Potassium Channels and Vascular Reactivity in Genetically Hypertensive Rats

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In hypertension, membrane potassium permeability and vascular reactivity are increased. This study characterizes a potassium-selective channel and contractions to barium, a potassium channel inhibitor, in vascular smooth muscle (tail artery) from spontaneously hypertensive stroke-prone rats (SHRSP) and normotensive Wistar-Kyoto (WKY) rats. Smooth muscle cells were isolated by enzymatic digestion, and potassium channel activity was characterized by using patch-clamp technique (inside-out configuration). Isometric contractile activity was evaluated in helically cut arterial strips by using standard muscle bath methodology. In membrane patches, a voltage-gated, calcium-insensitive, potassium-selective channel of large conductance (200 picosiemens) was observed. The channel did not conduct sodium or rubidium. Barium (10^-6 to 10^-4 M) produced a dose-dependent blockade of channel activity. These channel characteristics did not differ in SHRSP and WKY rat cells. After treatment with 35 mM KCl, barium (10^-5 to 10^-3 M) caused greater contractions in SHRSP arteries compared with arteries in WKY rats. The contractions to barium were markedly attenuated in calcium-free solution, and nifedipine and verapamil abolished contractions induced by barium in depolarizing solution. We conclude that increased vascular reactivity to barium in SHRSP arteries is not due to an alteration in the biophysical properties of the potassium channel studied. (Hypertension 1990;15:687-691)

Extensive evidence indicates that K^+ conductance is partly responsible for modulating cellular excitability in vascular smooth muscle.1,2 Electrophysiological studies with patch-clamp technique have demonstrated the presence of both voltage-dependent and Ca^{2+}-dependent K^+ channels in vascular tissue. Differences in the mechanisms of activation of these channels probably relate to their functional roles, with some channels playing a part in the maintenance of resting tone and others limiting or terminating contraction induced by physiological agonists.

In hypertension, it has been demonstrated that vascular reactivity and membrane K^+ permeability are both increased.3-4 Furthermore, several antihypertensive drugs that relax vascular smooth muscle appear to do so by opening membrane K^+ channels.2 The present study characterizes a K^+ selective channel by using patch-clamp technique in arterial smooth muscle cells isolated from stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive Wistar-Kyoto (WKY) rats. The inhibitory actions of barium (Ba^{2+}) on the channel were evaluated. Additionally, contractile activity to Ba^{2+} was characterized in arterial segments from SHRSP and WKY rats to evaluate functional properties related to membrane-associated K^+ channels.

Methods

Adult male and female SHRSP and WKY rats (16–20 weeks old) were obtained from rat colonies maintained in the Department of Anatomy and Cell Biology (University of Michigan, Ann Arbor, Michigan). The systolic blood pressures were measured by an indirect tail-cuff method using a pneumatic transducer (WKY, 118±4 mm Hg [n=12]; SHRSP, 176±4 mm Hg [n=12]; p<0.05).

On the day of an experiment, the rats (one SHRSP and one WKY rat) were anesthetized with sodium pentobarbital (50 mg/kg), and tail arteries were removed and placed in cold physiological salt solution (PSS). A 2–4 cm segment of each artery was cut helically into a strip (0.7×10 mm). The endothelium was removed from all segments by a rubbing procedure (confirmed by the absence of a relaxation response to acetylcholine). The strips were then mounted in an organ chamber containing PSS for measurement of isometric development, as described elsewhere.5 All preparations were allowed to equilibrate for 90 minutes before an experiment was...
begun. The PSS was maintained at 37°C and was aerated with a mixture of 95% O₂ and 5% CO₂. The composition of the PSS (mmol/l) was as follows: NaCl 130, KCl 4.7, KH₂PO₄ 1.18, MgSO₄·7H₂O 1.17, NaHCO₃ 14.9, CaCl₂·H₂O 1.6, dextrose 5.5, and CaNa₂-EDTA 0.03. The K⁺ and Ca²⁺ concentrations of the buffer were altered without compensating for changes in tonicity. All experiments were performed in the presence of 10⁻⁶ M phentolamine to block the actions of norepinephrine released from nerve endings by depolarizing conditions. Contractile responses to KCl (12–120 mM) and Ba²⁺ (10⁻⁵ to 10⁻³ M) were measured in all experiments.

The remaining section of each tail artery was used in studies of K⁺ channel activity by patch-clamp technique. To isolate cells, artery segments were incubated in digestion medium (mg/ml) (collagenase 0.5, trypsin inhibitor 0.3, papain 0.4, dithiothreitol 0.3, bovine serum albumin 7.5, pH 7.4) for 90 minutes at 37°C. Cells were then dispersed by trituration with fire-polished Pasteur pipettes of decreasing tip diameter.

