Role of Potassium Channels in Regulation of Brain Arteriolar Tone
Comparison of Cerebrum Versus Brain Stem

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Background and Purpose—Potassium channels are important regulators of resting tone in large cerebral arteries, but their activity and distribution may vary according to vessel location and species studied. In the cerebral microcirculation in vivo, however, these channels appear to be silent at rest. Our goal was to determine the activity of potassium channels of brain arterioles from 2 origins under basal conditions in vitro.

Methods—Penetrating cerebral (40.9±2.2 μm control diameter) and brain stem (36.2±1.2 μm) arterioles of rats were prepared from middle cerebral and basilar arteries, respectively. The internal diameter of cannulated and pressurized vessel was monitored with the inverted microscope before and after administration of potassium channel inhibitors. In addition, we studied the effect of nitric oxide synthase inhibition on potassium channel activity.

Results—Cerebral and brain stem arterioles were significantly constricted by 4-aminopyridine and low concentration of BaCl2 but not by glibenclamide. The addition of Nω-nitro-L-arginine to 4-aminopyridine further decreased diameters of both arterioles. Tetraethylammonium ion caused a significant constriction of brain stem but not cerebral arteriole. The brain stem arteriole was further constricted by additional Nω-nitro-L-arginine.

Conclusions—Voltage-dependent and inward-rectifying, but not ATP-sensitive, potassium channels are active under basal conditions of rat cerebral and brain stem arterioles. There is a regional difference in the activity of calcium-activated potassium channels, which, at rest, are open in brain stem but silent in cerebral arterioles. In addition, basal endogenous nitric oxide may not contribute to the activation of voltage-dependent and calcium-activated potassium channels. (Stroke. 2001;32:218-224.)

Key Words: arterioles □ brain stem □ microcirculation □ potassium channels □ rats

The brain circulation is characterized by autoregulation of blood flow over wide ranges of perfusion pressure.1,2 Interestingly, effective autoregulation is more potent in the brain stem than in the cerebrum.3-5 The basal vascular tone is known to be responsible for this regional difference in autoregulation in the brain.3-4 In general, development of the tone involves pressure- and/or stretch-dependent (myogenic tone) mechanisms.1,6,7 This basal tone is influenced by many regulatory systems, including endothelium-derived relaxing factors such as nitric oxide (NO),8,9 endothelium-derived contracting factors such as prostaglandin H2,10 and smooth muscle potassium (K V) channels.11,12

Recently, vascular smooth muscle K+ channels have drawn attention as a regulator of basal tone in cerebral arteries. K+ channels play a major role in the regulation of both membrane potential and arterial diameter under resting conditions.11-17 Furthermore, changes in K+ channel activity after subarachnoid hemorrhage may contribute to vascular insufficiency.11 Cerebral vessels may express at least 4 different types of K+ channels, including ATP-sensitive K+ (K ATP) channels, calcium-activated K+ (K Ca) channels, voltage-dependent K+ (K V) channels, and inward rectifiers K+ (K IR) channels.11-18 In vivo and vitro studies demonstrated that all types except K ATP channels may be active under resting conditions in large cerebral arteries.13-17,19 In addition, it is interesting that basal NO may hyperpolarize vascular muscle through activation of K+ channels in cerebral vessels.20,21 Thus, basal NO regulates vascular tone not only via K+ channel-independent mechanisms but also via K+ channel-dependent mechanisms, most likely by activating K Ca or K V channels.12,16,20-24 In the cerebral microcirculation, however, it has been reported that arteriolar K+ channels are present but are generally silent at resting states.11,12 These findings are based on in vitro studies reporting that K+ channel inhibitors did not affect the resting vessel diameter in pial arterioles in cranial window experiments.23,25,26 To our knowledge, there is no in vitro study concerning the basal activity of brain arteriolar K+ channels and...
comparing microvessels from different distributions, such as penetrating arterioles from the cerebrum and brain stem. Penetrating arterioles are important regulators of cerebral blood flow and may contribute as much as 23% of total arterial cerebrovascular resistance.27

The present study was therefore conducted to determine (1) the activity of K+ channels in isolated and pressurized brain arterioles under basal conditions in which the vessels had developed spontaneous tone, (2) regional differences between arterioles originating from cerebrum and brain stem, and (3) the role of basal NO in Kc and KC channels activity.

