiment. Blood gas tensions and pH were measured with oxygen and CO<sub>2</sub> electrodes and a pH meter. The rectal temperature of the animals was monitored continuously and kept constant with the aid of a heating blanket. The experimental protocols are approved by the institutional animal care committee.
The cerebral microcirculation of the parietal cortex was visualized through an acutely implanted cranial window, as described in detail previously. The space under the cranial window was filled with artificial cerebrospinal fluid (CSF) identical in composition to that of cats. One port of the window was connected to a pressure transducer for continuous monitoring of intracranial pressure. The intracranial pressure was maintained at 5 mm Hg by connecting another outlet of the window to a coiled plastic tube whose free end was placed at the appropriate height to give the desired pressure. Two ports of the cranial window were used as inlet and outlet, allowing topical application of various solutions. Pial arteriolar diameter was measured with an image-splitting device attached to a microscope. In each animal, several arterioles were observed, covering a wide range of vessel caliber. The responses of small and large arterioles (smaller and larger than 100 μm in diameter, respectively) were analyzed separately to identify any size-dependent differences in responses.

Glyburide, hydroxylysine, L-NNA, and L-lysine were obtained from Sigma Chemical Co. All agents were dissolved in artificial CSF except for glyburide, which was dissolved in ethyl alcohol to produce a stock solution. Appropriate dilutions from the stock solution were then prepared in artificial CSF.

The experimental design was as follows: The response to 2 levels of hypocapnic alkalosis of cerebral arterioles was tested in a control experiment without pretreatment. Hypocapnic alkalosis was induced by increasing the volume and frequency of the respirator. Each level of hypocapnia was maintained for at least 10 minutes to obtain steady-state responses. Measurements were made at PaCO₂ of 22 and 16 mm Hg. The experiment was repeated after topical treatment with 1 of 3 blockers of Kₐₐₜp channels in 2 species, suggest strongly that the response to CO₂ in cerebral arterioles is mediated by opening or closing of Kₐₐₜp channels. Accordingly, we conclude that hypercapnic acidosis opens these channels and hypocapnic alkalosis closes them. Results by others in isolated cerebral vessels also showed similar findings.

Results

Figures 1 to 3 show that hypocapnic alkalosis induced dose-dependent vasoconstriction of cerebral arterioles that was completely blocked by glyburide, hydroxylysine, or L-NNA, and that this blockade was reversed completely in the presence of L-lysine. Note that glyburide, hydroxylysine, and L-NNA did not cause significant changes in baseline arteriolar diameter.

Discussion

The principal finding of the experiments reported above is that 3 agents which block Kₐₐₜp channels in cerebral arterioles of the cat eliminated the vasoconstriction from hypocapnic alkalosis. These findings, together with earlier results which showed that the vasodilation from hypercapnic acidosis was also blocked by blockade of Kₐₐₜp channels in 2 species, suggest strongly that the response to CO₂ in cerebral arterioles is mediated by opening or closing of Kₐₐₜp channels. Accordingly, we conclude that hypercapnic acidosis opens these channels and hypocapnic alkalosis closes them. Results by others in isolated cerebral vessels also showed similar findings.
Electrophysiological and supporting pharmacological evidence showed the presence of \( K_{ATP} \) channels in smooth muscle from cerebral arteries of rabbits.\(^7\) In addition, based on the finding that glyburide caused substantial depolarization in cerebral arteries,\(^8\) it was suggested that ATP-sensitive potassium channels may be open under resting conditions in these vessels.\(^8\)

We are not aware of any studies in which blockade of \( K_{ATP} \) channels by glyburide interfered with vasoconstrictor responses in vivo. However, it was shown that serotonin and histamine, in isolated smooth muscle cells from cerebral arteries, decreased glyburide-sensitive inward potassium currents, suggesting that these agents are capable of closing down \( K_{ATP} \) channels.\(^7\) Similar findings have been shown in response to a number of vasoconstrictor agents in bladder smooth muscle\(^9\) as well as in coronary\(^10–12\) and mesenteric vascular smooth muscle.\(^13\)

Our studies are based exclusively on the use of pharmacological agents. The conclusion, therefore, that the vasorelaxation response to hypoxia is mediated by opening of \( K_{ATP} \) channels is dependent on the specificity of the agents we used to block these channels. In this respect, it is well established that glyburide is highly specific in blocking \( K_{ATP} \) channels in cerebral vessels. For example, several investigators\(^14–16\) found that the administration of glyburide did not affect responses due to agents which open calcium-activated potassium channels. Similarly, agents that are known to block calcium-activated potassium channels, such as iberiotoxin, charybdotoxin, and tetraethylammonium chloride, did not affect responses due to synthetic \( K_{ATP} \) channel openers.\(^14,15,17–19\)

