Increased Barium Influx and Potassium Current in Stroke-Prone Spontaneously Hypertensive Rats

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Abstract Arterial potassium permeability is increased in hypertension. In this study we conducted voltage-clamp experiments to determine whether the whole-cell K+ current is increased in a Ca2+-dependent manner in aortic smooth muscle cells from stroke-prone spontaneously hypertensive rats (SHRSP). Aortic cells from Wistar-Kyoto (WKY) rats and SHRSP demonstrated an outward rectifying current elicited by depolarization. The current was carried primarily by K+, because intracellular Cs+ replacement eliminated more than 97% of the current. The current density was higher (P<.05) in SHRSP cells at positive potentials. In the presence of LaCl3 (200 /mol/L) or tetraethylammonium (10 mmol/L), the residual current was similar in WKY and SHRSP cells. Also, the current density did not differ between WKY and SHRSP cells in which the intracellular Ca2+ concentration was clamped at zero. Fura 2 ratio measurement showed similar resting myoplasmic Ca2+ concentration (Ca2+o) in WKY and SHRSP cells (100±10 versus 117±9 nmo/L; P=.2). Under low extracellular Na+ conditions, which had a minimal effect on Ca2+o, Ba2+ replacement of Ca2+ caused a continuous and approximately linear increase in the fura 2 ratio, which was twofold faster in SHRSP cells. Because Ca2+ pumps do not transport Ba2+ and Na+Ca2+ exchange was inhibited by low extracellular Na+, this increase reflected unidirectional Ba2+ influx. These results suggest that (1) Ca2+-dependent K+ channel activity is increased in aortic smooth muscle cells from SHRSP, probably as a result of an increased Ca2+ influx that does not cause an increase in Ca2+o, and (2) increased Ca2+ cycling, which may underlie the increased K+ current, may occur across the sarclemma in SHRSP cells. (Hypertension. 1994;23[part 2]:1091-1095.)

Key Words • barium • muscle, smooth, vascular • fura-2 • lanthanum • tetraethylammonium compounds • potassium

Altered vascular membrane permeability to major cations (Ca2+, K+, and Na+) has been observed in hypertensive animals and humans with essential hypertension.13 Thus, abnormal ionic permeability is thought to be a primary defect in hypertension and may contribute to the increased sensitivity to vasoconstrictors observed in vessels from hypertensive animals.13 Calcium plays a crucial role in regulating the contractile state of vascular smooth muscles. Increased membrane permeability to Ca2+ in hypertension could cause an increase in average myoplasmic Ca2+ concentration (Ca2+i) and thus an increase in tension and total peripheral resistance. However, although 42Ca influx is shown to be increased in hypertension, measurement of Ca2+ by different groups has revealed either no change or an increase in the resting Ca2+i of vascular smooth muscle cells in hypertension.4-9 This inconsistency could be related to the differences in experimental conditions, animal models, and vessel types among the investigations. However, using fura 2 digital imaging, Erne and Hermmsayer10,11 have shown that the resting Ca2+ level at the subsarcolemmal region (peripheral Ca2+) was greater in cultured muscle cells from azogyn vein of spontaneously hypertensive rats (SHR), whereas Ca2+ and Ca2+ levels at the central region (central Ca2+) were not different between Wistar-Kyoto (WKY) rats and SHR. Immediately after stimulation with high K+, Ca2+ was also higher in SHR than WKY cells, and this difference was associated with a greater increase in Ca2+i concentration at the inner side of the sarclemma (Ca2+). Thus, changes in membrane Ca2+ handling in hypertension may lead only to a localized increase in Ca2+ without any detectable increase in Ca2+, especially under resting conditions.

The increase in Ca2+ after Ca2+ influx may activate Ca2+-dependent K+ (KCa) channels, which are known to exist in vascular smooth muscle cells.12 Consistent with this view, increased 42K (or 86Rb) efflux in arteries from hypertensive rats can be normalized by Ca2+ channel blockers.13,14 On the other hand, changes in K+ channel properties may also occur during hypertension, leading to a greater K+ efflux.9,15,16 To determine the mechanisms for the altered K+ efflux in hypertension, we measured whole-cell K+ current, Ca2+, and Ba2+ influx in the present study. Our results indicate that (1) the Ca2+-dependent K+ current is increased in vascular smooth muscle cells from hypertensive rats, and (2) Ba2+ (divalent cation) influx is enhanced in cells from stroke-prone SHR (SHRSP), whereas resting Ca2+ is not elevated.