Materials and Methods

Studies were approved by the Washington University Advisory Committee for Animal Resources. Adult male Sprague-Dawley rats (n = 36; weight, 350 to 450 g) were anesthetized with pentobarbital sodium (60 mg/kg IP) and killed. Brain was rapidly removed and placed in a cooled (4°C) dissection chamber filled with physiological salt solution (see below) containing 1% dialyzed bovine serum albumin. The following drugs were purchased: tetraethylammonium ion (TEA), glibenclamide, 4-aminopyridine (4-AP), BaCl2, NO-nitro-L-arginine (L-NNA), and pinacidil (Sigma).

To test the activity of various K+ channels in isolated brain arterioles under basal conditions, we applied 4 K+ channel inhibitors: TEA (a specific inhibitor of Kc channel), glibenclamide (a specific inhibitor of KATP channel), 4-AP (a specific inhibitor of Kc channel), and low concentration of BaCl2 (a specific inhibitor of KC channel). Some of the TEA- or 4-AP-treated arterioles were additionally treated with 10 μmol/L L-NNA (a NO synthase inhibitor). We also examined the effect of L-NNA followed by additional TEA or 4-AP.

In a separate series of experiments, we tested pinacidil (a KATP channel opener) to determine whether KATP channels were present in the brain stem arteriole. To activate KATP channels in these vessels, we also elevated extracellular K+ concentration ([K+]o) from 3 to 8 mmol/L. Isotonic K+ MOPS-buffered saline was prepared by substituting NaCl with an equimolar amount of KCl.

Each value represents the mean±SEM. One or 2 arterioles were studied from each animal. Single comparisons were made with Student’s paired or unpaired t test, as appropriate. For comparison of the various treatments, results were compared by ANOVA, followed by the Student-Newman-Keuls test. Values of P<0.05 were considered statistically significant.

| Passive diameter, Control Diameter, Tone, Dilation to Acidosis, and Constriction to Alkalosis in Isolated Rat Cerebral (n=24) and Brain Stem (n=36) Arterioles |
|---------------------------------|---------------------------------|
| Cerebrum | Brain Stem |
| Passive diameter, μm | 58.8±2.7 | 53.8±1.3† |
| Control diameter, μm* | 40.9±2.2 | 36.2±1.2† |
| Tone, % | 66.6±2.4 | 67.2±1.4† |
| Acidosis-induced dilation, % | 123.2±6.6 | 127.3±1.1† |
| Alkalosis-induced constriction, % | 71.6±2.6 | 72.6±0.9† |

Values are mean±SEM.

*Resting diameter=100%.

†P<NS, cerebral vs brain stem arterioles.

extraluminal solution was then warmed from room temperature to 37.5°C, and the organ bath was continuously perfused at a rate of 0.5 mL/min with a peristaltic pump (model 203, Scientific Industries, Inc.). Over approximately 45 minutes, spontaneous tone developed, and control diameter was measured. Vessels with poor tone (<20% decrease from the maximum diameter) were discarded for further studies. Before the experiment, vessel responsiveness was evaluated and compared by rapidly changing the pH of the extraluminal solution from 7.3 to 6.8 and from 7.3 to 7.65. Vessels with poor response (<15% diameter change) were discarded at this point.

The composition of the physiological salt solution (in mmol/L) was as follows: 144 NaCl, 3 KCl, 2.5 CaCl2, 1.4 MgSO4, 2.0 pyruvate, 5.0 glucose, 0.02 ethylenediaminetetraacetic acid, 2.0 3-(N-morpholino)propanesulfonic acid (MOPS), and 1.21 NaH2PO4. Solutions used for dissection and cannulation contained 1% bovine serum albumin. The following drugs were purchased: tetraethylammonium ion (TEA), glibenclamide, 4-aminopyridine (4-AP), BaCl2, NO-nitro-L-arginine (L-NNA), and pinacidil (Sigma).

