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_ATP channels. These findings suggested that this response is due to opening of K_ATP channels. Because the repose to CO₂ 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_ATP channels.

There are no studies of the effect of inhibition of K_ATP channels on the response to hypocapnic alkalosis.

Methods—We investigated the effect of 3 agents that in earlier studies were found to inhibit K_ATP channels—N⁶-nitro-L-arginine, hydroxylsine, 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_ATP 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_ATP channels inhibited dilation from hypercapnic acidosis demonstrate that the response to CO₂ in cerebral arterioles is mediated by the opening and closing of K_ATP channels. (Stroke. 1999;30:851-854.)

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

Several investigators have found that the cerebral vasodilation in response to hypercapnic acidosis is blocked by L-arginine analogs, such as N⁶-nitro-L-arginine (L-NNA) or N⁷-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_ATP) channels and that the vasodilation from hypercapnic acidosis is also blocked by known inhibitors of K_ATP 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_ATP channels.

Although many studies tested the effect of agents that block K_ATP 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 CO₂ 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_ATP 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 CO₂ of the animals was continuously monitored with a CO₂ 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, CO₂ partial pressures, and pH at appropriate intervals during the experiment. Blood gas tensions and pH were measured with oxygen and CO₂ 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.
K<sub>ATP</sub> Channel Blockade in Hypocapnic Constriction

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 PaCO<sub>2</sub> of 22 and 16 mm Hg. The experiment was repeated after topical treatment with 1 of 3 blockers of K<sub>ATP</sub> channels. These agents were applied topically by filling the 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.

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Electrophysiological and supporting pharmacological evidence showed the presence of $K_{ATP}$ channels in smooth muscle from cerebral arteries of rabbits. In addition, based on the finding that glyburide caused substantial depolarization in cerebral arteries, it was suggested that ATP-sensitive potassium channels may be open under resting conditions in these vessels. 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. Similar findings have been shown in response to a number of vasoconstrictor agents in bladder smooth muscle as well as in coronary and mesenteric vascular smooth muscle.

Our studies are based exclusively on the use of pharmacological agents. The conclusion, therefore, that the vasoconstrictor response to hypocapnia is mediated by closing 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 found that the administration of glyburide did not affect responses due to synthetic $K_{ATP}$ channel openers.

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 showed that $K_{ATP}$ channels in cerebral arterioles require binding of L-lysine or L-arginine to open in response to agonists, and tetraethyl-ammonium chloride, did not affect responses due to administration of glyburide. The specificity of the responses is also demonstrated by the fact that blockade induced by these agents is removed.

It is worthy of note that blockade of $K_{ATP}$ channels in cerebral arteries did not change basal diameter. Others have also found that blockade of $K_{ATP}$ channels in cerebral vessels does not cause a change in baseline vascular caliber. Electrophysiological studies 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. 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 inhibited by glyburide but not iberiotoxin. 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 CO$_2$.

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References
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The cerebral circulation is very sensitive to changes in arterial PCO₂ 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. 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 hydroxyl-lysine. 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, 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. 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 or it depolarizes cerebral vascular muscle. 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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References
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