ATP-Sensitive Potassium Channels in the Basilar Artery During Chronic Hypertension

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We examined the hypothesis that dilatation of the basilar artery in response to activation of ATP-sensitive potassium channels is impaired in stroke-prone spontaneously hypertensive rats (SHRSP). Changes in basilar artery diameter in response to aprikalim, a direct activator of ATP-sensitive potassium channels, were measured in anesthetized SHRSP and normotensive Wistar-Kyoto (WKY) rats through a cranial window. Topical application of aprikalim increased basilar artery diameter in WKY rats. Glibenclamide, a selective inhibitor of ATP-sensitive potassium channels, abolished aprikalim-induced vasodilatation. Thus, ATP-sensitive potassium channels are functional in the basilar artery of WKY rats in vivo. Aprikalim (10^-6 mol/L) dilated the basilar artery by 31±5% (mean ± SEM) in WKY rats but only 5±1% in SHRSP. The concentration-response curve to aprikalim in SHRSP was significantly shifted to the right, but the response to the highest concentration of aprikalim (10^-5 mol/L) was similar in SHRSP and WKY rats. Vasodilatation in response to norepinephrine was also impaired in SHRSP. Dilator responses of the basilar artery to forskolin, a direct activator of adenylate cyclase, and nitroprusside, a direct activator of guanylate cyclase, were normal in SHRSP. The findings suggest that dilatation of the basilar artery in response to direct activation of ATP-sensitive potassium channels is impaired in SHRSP compared with WKY rats in vivo. (Hypertension. 1993;22:677-681.)

KEY WORDS • cerebral arteries • forskolin • norepinephrine • potassium channels • rats, inbred SHR

Vasodilator responses are impaired in several models of experimental hypertension. Impairment of endothelium-dependent responses during chronic hypertension has been described both in vitro and in vivo. In contrast, endothelium-independent vasodilator responses are generally considered to be normal in hypertensive animal models.

Most previous studies of endothelium-independent mechanisms in hypertension have focused on responses to activation of guanylate cyclase, especially in response to nitrovasodilators, or activation of adenylate cyclase. Another major mechanism of vasodilatation involves hyperpolarization of smooth muscle, which can be produced by activation of several types of potassium channels.

Aprikalim, a direct activator of ATP-sensitive potassium channels, dilates the basilar artery in vivo, which suggests that ATP-sensitive potassium channels are functional in the basilar artery. The effect of chronic hypertension on the activity of ATP-sensitive potassium channels in cerebral blood vessels is not known. The first goal of this study was to test in vivo the hypothesis that dilatation of the basilar artery in response to activation of ATP-sensitive potassium channels by aprikalim is altered during chronic hypertension. We examined responses of the basilar artery to aprikalim in stroke-prone spontaneously hypertensive rats (SHRSP).

We have recently reported that dilator responses of the basilar artery to norepinephrine and forskolin in vivo are mediated in part by activation of ATP-sensitive potassium channels. Thus, a cyclic AMP (cAMP)-dependent mechanism may be involved in activation of these potassium channels. The second goal of the present study was to examine the hypothesis that altered activity of ATP-sensitive potassium channels may affect responses of the basilar artery to norepinephrine and forskolin in SHRSP.

Methods

Animal Preparation

Male Wistar-Kyoto (WKY) rats (386±7 g, n=15) and SHRSP (335±5 g, n=15) (6 to 9 months old) were anesthetized with pentobarbital sodium (50 mg/kg IP). The trachea was cannulated, and the animals were mechanically ventilated with room air and supplemental oxygen. Skeletal muscle paralysis was produced with gallamine triethiodide (5 to 10 mg/kg). Anesthesia was supplemented regularly at 20 to 25 mg/kg per hour. Depth of anesthesia was evaluated by applying pressure to a paw or the tail and observing changes in heart rate or blood pressure. When such changes occurred, additional anesthetic was administered. Catheters were placed in both femoral arteries to measure systemic...
arterial blood gases were monitored and maintained within normal limits throughout the experiments in both rat strains.

