Potassium channels modulate cerebral autoregulation during acute hypertension

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Potassium channels modulate cerebral autoregulation during acute hypertension. Am J Physiol Heart Circ Physiol 278: H2003–H2007, 2000.—We tested the hypothesis that constriction of cerebral arterioles during acute increases in blood pressure is attenuated by activation of potassium (K⁺) channels. We tested the effects of inhibitors of calcium-dependent K⁺ channels [iberiotoxin (50 nM) and tetraethylammonium (TEA, 1 mM)] on changes in arteriolar diameter during acute hypertension. Diameter of cerebral arterioles (baseline diameter = 46 ± 2 μm, mean ± SE) was measured using a cranial window in anesthetized rats. Arterial pressure was increased from a control value of 96 ± 1 mmHg to 130, 150, 170, and 200 mmHg by intravenous infusion of phenylephrine. Increases in arterial pressure from baseline to 130 and 150 mmHg decreased the diameter of cerebral arterioles by 5–10%. Greater increases in arterial pressure produced large increases in arteriolar diameter (i.e., “breakthrough of autoregulation”). Iberiotoxin or TEA inhibited increases in arteriolar diameter when arterial pressure was increased to 170 and 200 mmHg. The change in arteriolar diameter at 200 mmHg was 20 ± 3% and −1 ± 4% in the absence and presence of iberiotoxin, respectively. These findings suggest that calcium-dependent K⁺ channels attenuate cerebral microvascular constriction during acute increases in arterial pressure, and that increases in arteriolar diameter at high levels of arterial pressure are not simply a passive phenomenon.

cerebral arterioles; iberiotoxin; tetraethylammonium; calcium-dependent potassium channels

CEREBRAL BLOOD VESSELS AUTOREGULATE during increases and decreases in arterial pressure, resulting in maintenance of cerebral blood flow at a relatively constant level over a wide range of pressures (15). Several mechanisms have been proposed to mediate or modulate cerebral autoregulation, including myogenic and neural mechanisms as well as activation of potassium channels in vascular muscle (6, 11, 15, 29). For example, recent studies suggest that activation of potassium channels may mediate autoregulatory dilatation of cerebral blood vessels during reductions in arterial blood pressure (3, 22).

Acute increases in intravascular pressure produce graded depolarization of vascular muscle and constriction of cerebral arteries in vitro (4, 12, 18, 19). Mechanisms by which acute increases in pressure induce depolarization probably involve increases in intracellular calcium through voltage-dependent calcium channels (18, 19). As a membrane is depolarized, increases in intracellular calcium may activate calcium-dependent potassium channels (4, 18, 19). Membrane depolarization may activate calcium-dependent potassium channels as well as voltage-dependent potassium channels (26, 28). Activation of potassium channels in vascular muscle produces hyperpolarization, resulting in either relaxation or attenuation of contraction. Several lines of evidence suggest that calcium-dependent and voltage-dependent potassium channels are present in cerebral arteries and participate in regulation of vascular tone during increases in pressure in vitro (1, 4, 18, 19, 28). This concept is based almost entirely on studies of cerebral arteries in vitro and has not been tested in the cerebral circulation in vivo.

The goal of this study was to test the hypothesis that autoregulation of cerebral arterioles during acute increases in mean arterial blood pressure is modulated (attenuated) by activation of potassium channels. We determined whether iberiotoxin and tetraethylammonium (TEA) (inhibitors of calcium-dependent potassium channels) or 4-aminopyridine (4-AP, an inhibitor of voltage-dependent potassium channels) augment vasoconstrictor responses during increases in arterial pressure in vivo.

METHODS

Animal preparation. Sprague-Dawley rats (n = 53), 300–375 g, were anesthetized with pentobarbital sodium (50 mg/kg ip), which was supplemented regularly at ~20–25 mg·kg⁻¹·h⁻¹ iv. In addition, depth of anesthesia was evaluated by applying pressure to a paw and observing changes in heart rate or blood pressure. If such changes occurred, additional anesthesia was administered. The trachea was cannulated, and animals were ventilated mechanically with room air and supplemental oxygen. A catheter was placed in the femoral artery to measure pressure and obtain samples of arterial blood. A femoral vein was cannulated for infusion of drugs. The other femoral vein was cannulated for infusion of phenylephrine. Arterial blood gases were monitored and maintained within normal limits throughout the experiments (PH = 7.38 ± 0.004, PCO₂ = 38 ± 0.2 mmHg, PO₂ = 116 ± 1 mmHg). Baseline mean arterial pressure was 96 ± 1 mmHg.
A cranial window was prepared over the left parietal cortex and constantly superfused with artificial cerebrospinal fluid (CSF) (pH = 7.38 ± 0.005, PCO2 = 42 ± 0.4 mmHg, PO2 = 68 ± 1 mmHg, and temperature 37.5°C) as described in previous work (24, 25). Diameters of pial arterioles were measured using a microscope equipped with a television camera coupled to a video monitor. Images were recorded on videotape, and vessel diameters were measured with an image analyzer.