Figure 1. Effect of TEA and L-NNA on tone of cerebral (n=5) and brain stem (n=5) arterioles. *†Significant differences (P<0.05) from control and 3 mmol/L TEA, respectively. Note that TEA significantly reduced the control diameter of brain stem arteriole but not cerebral arteriole. L-NNA further constricted the TEA-treated brain stem arterioles.
Results

All vessels developed spontaneous tone and responded to pH challenge (summarized in the Table). There were no significant differences in passive diameter, spontaneous tone, dilatation to acidosis, and constriction to alkalosis between cerebral and brain stem arterioles.

Effect of K_Ca Channel Inhibitor

TEA did not cause a significant change in diameter of cerebral arteriole (96.1±2.3% of the control diameter) (n=5; Figure 1). On the other hand, TEA (1 and 3 mmol/L) significantly constricted the brain stem arteriole (83.1±2.5% and 74.4±2.4% of the control diameter, respectively) (n=5; Figure 1). TEA-induced constriction of brain stem arteriole started within 5 minutes and reached a stable maximum approximately 15 minutes after the solution was changed. Additional treatment with L-NNA (10 μmol/L) further decreased the diameter of brain stem arteriole (79.0±3.8% of the 3 mmol/L TEA–treated diameter). Since TEA showed no effect on cerebral arterioles, L-NNA was not tested in these vessels.

L-NNA decreased the control diameter of brain stem arterioles (76.7±4.0%), and additional TEA induced further significant constriction (79.6±4.9% of the 10 μmol/L L-NNA–treated diameter) (n=4; Figure 2A).

These results suggest the following: (1) K_Ca channels are either not present in cerebral arterioles or are silent during resting conditions in these vessels; (2) K_Ca channels are present in brain stem arteriole, and some of them are activated under basal conditions; and (3) basal NO may not contribute to K_Ca channel activity in brain stem arteriole.

Effect of K_ATP Channel Inhibitor

Glibenclamide (3 μmol/L) had no significant effect on the diameter of cerebral arteriole (99.0±3.2% of the control diameter) and brain stem arteriole (91.8±5.0%) arterioles within 30 minutes of application (n=5; Figure 3). Glibenclamide (3 μmol/L) tended to reduce the diameter of brain stem arteriole; however, this was not significant. A higher concentration of glibenclamide (10 μmol/L) also did not cause significant constriction of these vessels (n=5; Figure 3). These results indicate that K_ATP channels are either absent or silent in both arterioles under resting conditions.

Effect of K_V Channel Inhibitor

4-AP (0.1 and 1 mmol/L) significantly constricted cerebral arterioles (89.4±2.6% and 77.5±3.5% of the control diameter, respectively) (n=5; Figure 4). Additional treatment with L-NNA (10 μmol/L) further reduced the diameter of cerebral arterioles (67.8±3.4% of the 1 mmol/L 4-AP–treated diameter). In brain stem arterioles, 4-AP (0.1 and 1 mmol/L) produced a significant constriction (83.5±2.5% and 69.9±4.0% of the control diameter) (n=5; Figure 4). Brain stem arterioles treated with 1 mmol/L 4-AP were also constricted by 10 μmol/L L-NNA (68.8±2.5% of the 1 mmol/L 4-AP–treated diameter). The time course of 4-AP–induced constriction was similar to that of TEA-induced constriction.

Figure 2. A, Effect of L-NNA followed by TEA on the diameter of brain stem arterioles (n=4). B, Effect of L-NNA followed by 4-AP on the diameter of cerebral (n=4) and brain stem arterioles (n=4). *†Significant differences (P<0.05) from control and 1 mmol/L 4-AP or 3 mmol/L TEA, respectively. Note that 4-AP and TEA significantly constricted the L-NNA–treated arterioles.

Figure 3. Effect of glibenclamide on the diameter of cerebral (n=5) and brain stem arterioles (n=5). Glibenclamide had no effect on the basal diameter.
Cerebral and brain stem arterioles were significantly constricted by 10 mmol/L L-NNA (78.0 ± 2.5% and 75.9 ± 5.3% of the control diameter, respectively), and the subsequent application of 1 mmol/L 4-AP further decreased diameters of these vessels (73.1 ± 5.0% and 77.0 ± 2.7% of the 10 mmol/L L-NNA–treated diameter, respectively) (n = 4; Figure 2B).