Methods

Rat and Artery Preparation

Male WKY rats and SHRSP were obtained from Harlan Sprague Dawley Inc and from the University of Missouri, respectively. All rats used were 4 to 6 months old. Mean systolic blood pressure was 127±3 mm Hg for WKY rats and 209±3 mm Hg for SHRSP. Rats were killed by decapitation.
The animal use protocol was approved by an institutional committee at the University of Missouri. The thoracic aorta was removed, dissected, and placed in an ice-cold storage solution until cell isolation.17

**Smooth Muscle Isolation**

Smooth muscle cells were isolated according to the method of Sturek et al17,18 and of DeFeo and Morgan.19 The dispersion solution was a low-Ca2+ (0.5 mmol/L) storage solution containing 294 U/mL collagenase (Worthington), 10 U/mL elastase (Worthington), 2 mg/mL bovine serum albumin (fraction V, Sigma Chemical Co), 1 mg/mL soybean trypsin inhibitor (Worthington), and 0.4 mg/mL deoxyribonuclease I (Sigma). The vial was placed in a shaking water bath at 37°C. Forty minutes later the supernatant containing mostly endothelial cells was discarded. Fresh dispersion solution was then added, and after another 40 minutes the suspension, which contained a substantial number (80%) of relaxed smooth muscle cells, was collected. Drops of physiological salt solution (PSS) plus 0.2% bovine serum albumin were added to the suspension over a 15-minute period to bring the extracellular Ca2+ concentration close to 1.5 mmol/L. The PSS contained (mmol/L) NaCl 138, CaCl2 2, KCl 5, MgCl2 1, HEPES 10, and glucose 10 (pH 7.4). The cells were collected after 5 minutes of centrifugation at 150g and then resuspended in PSS plus 0.2% bovine serum albumin. Patch-clamp experiments were done immediately after cell collection.

**Whole-Cell Voltage Clamp With Simultaneous Fura 2 Ratio Monitoring**

The dialyzing pipette technique as described in detail previously21,22 was used to measure simultaneously the whole-cell currents and Ca2+. Unless otherwise indicated, soda lime glass pipettes (3 to 5 MΩ) were filled with a solution containing (mmol/L) KCl 120, NaCl 10, MgCl2 1, HEPES 20, Tris-GTP 0.5, MgATP 2, EGTA 0.2, and fura 2 pentapotassium salt 0.1 (pH 7.1). Liquid junction potential was corrected with the amplifier (List EPC-7) before the pipette touched the cell. After formation of a tight seal (1 to 20 GΩ), a background fura 2 fluorescence signal was recorded from the area covering the cell, and pipette capacitance was canceled. After the whole-cell configuration was achieved, a 3- to 6-minute period was allowed for fura 2 diffusion into the cell before any protocol was started. The current was filtered by an eight-pole low-pass Bessel filter at a cutoff frequency of 400 Hz and digitized at 600-microsecond intervals. The cell capacitance was not compensated. Data acquisition and analysis were done with aXOBASIC 1.0 (Axon Instruments). The current magnitude was corrected with membrane capacitance for each cell to account for the potential difference in cell size. The current density (picoamperes per picofarad) was then used to calculate group current reversed at -60±2 mV at 5 mmol/L extracellular K + (n=5 to 6), as measured by its tail current. This current could also be effectively inhibited (60% to 75%) by 10 mmol/L tetraethylammonium (TEA). These features are consistent with the observation of K+ currents. Similar to what was reported for vascular smooth muscle K+ current,16 this current reversed at -60±2 mV at 5 mmol/L extracellular K+ (n=5 to 6), as measured by its tail current.

**Data Analysis**

Results are presented as mean±SEM with n being the number of cells. Statistical comparisons were made using the unpaired Student's t test or ANOVA with a post hoc Bonferroni's test for both number of cells and number of rats. Significance was defined at a value of P<.05.

**Whole-Cell K+ Current**

Fig 1A shows typical recordings of outward currents elicited by a test potential of +30 mV from a holding potential of -80 mV. Replacing K+ with Cs+ in the pipette solution almost completely eliminated (>97%) the outward current. This current could also be effectively inhibited (60% to 75%) by 10 mmol/L tetraethylammonium (TEA). These features are consistent with the observation of K+ current. Similar to what was reported for vascular smooth muscle K+ current,
Comparison of the K⁺ current-voltage relations between WKY and SHRSP cells was done by progressively increasing the test potential in 10-mV steps to +80 mV at a constant holding potential of −80 mV. Simultaneous examination of the fura 2 ratio showed that such step depolarizations did not cause an increase in Ca²⁺. The current displayed outward rectification (Fig 1B), and the reversal potential was similar in WKY and SHRSP cells. As shown in Fig 1B, the current density was greater at positive potentials in SHRSP cells (P<.05). This difference was not observed in the presence of a Ca²⁺ entry blocker, LaCl₃ (200 μmol/L, Fig 2A), or a K⁺ channel blocker, TEA (10 μmol/L, Fig 2B). Although the K⁺ current was greater in SHRSP cells, Ca²⁺ influx between WKY and SHRSP cells. Numbers in parentheses are number of cells followed by number of rats.

