Remission of High Blood Pressure Reverses Arterial Potassium Channel Alterations

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Abstract Rat arterial muscle cells show an elevated Ca\textsuperscript{2+}-dependent K\textsuperscript{+} efflux during the established phase of hypertension. This association of enhanced K\textsuperscript{+} efflux with high arterial pressure implies that changes of in vivo blood pressure can alter the level of K\textsuperscript{+} channel current in arterial membranes. We directly tested this hypothesis by comparing K\textsuperscript{+} current density between patch-clamped aortic muscle membranes of normotensive Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and SHR treated with the angiotensin-converting enzyme inhibitor ramipril. In separate experiments, the acute effect of 10\textsuperscript{-6} mol/L ramiprilat (Upjohn Co), 2 mmol/L tetraethylammonium, and was predominantly blocked by 2 mmol/L tetraethylammonium. K\textsuperscript{+} current density in SHR aortic membranes was unchanged after 1 week of ramipril therapy, but it was reduced 42% (to 18±1 pA/pF) after 2 weeks of treatment. Parallel tension-recording studies showed that untreated SHR aortic segments but not aortic segments from WKY rats or ramipril-treated SHR constricted strongly after block of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels by tetraethylammonium. Our findings imply that Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current density in arterial muscle membranes shows a positive correlation with chronic arterial blood pressure levels. The enhanced expression of this K\textsuperscript{+} current in arterial membranes of hypertensive animals may be a homeostatic mechanism to regulate vascular excitability and hence prevent further arterial contraction during this disease. (Hypertension. 1994;23[part 2]:941-945.)

Key Words • hypertension, renovascular • ion channels • potassium channels • aorta • muscle, smooth, vascular

Calcium-dependent K\textsuperscript{+} efflux is increased in arteries from genetic and renal rat models of established hypertension.\textsuperscript{1,6} It has been proposed that this enhanced K\textsuperscript{+} current is a consequence of high blood pressure and is a compensatory mechanism activated during the development of hypertension to counteract arterial excitability and prevent further vasoconstriction.\textsuperscript{1,8} However, increased arterial K\textsuperscript{+} current has been described only in the established phase of hypertension,\textsuperscript{2} and there is little direct evidence to link changes in blood pressure amplitude with dynamic modulation of K\textsuperscript{+} current levels in arterial muscle membranes.

To explore the relation between blood pressure changes and arterial K\textsuperscript{+} current density, we compared K\textsuperscript{+} current density between patch-clamped aortic muscle cell membranes of adult spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto (WKY) rats, and SHR after antihypertensive treatment with the angiotensin-converting enzyme inhibitor ramipril. In addition, parallel measurements of tension were recorded in isolated vessel rings to determine whether K\textsuperscript{+} current suppressed aortic contraction in these preparations. Thus, we investigated for the first time whether a potential link exists between blood pressure regression and modulation of K\textsuperscript{+} current in arterial muscle membranes of hypertensive rats.

Methods

Ramipril Treatment

Adult (11 to 12 weeks old) WKY rats and SHR were obtained from Taconic Farms. Rats were housed in groups of three, with free access to food and water. Ramipril (Upjohn Co) addition to the drinking water (11 mg ramipril/500 mL) provided an average dose of 3.7 and 3.5 mg/kg per day for WKY rats and SHR, respectively. Untreated animals received standard drinking water. Four rat groups were studied for tension responses: untreated WKY rats (n=15), ramipril-treated WKY rats (n=10), untreated SHR (n=15), and ramipril-treated SHR (n=14). For patch-clamp analysis, additional untreated WKY rats (n=8), untreated SHR (n=9), and ramipril-treated SHR (n=11) were used. Systolic blood pressures were measured by the tail-cuff method for 5 days before ramipril treatment. Pressures were then measured the first 3 days after treatment was initiated and approximately every 2 days thereafter for a total of 1 or 2 weeks.

Tension Recording

Rats were anesthetized with pentobarbital (45 mg/kg IP), and aortas were removed, cleaned of connective tissue, and cut into 3-mm segments. Segments sutured with physiological saline solution were mounted in a tension-recording transducer system described previously for this preparation and were adjusted to length-tension optimum using 60 mmol/L KCl. Cumulative concentration-response curves to tetraethylammonium (TEA, Sigma Chemical Co) were performed in four parallel segments to evaluate the effect of this K\textsuperscript{+} channel blocker on basal tension. In separate experiments, the acute effect of 10\textsuperscript{-6} mol/L ramiprilat (Upjohn Co),\textsuperscript{9} the active metabolite of ramipril, was evaluated in aortas from four untreated SHR.