These results suggest the following: (1) KV channels are active under basal conditions of both arterioles, and (2) basal release of NO may not contribute to activation of KV channels.

**Effect of K IR Channel Inhibitor**

BaCl2 (30 and 100 μmol/L) significantly decreased the resting diameter of cerebral (90.0 ± 1.8% and 86.8 ± 3.3% of the control diameter) and brain stem arterioles (90.6 ± 2.5% and 81.4 ± 3.2%, respectively) (n = 5; Figure 5). Our results are consistent with the presence of K IR channels that are open under basal conditions of both arterioles.

**Effect of K ATP Channel Opener**

In brain stem arterioles, pinacidil (1 μmol/L) significantly diluted the vessel (n = 4; 117.8 ± 4.6%), and the pretreatment with 3 μmol/L glibenclamide almost abolished the dilation (100.7 ± 0.4%; P < 0.05). This demonstrates that K ATP channels are present in brain stem arterioles.

**Effect of [K+]o**

The small elevation of [K+]o (8 mmol/L) induced strong dilation of brain stem arterioles, and 30 μmol/L BaCl2 significantly attenuated this dilation from 159.5 ± 12.7% to 131.2 ± 14.3% (n = 4). This result supports the hypothesis that there are K IR channels in brain stem arterioles.

**Discussion**

The present study characterized, for the first time, the basal activity of K+ channels in rat brain penetrating arterioles from 2 distributions in vitro. The major findings are as follows: (1) KV and K IR channels are present and contribute to the resting tone of both arterioles. (2) K ATP channels are silent at resting states. (3) There is a regional difference in the basal activity of K Ca channels. In cerebral arteriole these channels are either absent or silent under resting conditions, while some are open in brain stem arteriole. (4) The basal production of NO may not contribute to the activity of either K Ca or K IR channels.

**Role of K Ca Channel**

The K Ca channel is abundantly present in vascular smooth muscle and is activated both by elevated concentrations of intracellular calcium and by membrane depolarization.11,12 It acts as the modulator of vasoconstrictor responses as well as the mediator of vasodilation. In addition, this type of K+ channel regulates resting membrane potential and influences the resting diameter of cerebral blood vessels, especially large vessels such as rat basilar,16,32 rat middle cerebral,33 and human pial14 arteries.

In contrast, it is generally accepted that K Ca channels are present but silent in cerebral arterioles in vivo.23,25,26 However, Régrigny et al34 recently reported that TEA induced significant constriction of rat pial arterioles in vivo. Thus, in the cerebral microcirculation, there may be regional or species-dependent differences in magnitude of influence of these channels on basal tone. Our results support this heterogeneity and indicate that the regulatory mechanism of basal tone in the territories of cerebral and brain stem arterioles may be different because K Ca channels in brain stem, but not in cerebral arterioles, are active under basal conditions. Since there are no specific activators of K Ca chan-
nuls,11 we cannot test for the presence of Kcn channels in cerebral arterioles. We can only speculate on the physiological significance of this regional difference. If Kcn channels are modulators of vasoconstrictor response,12 one consequence could be a difference in autoregulatory response between the cerebral and brain stem arterioles.4,5 During severe hypertension, autoregulation is more effective in the brain stem than cerebrum of cats.4,5 This potency of the brain stem circulation may be due to greater resistance of small vessels compared with the cerebrum. In addition, microvascular pressure in similar-sized arteries and arterioles is higher in the brain stem than in the cerebrum.3–5

This suggests that the basal tone of small vessels would be stronger in the brain stem than in the cerebrum. In the present study there was no difference in basal tone between the brain stem and the cerebral arterioles. Nevertheless, Kcn channels were active in brain stem arterioles in our experimental conditions. This may indicate that in these vessels Kcn channels serve as a negative feedback mechanism to regulate arteriolar tone and contribute to the enhanced autoregulation observed in the brain stem.4,5

The inactivity of Kcn channels at resting states has been reported in other microcirculatory beds such as cremasteric microcirculation.35,36 Although the lack of activity of these channels at basal tone in the microcirculation remains unclear, this inactivity could be explained by a low voltage sensitivity, a low calcium sensitivity, or a high calcium set point of the channel.35–37 Recently, in cremasteric microcirculation, Jackson and Blair35 proposed that the high calcium set point was responsible for the inactivity of Kcn channels by pharmacological and patch-clamp techniques. Thus, Kcn channels in cremasteric arteriolar muscle cells require relatively higher concentrations of calcium than usual to be active at physiological membrane potentials.