**Ca²⁺ and Ba²⁺ Influx**

We also examined the fura 2 fluorescence ratio in cells without simultaneous voltage clamp to determine whether the resting Ca²⁺ was increased in SHRSP cells. Consistent with the findings from simultaneous measurement of the current and fura 2 ratio, the resting Ca²⁺ was not different between WKY and SHRSP cells (ratio and Ca²⁺, respectively, 0.72±0.02 and 100±10 nmol/L versus 0.76±0.02 and 117±9 nmol/L; Fig 3A). It is possible that in SHRSP cells, Ca²⁺ influx might be enhanced but it is matched closely with an increased efflux, resulting in an increase only in Ca²⁺ but not necessarily Ca²⁺. To investigate this possibility, we measured the rate of Ba²⁺ influx. Ba²⁺ passes through most Ca²⁺-permeable channels with a greater permeability via voltage-gated Ca²⁺ channels than Ca²⁺, and it is also transported by the Na⁺-Ca²⁺ exchanger. However, it is not transported by Ca²⁺ pumps in the sarcosome and sarcoplasmic reticulum. Thus, we replaced Ca²⁺ in PSS with equimolar Ba²⁺ (2 mmol/L) and lowered the Na⁺ concentration to 19 mmol/L. As the Na⁺-Ca²⁺ exchanger in vascular smooth muscle cells was effectively inhibited by lowering the extracellular Na⁺ to 19 mmol/L, the rate (or slope) of the fura 2 ratio increase under such conditions should be directly related to the rate of Ba²⁺ entry into the cells. As shown in Fig 3B and 3C, reduction of the extracellular Na⁺ concentration to 19 mmol/L with choline substitution imposed only a minimal effect on the

**Fig 3. Plots and tracing show myoplasmic Ca²⁺ concentration (Ca²⁺) and Ba²⁺ influx in cells from Wistar-Kyoto (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP).** A, Basal fura 2 ratio and Ca²⁺ in cells superfused with physiological salt solution (PSS); n=39 cells and 6 WKY rats and 68 cells and 8 SHRSP. Inset, in vitro Ca²⁺ calibration curve used for converting ratio values to Ca²⁺ concentrations (each point is mean±SEM of five measurements). In vitro calibration was conducted by addition of fura 2 pentapotassium salt (0.1 mmol/L) to a mock intracellular solution containing (mmol/L) KCl 120, NaCl 10, HEPES 20, and MgCl₂ 1 and four different Ca²⁺ concentrations. Desired Ca²⁺ concentration was obtained by addition of an appropriate ratio of KCl-EGTA and CaK₂EGTA to achieve a final EGTA concentration of 10 mmol/L. Fura 2 ratio was measured from 2-μL droplets of the solutions placed on coverslips. Curve is fitted by a second polynomial equation, [Ca²⁺] = a rᵇ + b rᶜ + c rᵈ + d, where r is the fura 2 ratio. B, Typical traces of fura 2 ratio measurement in rat aortic cells. At arrows, one cell was exposed to 2Ca₁₉Na solution and the other to 2Ba₁₉Na (replacement of Ca²⁺ in PSS with 2 mmol/L Ba²⁺ and reduction of Na⁺ to 19 mmol/L with choline substitution). Fura 2 ratio increased with about a 1.5-minute delay in most cells. Thus, slope was determined from the change during 3 to 6 minutes. Occasionally, the ratio began to increase early, in which case slope was determined starting from the time point of the increase. C, Ba²⁺ influx determined by the rate of fura 2 ratio increase in WKY and SHRSP cells. *P<.05 compared with 2Ca₁₉Na; †P<.05 for comparison between WKY and SHRSP cells. Numbers in parentheses are number of cells followed by number of rats.
fura 2 ratio. Ba2+ replacement of Ca2+ led to a continuous and approximately linear increase in the fura 2 ratio, which occurred with about a 1.5-minute delay. The rate of increase (Ba2+ influx) was approximately 2.5-fold greater in SHRSP cells compared with WKY cells (0.025 ± 0.005 versus 0.063 ± 0.012, WKY versus SHRSP, \( P < .05 \), Fig 3C).