Patch-Clamp Measurements

Other aortas were cut into 1-mm segments and enzymatically dissociated into single aortic muscle cells for patch-clamp measurement of whole-cell K\textsuperscript{+} current. The cell dissociation
procedure and patch-clamp acquisition and analysis station were identical to those used earlier for this preparation. Cells were dialyzed with pipette solution containing a high ionized calcium concentration ([Ca\textsuperscript{2+}]) to amplify Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current. This pipette solution was composed of (mmol/L): K\textsuperscript{+} glutamate 130, MgCl\textsubscript{2} 1, EGTA 0.1, HEPES 10, and [Ca\textsubscript{2+}] 10\textsuperscript{-4} mol/L (pH 7.2). The level of [Ca\textsubscript{2+}] in the pipette solution was verified by the intracellular fluorescent indicator fura 2. The final [Ca\textsubscript{2+}] inside dialyzed, patch-clamped aortic muscle cells was not determined. Bath solution consisted of (mmol/L): NaCl 135, KCl 4.7, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, HEPES 5, and glucose 10 (pH 7.4).

Families of whole-cell K\textsuperscript{+} currents were generated by progressive depolarizing pulses (10 mV, 400 milliseconds) from a holding potential of −60 to +60 mV. Immediately after membrane rupture, K\textsuperscript{+} current amplitude generally increased, perhaps secondary to cell dialysis with high Ca\textsuperscript{2+}-containing pipette solution. Thus, K\textsuperscript{+} current amplitudes were recorded 2 to 5 minutes after conversion to the whole-cell recording mode to facilitate adequate cell dialysis and the measurement of stable K\textsuperscript{+} current amplitudes. Membrane area was estimated by integrating capacitive currents generated by 10-mV hyperpolarizing pulses after electronic cancellation of the pipette-patch capacitance. These signals were passed through an eight-pole Bessel filter with a cutoff frequency of 5 kHz and were digitized at 1 kHz. Peak K\textsuperscript{+} current amplitude was calculated individually for each cell and expressed in picoamperes per picofarad to normalize for differences in cell size.

Data Analysis

Data are expressed as mean±SEM for all aortic preparations. Patch-clamp results were obtained from 131 cells. For each aorta, current densities from an average of five cells were combined to obtain the mean value. Significance of differences between control and matched drug responses or differences between rat strains were determined by ANOVA with repeated measures. Controls were defined as untreated rats that were killed at days 0, 7, or 14 of the treatment regimen.

Results

Effect of Ramipril on Blood Pressure

Fig 1 shows that ramipril treatment of adult SHR for 2 weeks reduced systolic blood pressure from 184±3 to 140±7 mm Hg. Also, at the end of 2 weeks systolic blood pressure of ramipril-treated SHR was not different from that of untreated WKY rats. Ramipril reduced systolic pressure in WKY rats by 15 mm Hg compared with untreated WKY rats.

Effect of Tetraethylammonium on Basal Tension

Tension recording was used to assay aortic reactivity to TEA. This drug preferentially blocks maxi-K\textsuperscript{+} channels over other K\textsuperscript{+} channel types, with 2 mmol/L TEA reducing single-channel maxi-K\textsuperscript{+} current by 75% to 90% in this preparation. Experiments were based on the premise that the presence of TEA-induced arterial contraction indicated a high level of maxi-K\textsuperscript{+} channel current, whereas the absence of TEA responsiveness indicated reduced maxi-K\textsuperscript{+} channel opening. With this in mind, recordings in Fig 2A show that TEA (0.1 to 5 mmol/L) induced only small constrictions in untreated and ramipril-treated WKY aortas but dose-dependently contracted aortas of untreated SHR. However, after 1 and 2 weeks of ramipril treatment in SHR, these contractions to cumulative increases in TEA were suppressed and completely abolished, respectively. Fig 2B and 2C show the mean data for all rat groups. Acute application or a 30-minute preincubation with 10\textsuperscript{-6} mol/L ramiprilat, the active metabolite of ramipril, did not affect the amplitude of TEA-induced contractions in aortas from untreated SHR (n=4, data not shown). This finding implied that the absence of TEA-induced contractions in aortas of ramipril-treated SHR probably was not due to a direct dilator effect of ramiprilat on the vascular muscle.