Single channel currents were measured with standard patch-clamp technique. A Dagan 8900 Patch Clamp/Whole Cell Clamp (Dagan Corp., Minneapolis, Minnesota) was used to voltage-clamp membrane patches in the inside-out configuration. Data were digitized with a modified digital audio processor (Sony PCM-701ES, Medical Systems Corp., Greenvale, New York) and recorded on videocassette tape for analysis at a later time. Electrodes were prepared from borosilicate glass (Kimble R-6, Richland Glass, Richland, New Jersey).

The pipette-filling solution (PFS) always contained (mM): KCl 145, HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]) 10, glucose 10, and KOH to bring pH to 7.4. In the bath PFS or a Ca²⁺-buffered solution was used that contained (mM): KCl 113, EGTA 2.02, K₂-PIPES (piperazine-N,N’-bis[2-ethanesulfonic acid]) 20.2, pH 7.0. Sufficient CaCl₂ was added to yield calculated free Ca²⁺ concentrations of 100–1,000 nM as described by Chang et al.⁶ Ba²⁺ in the form BaCl₂ (10⁻⁵ to 10⁻³ M) was added to the bath PFS.

Unless otherwise stated, data are reported as the mean±SEM. ED₅₀ values (agonist dose producing a half-maximal response) were determined after logit transformation of normalized, dose-response curves. Statistical comparisons were performed by Student's t test with the Bonferroni adjustment for multiple procedures. A p value less than 0.05 was considered significant.

Results

Contractile Responses to Potassium Chloride and Ba²⁺

Cumulative addition of KCl (12–130 mM) to the muscle bath caused contractions in tail arteries from SHRSP and WKY rats. Maximal force generation in tail arteries from SHRSP (525±36 mg, n=12) was not different from that in WKY rat arteries (524±36 mg, n=12). Arteries from SHRSP were more sensitive to the contractile properties of potassium chloride than arteries from WKY rats, as indicated by lower ED₅₀ values (SHRSP, -log ED₅₀ value=1.368±0.016, antilog=42.9 mM, n=12; WKY, -log ED₅₀ value=1.301±0.017, antilog=50.0 mM, n=12; p<0.05).

Addition of Ba²⁺ (10⁻⁵ to 10⁻³ M) to the muscle bath had no effect on the resting force of tail artery strips from SHRSP or WKY rats. In contrast, when the arteries were first contracted with KCl (18–50 mM), Ba²⁺ was then able to induce small contractions in arteries from both rat groups (Figures 1 and 2). Arteries from SHRSP developed significantly greater force to Ba²⁺ chloride at 3×10⁻⁴ and 10⁻³ M in the presence of 35 mM KCl (Figure 2). When the K⁺ concentration of the bathing solution was varied from 18 to 50 mM, the magnitude of contractile responses to 10⁻³ M Ba²⁺ increased in a direct manner (Figure 2, inset). Force development to Ba²⁺ (10⁻³ M) in SHRSP arteries was statistically greater than that in WKY rat arteries at K⁺ concentrations of 35 and 50 mM. Contractile responses to KCl in these experiments were as follows: 1) 18 mM KCl: SHRSP, 27±6 mg; WKY, 13±7 mg; 2) 25 mM KCl: SHRSP, 67±18 mg; WKY, 53±16 mg; 3) 35 mM KCl: SHRSP, 195±28 mg; WKY, 124±29 mg; 4) 50 mM KCl: SHRSP, 334±39 mg; WKY, 279±57 mg (n=6 in all groups).

The Ca²⁺-dependence of contractile responses to Ba²⁺ were examined in arteries incubated in Ca²⁺-free depolarizing solution (35 mM KCl) containing 1.0 mM EGTA (Figure 1) and in arteries treated with 10⁻⁶ M nifedipine or 10⁻⁴ M verapamil. Further, the effect of Ba²⁺ on contractile responses to caffeine were examined to determine if the cation had an effect on subcellular stores of activator Ca²⁺. Contractile responses to Ba²⁺ in Ca²⁺-free, depolarizing solution in arteries from SHRSP (10±6 mg, n=6) were not significantly different from WKY rat values (5±2 mg, n=6). Nifedipine and verapamil completely blocked contractions to 35 mM KCl and subsequent responses to Ba²⁺ (10⁻³ M) in both normal PSS and in strips incubated in Ca²⁺-free PSS. Ba²⁺ (10⁻³ M) did not alter contractile responses to caffeine (20 mM) in arteries from SHRSP or WKY rats (Figure 1). Contractile responses to caffeine in arteries from SHRSP (Ca²⁺-free PSS, 200±17 mg; Ca²⁺-free depolarizing PSS, 196±20 mg; n=6) were greater than the responses in WKY rat arteries (Ca²⁺-free PSS, 101±12 mg; Ca²⁺-free depolarizing PSS, 102±13 mg; n=6).