**Discussion**

Although increased membrane permeability to K+ has been well documented in vascular tissue in hypertension, the conductance mechanisms for the alteration are not fully understood because of limited electrophysiological studies. The present study demonstrated an increase in whole-cell K+ current in freshly isolated SHRSP aortic cells compared with WKY cells. This increase was detected only at positive membrane potentials and was not observed in the presence of LaCl3 or TEA+ or in cells in which the internal Ca2+ level was clamped at zero. Thus, our results are consistent with the findings by Rusch et al16 who observed an enhanced whole-cell K+ current in aortic smooth muscle cells from SHR after stimulation with the Ca2+ ionophore A23187. However, we observed an increase in the absence of any pharmacologic intervention. This discrepancy might be related to differences in animal type (SHRSP versus SHR), cell isolation method, and experimental conditions (eg, inclusion of GTP and ATP in our pipette solution but not in their study). Because the increased K+ current in SHRSP cells could be reduced by blocking Ca2+ influx with La3+, this increase is more likely to be caused by changes in Ca2+ activation of Kc channels rather than changes in the voltage dependence of Kc channels. However, our results do not exclude possible changes in the intrinsic gating properties of K+ channels. Recently, England et al29 showed in single-channel studies that the opening probability and Ca2+ sensitivity of a 225-pS Ca2+- and voltage-dependent K+ channel were increased in SHR cells even at 10-7 mol/L Ca2+. In cultured aortic smooth muscle cells from SHR, Shoemaker and Worrell31 also noted an increased Ca2+ sensitivity of a 55-pS Kc current. These studies suggest that alterations in the Kc channel property accounts not only when the major Ca2+ efflux pathways were inhibited, yet this increase is matched closely by an increased Ca2+ influx. This possibility can be tested by blocking Ca2+ efflux pathways, while leaving influx pathways unaffected. For this purpose, Ba2+ is a good tool, because it passes through most Ca2+-permeable channels, binds with fura 2 in a manner similar to that of Ca2+, and is neither extruded by the sarcolemmal Ca2+ pumps nor sequestered into the sarcoplasmic reticulum.21-23,27 Under a low extracellular Na+ condition that itself did not impose a significant effect on Ca2+, Ba2+ influx (Fig 3C) was greater in SHRSP cells than WKY cells. This result is consistent with the concept that the membrane permeability to Ca2+ (divalent cations) is increased in hypertension. Furthermore, a significant increase in the fura 2 ratio in SHRSP cells was apparent only when the major Ca2+ efflux pathways were inhibited. Thus, our results suggest that increased Ca2+ cycling may occur across the sarcolemma in hypertension. A "Ca2+-cycling hypothesis" has been proposed to maintain a high level of cell membrane-related activities without a substantial decrease in Ca2+ and thus preventing the cytotoxic effects of Ca2+. Ca2+ cycling may also provide an explanation for the observation of Erne and Hermensmyer9,11 that the resting Ca2+ level was increased only at the subsarcolemmal region, whereas Ca2+ remains unchanged in hypertension.

It is not certain why the Ba2+ influx-induced fura 2 ratio increase occurred with a delay. As reported by Schilling et al,21 the affinity of fura 2 for Ba2+ is threefold to fourfold lower than that for Ca2+ (Kd, 780 versus 212 nmol/L). Because of this relatively low affinity of fura 2 for Ba2+, changes in the fura 2 ratio may not be detected until enough Ba2+ has accumulated within the cells and bound to fura 2.

Ba2+ is also known to inhibit K+ channels that are important in controlling membrane potential.20,22 Thus, the increased Ba2+ influx in SHRSP cells may result from a greater blockade of K+ channels and thus a larger depolarization in these cells. This probably is not the case, based on a study by Furspan and Webb,29 who found no difference in Ba2+ inhibition of K+ currents in isolated tail artery cells from WKY rats and SHRSP. The importance of K+ channels in controlling smooth muscle functions may differ during normotensive and hypertensive states. Inhibition of K+ channels by TEA caused contraction of SHR aorta but not WKY aorta.16 In tail artery, Ba2+ (1 mmol/L) had no effect on resting tension, yet it potentiated 30 mmol/L KCl-induced contraction more in SHRSP than WKY.20 Based on these observations, we cannot fully exclude the possibility that inhibition of K+ channels is involved in potentiation of Ba2+ influx in SHRSP cells. With the use of an alternate approach to 45Ca influx and voltage clamp, our present study has provided evidence that the activities of Ca2+ channels (Ca2+ influx-permeable channels) are increased in hypertension and that this membrane defect is maintained in freshly isolated arterial smooth muscle cells.

In conclusion, we demonstrated in this study an increased activity of Ca2+-dependent K+ channels and an increased Ba2+ (divalent cation) influx in aortic muscle cells from SHRSP. These changes were noted despite no detectable elevation in resting Ca2+ in SHRSP cells. Our results suggest increased Ca2+ (or divalent cation) cycling across the sarcolemma and a localized increase in Ca2+.

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