Aortic Membrane K\textsuperscript{+} Current Density

We measured whole-cell K\textsuperscript{+} current to verify that its density was different between WKY and SHR aortic muscle membranes and that this difference could be abolished by blood pressure reduction with ramipril. Families of whole-cell K\textsuperscript{+} currents were generated by progressive 10-mV depolarizing steps from −60 to +60 mV, as shown by the voltage ramp in Fig 3. The current-voltage (I-V) curve in Fig 3A shows that the plot of K\textsuperscript{+} current density as a function of membrane potential was shifted to the left in aortic membranes of untreated SHR compared with untreated WKY rats, with the maximum K\textsuperscript{+} current density showing a 2.6-fold increase at +60 mV. The current traces (bottom panels of Fig 3B and 3C) and I-V curves (top panels of Fig 3B and 3C) show that most of the K\textsuperscript{+} current amplitude difference (92% at +60 mV) between aortic membranes of untreated WKY rats and SHR was abolished by the maxi-K\textsuperscript{+} channel blocker TEA (2 mmol/L). The remaining TEA-insensitive current was attributed to other K\textsuperscript{+} channel types, or alternatively, to incomplete block of maxi-K\textsuperscript{+} channel current by 2 mmol/L TEA, leaving a residual current component.

In further studies, aortic K\textsuperscript{+} current density was compared between untreated and ramipril-treated SHR. Fig 4 shows scatterplots of maximum K\textsuperscript{+} current density (at +60 mV) as a function of individual cell capacitance. Each symbol represents patch-clamp results from one cell. Graphs compare single-cell measurements between untreated SHR versus untreated WKY rats (Fig 4A), SHR treated with ramipril for 1 week (Fig 4B), and SHR treated with ramipril for 2 weeks (Fig 4C). Corresponding I-V curves, which represent mean values for each cell isolation, are shown below each scatterplot. Fig 4A shows that maximum current density in aortic membranes of untreated SHR was higher than in untreated WKY rats (31±3 and 12±1 pA/pF, respectively), and this was reflected as a leftward shift of the I-V curve in SHR. Compared with untreated SHR, aortic membranes of SHR treated with ramipril for 1 week showed an unchanged K\textsuperscript{+} current density of 31±2 pA/pF, as indicated in Fig 4B. There was a tendency for K\textsuperscript{+} current density to be elevated at
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Untreated  1-wk RMPL  2-wk RMPL

5.0 -0.5 1.0 2.0 5.0 -0.5 1.0 2.0 5.0

^ 1.0 2.0 5.0 ^ 1.0 2.0 5.0

10 min

Fig 2. Traces and plots show effect of ramipril (RMPL) treatment for 1 or 2 weeks on concentration-response curves to tetraethylammonium (TEA, 0.1 to 5 mmol/L) in rat aorta. A, Aortas from untreated or ramipril-treated Wistar-Kyoto (WKY) rats contracted only slightly to TEA, whereas aortas from untreated spontaneously hypertensive rats (SHR) showed large contractions. After ramipril treatment for 1 or 2 weeks, SHR contractions were repressed (lower right traces). B and C, Collective data show effect of ramipril treatment on TEA concentration-response curves in WKY rats and SHR. Points represent mean±SEM for an average of nine rats.

Discussion

Arterial muscle membranes from genetic, renal, and salt-sensitive hypertensive rats show an elevated K⁺ efflux. It has been proposed that this enhanced K⁺ current regulates vascular excitability during hypertension by limiting voltage-dependent Ca²⁺ influx and promoting vasodilatation. Only recently, however, has the TEA-sensitive, Ca²⁺-activated "maxi-K⁺ channel" been identified as the likely molecular pathway for this elevated current. At the same membrane potential and [Ca],, this K⁺ channel type appears to show a higher open state probability in cell-attached and inside-out aortic membrane patches from adult SHR compared with WKY rats. However, despite clarification of its single-channel nature, the link between high blood pressure and the etiology of elevated arterial K⁺ current remains unclear. With regard to genetic hypertension, only K⁺ flux in vascular membranes from adult SHR has been measured, and therefore it is unknown whether K⁺ channel changes are a consequence of elevated blood pressure or precede blood pressure elevation as an expression of genetic diversity between rat strains.