The specificity of the responses is also demonstrated by the fact that the blockade induced by the 3 blocking agents we used was readily removed by a low concentration of \( L \)-lysine. Earlier studies\(^4\) showed that \( K_{ATP} \) channels in cerebral arterioles require binding of \( L \)-lysine or \( L \)-arginine to open in response to agonists, such as pinacidil. The 3 agents we used to block these channels, namely, glyburide, \( L \)-NNA, and hydroxylysylamine, evidently block these channels by displacing \( L \)-arginine or \( L \)-lysine from the channel.\(^4\)

In the presence of micromolar concentrations of \( L \)-lysine or \( L \)-arginine in the fluid bathing the vessels, the blockade induced by these agents is removed.\(^4\)

It is worthy of note that blockade of \( K_{ATP} \) channels in cerebral arteries did not change baseline diameter. Others\(^20\) have also found that blockade of \( K_{ATP} \) channels in cerebral vessels does not cause a change in baseline vascular caliber. Electrophysiological studies\(^8\) have shown that blockade of these channels causes a large depolarization of isolated cerebral arteries without a change in basal tone. The surprising absence of change in basal tone was ascribed to the fact that the depolarization may not have reached the threshold for activating vasoconstrictor mechanisms.\(^8\)

In our in vivo experiments, another reasonable explanation for the absence of a change in baseline diameter is the fact that agents which are present in the vicinity of vessels under resting conditions may have competing influences on \( K_{ATP} \) channels, some of them acting on these channels to cause vasodilation and others to cause vasoconstriction. The blockade of the channels by elimination of opposing actions on these channels may result in no net change in baseline diameter.

The relaxation of isolated basilar arteries in response to acidosis was blocked by glyburide but unaffected by charybdotoxin.\(^5\) Also, the dilation of isolated coronary arteries due to acidosis was inhibited by glyburide but not charybdotoxin.\(^6\) In unpublished studies we found that charybdotoxin did not modify the cerebral arterial dilatation due to hypercapnia in rats (authors’ unpublished data, 1998). Thus, the available evidence does not support participation of calcium-activated potassium channels in the cerebral vascular response to \( \text{CO}_2 \).

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References

The cerebral circulation is very sensitive to changes in arterial \( P_{CO_2} \) during hypercapnia and hypocapnia. Although it has been known for many years that these stimuli alter cerebral vascular resistance, mechanisms that mediate these responses have not been completely defined.

Some studies have suggested that activation of one type of potassium channel, the ATP-sensitive potassium channel \( K_{ATP} \), may contribute to dilation of cerebral blood vessels during hypercapnia.\(^1\)\(^2\) The present study presents new data which suggest that these potassium channels are involved in the vascular response to hypocapnia. This conclusion is based in part on the finding that glibenclamide, which inhibits \( K_{ATP} \), blocked constriction of cerebral arteries and arterioles in responses to hypocapnia. In addition, the response to hypocapnia was attenuated by \( N^G\)-nitro-L-arginine and hydroxyllysine. These latter substances are not traditionally used as inhibitors of \( K_{ATP} \), but they inhibit dilation of cerebral vessels in response to activators of these potassium channels in the feline model used in these experiments. The finding that three structurally unrelated compounds produced similar results provides strong evidence that inhibition of responses to hypocapnia did not reflect some nonspecific effect.

Although many studies have examined effects of glibenclamide on vasodilator stimuli,\(^4\)\(^5\) almost none have examined effects of this drug on constrictor responses in the cerebral circulation. Implicit in the interpretation of the present findings, that glibenclamide (and other inhibitors) attenuate vasoconstriction during hypocapnia, is the assumption that \( K_{ATP} \) are active (open) under basal conditions. This assumption is not consistent with the finding of many studies (including the data in the authors’ study) that glibenclamide does not alter resting tone of cerebral blood vessels, which suggests that \( K_{ATP} \) are not open under basal conditions.\(^4\)\(^5\) As the authors note, however, other mechanisms also influence vascular tone, and perhaps these other mechanisms maintain vessel diameter constant during application of glibenclamide in vivo.

Measurement in vivo of membrane potential, a variable that is very sensitive to activity of potassium channels, during application of glibenclamide would help greatly to determine whether \( K_{ATP} \) are open under basal conditions and thus have the potential to close and produce vasoconstriction. Unfortunately, in vivo measurements of membrane potential in cerebral blood vessels have not been reported. Previous studies in vitro have reported that glibenclamide does not alter resting membrane potential\(^7\) or it depolarizes cerebral vascular muscle.\(^8\) The latter effect would be consistent with inhibition of activity of \( K_{ATP} \). The present study is the first to examine the effects of glibenclamide on responses to a vasoconstrictor stimulus in brain in vivo. Additional studies will be needed to determine whether this effect of glibenclamide is observed in other models and during other vasoconstrictor stimuli.

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