A craniotomy was prepared over the ventral brain stem. A portion of the dura mater was opened and suffused with artificial cerebrospinal fluid (temperature, 37°C; ionic composition [mmol/L], NaCl, 132; KCl, 2.95; CaCl₂, 1.71; MgCl₂, 0.65; NaHCO₃, 24.6; d-glucose, 3.69) that was bubbled continuously with appropriate gases to produce normal levels of pH and PCO₂. Basilar artery diameter was measured using a microscope equipped with a television camera coupled to a video monitor and image-shearing device (model 908, Instrumentation for Physiology & Medicine, San Diego, Calif). The images were recorded on videotape for later analysis.

**Experimental Protocol**

We examined responses of the basilar artery to topical suffusion of aprikalim (10⁻² to 10⁻⁵ mol/L), forskolin (10⁻⁴ to 10⁻³ mol/L), norepinephrine (10⁻⁸ to 10⁻³ mol/L), and sodium nitroprusside (10⁻⁴ to 10⁻⁶ mol/L). Agonists were mixed in artificial cerebrospinal fluid and suffused over the craniotomy for 5 minutes. Basilar artery diameters were measured immediately before and during the last minute of application of each agonist. After application of each agonist, the artery returned to baseline diameter within a few minutes before application of a subsequent agonist. The agonist sequence of agonists was randomized. Glibenclamide (10⁻⁶ mol/L) was suffused 5 minutes before and during application of agonists. Glibenclamide and aprikalim were dissolved in dimethyl sulfoxide. Control experiments were performed in the presence of the vehicle, 0.05% dimethyl sulfoxide.

**Statistical Analysis**

All values are expressed as mean±SEM. An unpaired t-test was used to compare absolute values under control conditions and during interventions, and Wilcoxon's test was used to compare percentage changes. A value of *P*<.05 was considered significant.

**Results**

Mean arterial pressure was 95±5 mm Hg in WKY rats and 203±10 mm Hg in SHRSP. Under control conditions, baseline basilar artery diameter was smaller in SHRSP (209±6 μm) than in WKY rats (277±19 μm) (*P*<.05).

**Responses to Aprikalim**

In WKY rats, topical application of aprikalim increased basilar artery diameter, with a maximum response of 44±4% (Fig 1). Application of glibenclamide (10⁻⁶ mol/L) alone for 5 minutes did not produce any significant change in basilar artery diameter (*P*>.05) but almost completely inhibited dilatation of the basilar artery in response to aprikalim (*P*<.05) (data not shown). Thus, ATP-sensitive potassium channels are functional in the basilar artery of WKY rats in vivo. The findings are similar to those observed in Sprague-Dawley rats.¹¹

In SHRSP, the concentration-response curve of the basilar artery to aprikalim was shifted to the right compared with that in WKY rats (Fig 1). Aprikalim (10⁻⁴ mol/L) dilated the basilar artery by 31±5% in WKY rats but only 5±1% in SHRSP (*P*<.05 versus WKY rats). The response to the highest concentration of aprikalim (10⁻⁵ mol/L), however, was similar in SHRSP and WKY rats (Fig 1).

**Responses to Forskolin, Norepinephrine, and Nitroprusside**

Dilatation of the basilar artery in response to norepinephrine and forskolin appears to be mediated in part by activation of ATP-sensitive potassium channels.¹² We examined responses of the basilar artery to norepinephrine and forskolin in SHRSP and WKY rats. Norepinephrine caused marked vasodilatation in WKY rats, and the response was impaired significantly in SHRSP (Fig 2, *P*<.05). In contrast, forskolin produced similar dilatation of the basilar artery in WKY rats and SHRSP (Fig 3, *P*>.05). Responses of the basilar artery to sodium nitroprusside were similar in WKY rats and SHRSP (Fig 4, *P*>.05).