Experimental protocol. In group 1 (control), arteriolar diameter was measured under control conditions and during increases in mean arterial pressure to 130, 150, 170, and 200 mmHg by infusion of increasing concentrations of phenylephrine (4–80 mg/min iv). At each level of arterial pressure, a steady state was reached in about 2 min, and pressure was maintained constant for 3 min. Group 1 functioned as a time control to establish the effects of increases in arterial pressure on the diameter of cerebral arterioles.

In group 2 (TEA), arteriolar diameter was measured during control conditions and during increases in arterial pressure (following the same protocol as group 1) in the presence of TEA (1 mM). The concentration of TEA was chosen based on previous studies (26, 27, 31, 32). The cranial window was treated with TEA for 15 min before and during all increases in blood pressure. The purpose of these experiments was to determine whether TEA inhibited increases in arteriolar diameter during increases in arterial pressure.

In group 3 (iberiotoxin), arteriolar diameter was measured under control conditions and during increases in arterial pressure (following the same protocol as group 1) in the presence of iberiotoxin (50 nM). The concentration of iberiotoxin was chosen based on previous studies (26, 27, 31, 32). Iberiotoxin was applied topically, following the same protocol as in group 2 with TEA. The purpose of these experiments was to determine whether iberiotoxin, a highly selective inhibitor of calcium-sensitive potassium channels, altered increases in arteriolar diameter during increases in arterial pressure.

In group 4 (4-AP), arteriolar diameter was measured under control conditions and during increases in arterial pressure (following the same protocol as group 1) in the presence of 4-AP (200 µM). The concentration of 4-AP was chosen based on previous studies (26, 30). The 4-AP was applied topically, following the same protocol as in group 2 with TEA. The purpose of these experiments was to determine whether 4-AP, an inhibitor of voltage-dependent potassium channels, attenuates increases in arteriolar diameter during increases in arterial pressure.

In group 5 (U-46619), arteriolar diameter was measured under control conditions and during topical application of the thromboxane A2 analog, U-46619 (0.1 and 1 µM). After a 60-min recovery period, application of U-46619 was repeated in the presence of vehicle (time control), TEA (1 mM), or iberiotoxin (50 nM). The U-46619 was used to determine whether TEA or iberiotoxin produced augmentation of vasoconstriction in response to stimuli other than increases in arterial pressure. The U-46619 was used because it produces concentration-dependent, reproducible constriction of cerebral arterioles in the rat (8, 23).

Statistical analysis. Values are presented as means ± SE. Values were analyzed using ANOVA with repeated measures followed by the Student-Newman-Keuls test to detect individual differences. A paired t-test was used for comparison of percent change of diameter in the absence and presence of inhibitors. Values of P < 0.05 were considered to be significant.

RESULTS

Responses under control conditions. Baseline diameter of cerebral arterioles for all groups was 46 ± 2 µm. When mean arterial pressure was increased from a control value of 96 ± 1 mmHg to 130 and 150 mmHg, there was a decrease in diameter of cerebral arterioles of 5 ± 1 and 8 ± 1%, respectively (n = 11) (Fig. 1). When arterial pressure was increased to 170 and 200 mmHg, there was a large increase in arteriolar diameter (Fig. 1A).

Effects of TEA, iberiotoxin, and 4-AP on responses to increases in arterial pressure. Application of TEA and iberiotoxin to the cranial window did not alter arterial blood pressure. Diameter of cerebral arterioles was not altered significantly by TEA (P > 0.05) or iberiotoxin (P > 0.05). TEA tended to augment constriction of cerebral arterioles during increases in arterial pressure to 130 and 150 mmHg, but these effects were not statistically significant (n = 8) (Fig. 1B). In contrast to the modest effects observed during more moderate increases in arterial pressure, TEA significantly inhibited increases in diameter of cerebral arterioles that occurred when arterial pressure was increased to 170 and 200 mmHg (Fig. 1B).