To address this question, the purpose of the present study was twofold: (1) to verify an enhanced whole-cell K⁺ current in SHR compared with WKY aortic muscle cells and consequently (2) to correlate blood pressure remission in WKY with changes in K⁺ current density in aortas of the same animals. Initial patch-clamp comparison between membranes from untreated WKY rats and SHR showed an elevated, TEA-sensitive K⁺ current density in SHR, and only aortic segments from untreated hypertensive animals constricted strongly to less than or equal to 2 mmol/L TEA, a blocking concentration more selective for the maxi-K⁺ channel than other K⁺ channel types. These findings support the concept that a TEA-sensitive, maxi-K⁺ channel current counteracts arterial excitability in the established phase of genetic hypertension. However, there were two differences between these studies and previous experiments from our laboratory. First, a faster sampling rate for acquisition of capacitive transients in the present study permitted better time resolution of membrane capacitance. Second, we previously used a Ca²⁺ ionophore to increase [Ca], and amplify Ca²⁺-dependent K⁺ current in patch-clamped aortic cells, whereas in the present study we dialyzed cells directly with high (10⁶ mol/L) Ca²⁺-containing pipette solution to permit better control of [Ca],. Despite these different conditions, both studies indicated a twofold to threefold increase of K⁺ current density in SHR aortic membranes compared with similar WKY rat preparations.
The novel finding of the present study was that antihypertensive treatment reversed TEA-induced excitability and lowered K⁺ current density in SHR arterial muscle. Aortas from ramipril-treated SHR showed depressed or absent TEA-induced constrictions as early as 1 week after the beginning of treatment, in contrast to the large TEA-induced constrictions in aortas of untreated SHR. This insensitivity to TEA after antihypertensive treatment might reflect a downregulation of K⁺ current caused by blood pressure reduction, resulting in fewer maxi-K⁺ channel openings susceptible to TEA block. We did not detect a change in K⁺ current density of SHR aortic membranes after 1 week of ramipril therapy, which was perplexing in view of the reduced contractile response to TEA in aortas of similar SHR (Fig 2A and 2C). However, K⁺ current density clearly was attenuated after 2 weeks of antihypertensive treatment. One interpretation of these findings is that arterial K⁺ current downregulates in response to blood pressure reduction, with a time course for altered expression of 1 to 2 weeks as detected by our methods. Alternatively, because ramipril lowers angiotensin II (Ang II) levels concurrently with blood pressure remission in SHR, one could argue that low Ang II rather than remission of high blood pressure reduced K⁺ current density under our conditions. However, a predominant, direct action of Ang II on K⁺ channel expression seems unlikely, because vascular muscle cells from distal aortic segments of aortic-coarcted rats, which are exposed to high Ang II but normal arterial pressure, show normal K⁺ current density. The latter findings suggest that K⁺ current density correlates more closely with blood pressure amplitude than with circulating Ang II levels, although circulating and intravascular concentrations and actions of Ang II may not be comparable. Conceivably, future monitoring of arterial K⁺ current density in ramipril-treated WKY rats, whose blood pressure is minimally affected by ramipril administration, will help to delineate whether Ang II can modulate K⁺ current expression independent of its antihypertensive effect.

It also was our intention to examine the long-term effects of ramipril on arterial K⁺ current expression in SHR, and we treated another group of SHR with ramipril for 4 to 5 weeks to provide chronic blood pressure normalization. However, the resulting aortic muscle cells from long-term ramipril-treated SHR appeared small and did not permit stable, tight-seal formation for precise measurement of K⁺ current. This observation may reflect the trend for ramipril-treated cells to show reduced membrane capacitance, indicative of smaller cell size.

In summary, our findings imply that in vivo blood pressure may regulate arterial ion channel currents. At the single-cell level, changes in the expression of K⁺ channel current may provide a powerful cellular mechanism for regulating arterial contraction and preventing further blood pressure increases. We do not understand the pathway by which blood pressure alterations are transformed into K⁺ current changes, although single-channel studies suggest that maxi-K⁺ channel density or Ca²⁺ sensitivity may change proportionally to arterial pressure levels. In this regard, a better understanding of the structure-function relation of the maxi-K⁺ chan-
nel15 and the use of 125I-charybdotoxin to estimate maxi-K+ channel density16-17 may help to pinpoint the molecular basis of altered K+ current in arterial muscle membranes in hypertension.

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