**Discussion**

There are three major new findings in the present study. First, dilatation of the basilar artery in response to aprikalim is impaired in SHRSP in vivo. Thus, activity of ATP-sensitive potassium channels in the basilar artery is altered during chronic hypertension. Second, dilatation of the basilar artery in response to forskolin is not impaired in SHRSP in vivo. Thus, activation of ATP-sensitive potassium channels in the basilar artery by a CAMP-dependent mechanism may not be impaired during chronic hypertension. Third, dilatation of the basilar artery in response to norepinephrine, which may be mediated in part by activation of ATP-sensitive potassium channels, is impaired in SHRSP.

**Altered Responses to Aprikalim**

Aprikalim, a direct activator of ATP-sensitive potassium channels,¹³ dilates the basilar artery (see Refer-
Fig 2. Line graph shows responses of basilar artery in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) to norepinephrine (10⁻⁸ to 10⁻⁵ mol/L). Values are mean±SEM in six WKY rats and six SHRSP. *P<.05 vs responses in WKY rats.

Fig 3. Line graph shows responses of basilar artery in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) to forskolin (10⁻⁸ to 10⁻⁵ mol/L). Values are mean±SEM in six WKY rats and six SHRSP.

Fig 4. Line graph shows responses of basilar artery in Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) to sodium nitroprusside (10⁻⁸ to 10⁻⁶ mol/L). Values are mean±SEM in six WKY rats and six SHRSP.
Responses to Forskolin, Norepinephrine, and Nitroprusside in SHRSP

A proposed mechanism of dilatation of the basilar artery in response to forskolin and norepinephrine is summarized in Fig 5. Dilatation of the basilar artery in response to forskolin, a direct activator of adenylate cyclase, appears to be mediated in part by activation of ATP-sensitive potassium channels, suggesting that the cAMP-dependent mechanism may activate potassium channels. Norepinephrine-induced dilatation of the basilar artery is mediated in part by activation of ATP-sensitive potassium channels, and a cAMP-dependent mechanism may also be involved in activation of potassium channels by norepinephrine.

Dilatation of the basilar artery in response to forskolin was not reduced in SHRSP. Preservation of responses to forskolin in the basilar artery of SHRSP is similar to findings obtained in femoral and mesenteric arteries in spontaneously hypertensive rats in vitro. Because cAMP concentration cannot be measured in the basilar artery in vivo, we cannot exclude the possibility that a compensatory increase in adenylate cyclase activity masks impaired responsiveness of potassium channels to cAMP. Although forskolin is known to activate adenylate cyclase, it may exert other actions under some conditions. The findings, however, suggest that the cAMP-responsive site on ATP-sensitive potassium channels may not be altered in SHRSP. The findings that there are similar responses to the highest concentration of aprikalim in SHRSP and WKY rats also support this conclusion.

In the present study, dilatation of the basilar artery in response to norepinephrine, which is mediated by activation of \( \beta \)-receptors, was impaired in SHRSP. The number of \( \beta \)-adrenergic receptors appears to be diminished in aorta of spontaneously hypertensive rats. Because forskolin-induced dilatation of the basilar artery is similar in SHRSP and WKY rats, it appears that the cAMP-dependent mechanism is intact. Thus, impaired responses of the basilar artery to norepinephrine in SHRSP may also be due to a reduced number of \( \beta \)-receptors. We also cannot exclude the possibility that increased expression of \( \alpha \)-adrenergic receptors may contribute to reduced vasodilator responses to norepinephrine in SHRSP.

In summary, dilator responses of the basilar artery to aprikalim, a direct activator of ATP-sensitive potassium channels, are impaired in SHRSP in vivo. This impairment may be due to a reduced affinity of the potassium channels to aprikalim. Forskolin-induced vasodilation was not impaired in SHRSP. These findings suggest that responsiveness of potassium channels to a cAMP-dependent mechanism, which seems to occur at a different site than activation by aprikalim, may not be impaired during chronic hypertension.

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References


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