Similar to the findings with TEA, iberiotoxin tended to augment constriction of cerebral arterioles during more moderate increases in arterial pressure, but these effects were not statistically significant (n = 7) (Fig. 1C). Iberiotoxin significantly inhibited increases in the diameter of cerebral arterioles that occurred when arterial pressure was increased to 170 and 200 mmHg.
A major finding of this study is that in the presence of TEA or iberiotoxin, arteriolar diameter did not increase, even when arterial pressure was raised to 200 mmHg (Fig. 1C). Application of 4-AP to the cranial window did not alter arterial blood pressure and did not alter constriction of cerebral arterioles during moderate increases in arterial pressure (n = 11). When arterial pressure was increased to 150 mmHg, for example, arteriolar diameter was reduced by 8 ± 1% in the absence and 10 ± 3% in the presence of 4-AP. The 4-AP tended to inhibit increases in diameter of cerebral arterioles that occurred when arterial pressure was increased to 170 and 200 mmHg, but these changes were not statistically significant (P > 0.05; Fig. 1D).

Effect of TEA and iberiotoxin on responses to U-46619. U-46619 (0.1 and 1 µM) produced constriction of cerebral arterioles that was reproducible. For example, 0.1 µM U-46619 constricted cerebral arterioles by 14 ± 2 and 17 ± 2% during the first and second applications, respectively. Both TEA and iberiotoxin tended to increase vasoconstrictor responses to U-46619, but only the effects of iberiotoxin were statistically significant (Fig. 2). Although TEA and iberiotoxin may produce some augmentation of vasoconstrictor responses to U-46619, overall these effects appear to be less than those observed during increases in arterial pressure to 170 and 200 mmHg.

**DISCUSSION**

The major findings in the present study are that TEA and iberiotoxin significantly attenuate increases in the diameter of cerebral arterioles that occur during severe increases in arterial blood pressure. These data suggest that cerebral autoregulation during increases in arterial pressure is modulated by the activity of calcium-dependent potassium channels. To our knowledge, this is the first study to examine the functional role of calcium-dependent potassium channels in autoregulation of the cerebral circulation during acute hypertension in vivo.

Effect of increasing arterial blood pressure on cerebral arterioles. In the present study, increases in arterial pressure from control levels of about 100 mmHg to 130 and 150 mmHg produced a reduction in the diameter of cerebral arterioles of up to ~10%. This finding is consistent with previous studies in cats and rats in which similar increases in arterial pressure resulted in reductions in pial arteriolar diameter of the same magnitude (5–10% decrease in vessel diameter) (13, 14, 21). The results are also consistent with previous studies in which autoregulation of cerebral blood flow to the cerebrum was measured during acute increases in arterial pressure (5, 9, 13–15, 21, 33). Thus cerebral arterioles autoregulate during moderate increases in blood pressure.

During larger increases in arterial pressure, above ~150 mmHg, there was a large increase in the diameter of cerebral arterioles. This “breakthrough” of autoregulation at very high levels of arterial pressure has been described by many investigators previously. The magnitude of the increase in steady-state diameter of cerebral arterioles at 170 and 200 mmHg is consistent with previous studies in rats (13, 14). The present findings are also consistent with previous studies in which increases in cerebral blood flow to the cerebrum were measured during large increases in arterial pressure (9, 13–15, 21, 33).

In this study and in previous studies (24, 25), we and others have used phenylephrine to induce acute hypertension. Infusion of phenylephrine intravenously does not have direct effects on pial vessels because of the presence of the blood-brain barrier and because there are few functional α-adrenergic receptors in smooth muscle of cerebral vessels in the rat (17). Thus the vasoconstrictor response of cerebral vessels during infusion of phenylephrine appears to be mediated by increases in arterial pressure, not by a direct effect of phenylephrine.

We used pentobarbital sodium for anesthesia in these experiments, and we have considered the possibility that the use of this agent may influence the results of the experiments. The use of any anesthesia is a potential limitation in studies of vascular response. However, it would be difficult to perform these studies and obtain accurate measurements of cerebral arteriole diameters in awake animals. In studies of autoregulation, pentobarbital sodium (or other related barbiturates) has been used commonly (see Refs. 7, 13, 14, and 21 for some examples). In all of these experiments, the vascular preparations exhibited fairly typical autoregulatory responses (vasodilatation with reductions in pressure, vasoconstriction to moderate increases in pressure). Although pentobarbital sodium reduces baseline cerebral metabolism and blood flow, the effectiveness of autoregulation is similar in awake and pentobarbital sodium-anesthetized animals (7).

**Role of potassium channels during acute hypertension.** Acute increases in intravascular pressure produce graded membrane depolarization, increases in intracellular calcium, and contraction of cerebral vascular muscle in vitro (19). This relationship is very steep, so that changes in membrane potential of only a few millivolts are associated with significant changes in vascular tone (10, 19, 26). Membrane depolarization
also increases the frequency of calcium sparks that activate calcium-dependent potassium channels (16). Formation of calcium sparks and activation of calcium-dependent potassium channels are thought to represent a negative-feedback mechanism that limits tonic membrane depolarization and constriction of cerebral blood vessels in response to increases in blood pressure and other vasoconstrictor stimuli (4, 16, 18, 19).