Role of KATP Channel
KATP channels are activated by several stimuli, such as reductions in intracellular ATP, PO2, and pH.11,12 This type of K+ channel is distributed in both large cerebral arteries and pial arterioles.11,12,18,26 It is generally known that KATP channels are not activated under resting conditions in both cerebral arteries and arterioles.26,32,38 In rat cerebral penetrating arterioles, similar to our preparation, it was found that KATP channels are present but inactive under resting conditions.39,40 In our study we confirmed the presence of KATP channels in brain stem arteriole using the KATP channel opener pinacidil and inhibitor glibenclamide. Thus, KATP channels are distributed in both arterioles, but they are silent under basal conditions. On the other hand, Nagao and coworkers18 reported that glibenclamide significantly caused substantial depolarization in rabbit vertebral arteries, suggesting that some KATP channels may be open under resting conditions. These data suggest that the basal activity of KATP channels in the anterior circulation is different from that in the posterior circulation. In addition, they proposed that the distribution of KATP channels decreased along the vascular tree from vertebral to superior cerebellar arteries because there was a regional heterogeneity in the sensitivity to an opener of KATP channels.

In other organs, glibenclamide depolarized the membrane and/or decreased the diameter in both arteries and arterioles such as the rabbit mesenteric artery41 and the hamster cremaster arteriole,36 supporting the hypothesis that the activity of KATP channels contributes to and influences the basal tone. Thus, there is also heterogeneity of basal activity of KATP channels among species and tissues.

Role of KV Channel
KV channels are activated by membrane depolarization, similar to Kcn channels; however, this activation does not depend on the intracellular calcium concentration.11,17,19,40,42–44 Low concentration of BaCl2 (<50 μmol/L) has been shown to be a selective antagonist of KIR channels.17,44 Edwards et al12,43 previously showed that K+ current of KIR channel will be outward at membrane potentials of ≤−50 mV. We reported that the resting membrane potential of isolated rat cerebral arteriole was approximately −40 mV.46 suggesting that K+ current of KIR channel may be outward in resting conditions. KIR channels may participate in regulation of cerebral vascular tone.17 In this study BaCl2 (30 μmol/L) significantly constricted both arterioles. This result indicates that KIR channel also appears to be present and open in the resting states of both arterioles.

Small elevations of [K+]o cause dilation of cerebral vessels via stimulation of KIR channels.17,19,40,44 Recently, Nguyen et al showed that K+ ion activated KIR channels, resulting in dilation of rat cerebral penetrating arterioles. In the brain stem arteriole, the elevation of [K+]o also caused the dilation that was inhibited by low concentration of BaCl2. These data further support the hypothesis that KIR channels are present in both arterioles.

Role of NO in Potassium Channel Activity
We previously showed that basal production of NO contributed to the resting diameter of rat cerebral arterioles.8 Recently, it was demonstrated that the vasodilator response to both basal and agonist-induced NO may cause hyperpolarization via activation of K+ channels, especially Kcn and KATP channels in cerebral arteries.12,16,21,22,24 Thus, there are 3 possible ways in which basal NO can regulate arteriolar tone: (1) via a K+ channel–independent mechanism, (2) directly or indirectly via stimulation of K+ channels, or (3) through both mechanisms.11,12 In the present study the NO
synthase inhibitor L-NNA further constricted the arteriole treated with TEA or 4-AP, and the L-NNA–treated arterioles were also constricted by 4-AP or TEA. We reported that L-NNA constricted rat cerebral arterioles without increasing intracellular calcium levels, and Nω-monomethyl-L-arginine (another inhibitor of NO synthase) caused constriction without affecting membrane potential. These findings suggest that a possible stimulation of Kv or Kir channels, if any, might not have a major role in regulation of arteriolar tone by basal NO because the activity of K⁺ channels contributes to both membrane potential and intracellular calcium concentration. In summary, Kv and Kir, but not KATP, channel inhibition produces a substantial constriction in both cerebral and brain stem arterioles, while Kir channel inhibition constricts brain stem but not cerebral arterioles. These findings suggest that Kv and Kir channels are active and KATP channels are silent under resting conditions in both arterioles. Kir channels appear to be active at basal tone in brain stem but not cerebral arterioles. In addition, basal NO may not contribute to the activity of either Kir or Kv channels. Thus, the basal activity of several K⁺ channels may regulate the spontaneous tone in rat brain microcirculation independent of basal NO.