K⁺ Channel Activity

We were able to identify and characterize a large conductance K⁺ channel in inside-out patches of the plasma membrane of tail artery vascular smooth muscle cells from both strains of rat. The number of channels per patch varied considerably (1–5) for cells from both SHRSP and WKY rats. K⁺ currents were recorded that had a slope conductance (psiemens) of 194.8±1.49 (n=5) and 201.3±1.44 (n=5) in SHRSP and WKY rat tail artery cells, respectively (Figure 3).
The channel did not exhibit rectification and was highly selective for K⁺ (K⁺ > Na⁺, Rb⁺). Channel activity did not respond to varying Ca²⁺ concentrations in the bath (10⁻⁹ to 10⁻⁶ M). This channel, however, exhibited pronounced voltage dependence. Channel activity increased as holding potential (bath) was made more positive. There was little or no activity at the equivalent of resting membrane potential in whole cells. There were no apparent differences in the voltage dependence of channels from WKY rat and SHRSP tail artery cells (Figure 3). The channel was blocked in a concentration-dependent
manner by BaCl\(_2\) (10\(^{-6}\) to 10\(^{-4}\) M) (Figure 4). The effect of Ba\(^{2+}\) did not differ in its effect on channels from WKY rat and SHRSP cells (Figure 4).

**Discussion**

This study demonstrates that the contractile properties of Ba\(^{2+}\) in tail arteries from SHRSP are augmented compared with those in tail arteries from WKY rats. This augmented responsiveness does not appear to be related to a difference in the biophysical properties of a voltage-gated, Ca\(^{2+}\)-insensitive, K\(^+\)-selective channel of large conductance. This channel was observed in membrane patches isolated from smooth muscle cells from both SHRSP and WKY rats.

The cellular mechanism that explains augmented responsiveness to Ba\(^{2+}\) in SHRSP arteries is not clear. Ba\(^{2+}\) is rather nonselective in that it blocks most K\(^+\) channels. In many of these channels, it blocks at the K\(^+\)-selectivity filter, which is approximately halfway through the channel. Based on patch-clamp studies, it has been noted that Ba\(^{2+}\) is more potent (10,000-fold) at the inner surface of the membrane compared with the outer surface. Presumably, this indicates that Ba\(^{2+}\) ions can first enter the cell through Ca\(^{2+}\) channels before blocking K\(^+\) channels.

**Figure 3.** Plotting showing current-voltage relation for a K\(^+\) channel from tail artery cells of stroke-prone spontaneously hypertensive rats (SHRSP) (right panel) and Wistar-Kyoto (WKY) rats (left panel). Lines represent the average of five current-voltage plots; five cells per five rats. SEMs for each point are smaller than the symbol before reduction.

**Figure 4.** Recordings showing representative response of K\(^+\) channel to Ba\(^{2+}\) (10\(^{-6}\) to 10\(^{-4}\) M). Effect of Ba\(^{2+}\) on channel activity was similar for channels from both strains of rat. BaCl\(_2\) was added to the bath solution additively. In this experiment, the patch from the stroke-prone spontaneously hypertensive rat (SHRSP) cell contained one channel, and the Wistar-Kyoto (WKY) rat cell contained at least three channels.
channels from the inside. This might explain the differences in Ba$^{2+}$ sensitivity observed in the present study with respect to K$^+$ channels in membrane patches (inside-out configuration) and contractile activity in intact arterial segments.

We speculate that the augmented responsiveness to Ba$^{2+}$ in SHRSP arteries might be related to an alteration in Ca$^{2+}$ permeability. Ba$^{2+}$ did not cause contraction in the resting state, indicating that it was not able to enter the cell and sufficiently block K$^+$ channels to cause membrane depolarization. If the arteries were first treated with depolarizing solution, however, the arteries were able to subsequently contract to Ba$^{2+}$. Because Ca$^{2+}$ permeability is increased in arteries from hypertensive rats,$^4,8$ it might be that in the depolarized state, more Ba$^{2+}$ enters the cell, resulting in a greater block of K$^+$ channels in the hypertensive rat arteries. Further support for this hypothesis is that the contractile properties of Ba$^{2+}$ were markedly attenuated in Ca$^{2+}$-free depolarizing solution, and nifedipine and verapamil completely blocked the contractions. Furthermore, Lamb and Webb$^9$ reported that $10^{-4}$ M Ba$^{2+}$ caused added depolarization of tail artery that had been depolarized with norepinephrine.

This study characterized augmented responsiveness to Ba$^{2+}$ in tail arteries from SHRSP compared with normotensive WKY rat values. This augmented contractile activity is not due to a specific change in the biophysical properties of a K$^+$ channel characterized in isolated membrane patches. We speculate that the augmented responsiveness might be due to an increased entry of Ba$^{2+}$ into the smooth muscle cells through Ca$^{2+}$ channels, with subsequent block of the K$^+$ channels from the inside.

**References**


**Key Words** • barium • potassium • vascular smooth muscle • stroke-prone spontaneously hypertensive rats
Potassium channels and vascular reactivity in genetically hypertensive rats.
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Hypertension. 1990;15:687-691
doi: 10.1161/01.HYP.15.6.687

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