In the present study, we found that two inhibitors of calcium-dependent potassium channels (TEA and iberiotoxin) tended to augment constriction of cerebral arterioles during moderate acute increases in arterial pressure (to 130 and 150 mmHg), but this effect was not statistically significant. Although these findings do not exclude some role for activity of calcium-dependent potassium channels, the data suggest that these potassium channels do not exert a major influence on the tone of cerebral arterioles in vivo during moderate increases in arterial pressure.

In contrast to the modest effects observed during more moderate increases in arterial pressure, TEA and iberiotoxin significantly inhibited increases in the diameter of cerebral arterioles that occurred during greater elevations in arterial pressure. In the presence of these inhibitors, arteriolar diameter did not increase even when arterial pressure was raised to 200 mmHg. These results suggest that activity of calcium-dependent potassium channels has a major influence on vascular tone during large increases in arterial pressure and that modulation of cerebral autoregulation by these ion channels varies depending on the level of acute hypertension. This result is consistent with data obtained using pressurized cerebral arteries in vitro (20).

In contrast to the data with inhibitors of calcium-dependent potassium channels, 4-AP had no significant effect on the diameter of cerebral arterioles during moderate or severe increases in arterial pressure. The 4-AP tended to inhibit increases in the diameter of cerebral arterioles that occurred when arterial pressure was increased by 200 mmHg, but the changes were not statistically significant. Although these findings do not exclude some role for activity of voltage-dependent potassium channels, the data suggest that this subgroup of potassium channels does not exert a major influence on cerebral microvascular tone during acute increases in arterial pressure.

Use of potassium-channel inhibitors. To our knowledge, measurements of the activity of potassium channels or the membrane potential of cerebral vascular muscle in vivo have not been reported. Thus although direct electrophysiological data on the activity of potassium channels in vivo in the cerebral circulation is lacking, an estimation of the functional importance of these channels in intact cerebral arterioles can be made using pharmacological inhibitors such as TEA and iberiotoxin. The conclusions from such studies are dependent on the selectivity of these agents. Available data suggest that iberiotoxin is a highly selective inhibitor of calcium-dependent potassium channels (10, 26), and TEA is considered to be a selective inhibitor of these channels in the concentration range used in these experiments (26). Although structurally dissimilar, both TEA and iberiotoxin had similar effects in the present experiments. Our findings are also consistent with previous studies of the effects of the same inhibitors on cerebral blood vessels in vitro (4, 18, 19).

Because iberiotoxin and TEA attenuated cerebral vascular dilation during increases in arterial pressure, it is important to consider whether these inhibitors exert nonspecific effects on responses to vasoactive stimuli. At the electrophysiological levels, both iberiotoxin and TEA are considered to exert a high degree of selectivity at the concentrations used (26). In relation to vascular responses, we and others have observed that TEA and iberiotoxin do not alter responses of cerebral arterioles to several vasodilator stimuli, including papaverine, acetylcholine, aprikalim, pinacidil, and cromakalim (2, 27, 31, 34, 35). In the present experiments, we also examined effects of these inhibitors on vasoconstrictor responses using U-46619. We found that both TEA and iberiotoxin tended to increase vasoconstrictor responses to U-46619, although only the effect of iberiotoxin was statistically significant. We suspect that this modest augmentation of response to U-46619 is an effect on calcium-dependent potassium channels and not the result of a nonspecific effect of iberiotoxin. Because constriction of cerebral arterioles in response to U-46619 presumably is associated with increases in levels of intracellular calcium, it is not surprising that vasoconstrictor responses may be increased by inhibitors of calcium-dependent potassium channels. The finding that TEA and iberiotoxin had no significant effect on baseline diameter of cerebral arterioles is consistent with previous studies (2, 3, 27, 31, 32, 34, 35) and also provides some evidence against nonspecific effects of these inhibitors in brain microvessels. Thus TEA and iberiotoxin may produce some augmentation of vasoconstrictor response to U-46619, although the effects appear to be modest. Available evidence from this study and work in the literature suggest that these inhibitors are selective at the concentrations used in these experiments.

In conclusion, increases in arteriolar diameter during acute increases in arterial pressure are attenuated by TEA and iberiotoxin. These data suggest that autoregulatory constriction of cerebral blood vessels during acute increases in arterial pressure is attenuated by activation of calcium-dependent potassium channels, and that marked dilatation (i.e., breakthrough) of cerebral vessels at high levels of arterial blood pressure is not simply a passive mechanism.

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