Acknowledgments

This study was supported by National Institutes of Health grants HL57540 and NS30555. We are very grateful to Robyn L. Reese and Christine M. Pluchinsky for expert technical assistance.

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Activity of potassium (K⁺) channels is a major regulator of membrane potential of vascular muscle, and is therefore an important determinant of vascular tone. At least 4 types of K⁺ channels are known to be expressed in cerebral arteries: calcium-activated (K_Ca), voltage-dependent (K_V), ATP-sensitive (K_ATP), and inwardly rectifying (K_IR) K⁺ channels. Activation of K⁺ channels may influence resting vascular tone and modulate responses to vasoconstrictors, and it is believed to play a major role in the mechanism(s) underlying cerebral vasodilation in response to a variety of stimuli, including hypoxia and hypercapnia, receptor-mediated agonists, potassium ion, reactive oxygen species, and second messengers. 1–3

Although studies using pharmacological inhibitors have provided evidence that several K⁺ channels influence cerebral vascular tone under basal conditions in vitro and in vivo, 4–6 little is known as to whether there are regional variations in the expression and functional importance of specific K⁺ channels in the cerebral circulation, as has been reported in the pulmonary circulation. 7 In the accompanying article, Horiuchi et al have examined the role of K⁺ channels in responses of isolated and pressurized arterioles from brain stem and cerebrum using inhibitors of all major groups of K⁺ channels. Based on the observations that 4-aminopyridine (4-AP) and barium ion produce constriction of arterioles from both regions, it was concluded that K_Ca and K_IR channels, respectively, were active under resting conditions in these vessels. A lack of effect of glibenclamide, an inhibitor of K_ATP channels, suggested no influence of this type of ion channel under resting conditions. All of these results are consistent with previous findings. 1–6 A new finding in the study was the observation that tetraethylammonium ion (TEA) caused constriction of arterioles from brain stem, but not cerebrum, suggesting that regional differences in basal K_Ca channel expression or activity may be present within the cerebral circulation. The mechanism that accounts for this regional difference in the influence of K_Ca channels was not studied. One possibility is that expression of the genes which encode the 2 proteins that together constitute the K_Ca channel vary in different brain regions.

A second goal of these studies was to examine the interaction of nitric oxide (NO) with K⁺ channels in cerebral arterioles. Because cerebral vasodilator responses to NO may be attenuated by application of inhibitors of some K⁺ channels, 3,5 it might be expected that at least part of the activity of these channels under resting conditions results from activation by NO produced by endothelium under basal conditions. The finding that TEA and 4-AP caused similar vasoconstrictor responses whether administered in the presence or absence of an inhibitor of NO synthase suggested to the authors that activity of K_Ca and/or K_V channels may not be influenced by NO produced under basal conditions in normal arterioles.

Production and/or activity of NO by endothelium is known to be diminished under pathophysiological conditions. 2 An implication of the present study is that the depolarized state of vascular muscle observed in disease states (eg, hypertension, diabetes, subarachnoid hemorrhage) may not be directly caused by the decreased levels of NO within the vessel wall. Because recent studies suggest that K⁺ channels in the cerebral circulation may become functionally more important under pathophysiological conditions, 3 it will be of interest to examine the interaction of NO and K⁺ channels in cerebral vessels in disease states as well.

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*Stroke.* 2001;32:218-224
doi: 10.1161/01.STR.32.1.218

*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

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