Calcium Currents Are Altered in the Vascular Muscle Cell Membrane of Spontaneously Hypertensive Rats

Nancy J. Rusch and Kent Hermsmeyer

Calcium currents were recorded during whole-cell voltage clamp in cultured azygos venous muscle cells from 1-3-day-old normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Different holding potentials were used to separate total cell current into its transient (T) and sustained or long-lasting (L) components. In recordings from 30 WKY and 30 SHR vascular cells, total cell calcium current was the same between cells from normotensive (167±20 pA) and hypertensive (139±15 pA) rats. However, the relative proportion of T and L calcium currents was different between WKY and SHR cells. In WKY cells, the peak amplitude of the L current was less than that of the T current (42±30% of total current), whereas in SHR cells, the L current was greater (62±3% of total current). Calcium currents in vascular muscle cells from SHR were activated and inactivated at more positive potentials than in cells from WKY. This study directly compares transmembrane calcium current in isolated cells from WKY and SHR blood vessels and shows that the proportions of T and L calcium channels activated by depolarization are altered in this genetic model of hypertension. (Circulation Research 1988;63:997-1002)

Despite the recent emphasis on the role of calcium in the pathophysiology of hypertension, only a limited number of studies have explored the regulation of calcium in vascular muscle as a possible etiological mechanism (for review, see Kwan1). Cellular mechanisms of calcium metabolism reported to be associated with hypertension in the spontaneously hypertensive rat (SHR) include changes in intracellular release and reuptake of calcium and alterations in sarcolemmal transport of and permeability to the ion.2-6

The recent introduction of the tight-seal patch-clamp technique7 for the measurement of transmembrane calcium currents in vascular muscle cells8-13 enables the implementation of a powerful tool to directly compare the properties of voltage-dependent calcium channels in cells from normotensive and hypertensive animals. In the experiments described here, we used the whole-cell voltage-clamp technique7 to measure calcium currents and compared the voltage-dependent calcium channels in cultured vascular muscle cells from normotensive Wistar-Kyoto rats (WKY) and genetically matched spontaneously hypertensive rats (SHR). Recent studies have characterized two types of calcium current in vascular muscle8-1012 and a related cell line,14 called transient (T) and sustained or long-lasting (L), to describe their inactivation kinetics during maintained depolarization. Our results suggest that the proportion of T and L calcium channels is altered in the vascular muscle membrane from the SHR, which would change the voltage-dependent calcium currents in blood vessels of spontaneously hypertensive rats.

Materials and Methods

Cell Culture

Primary cultures of neonatal venous muscle cells were used. Azygos veins of 15–20 decapitated 1–4-day-old rats from the Wistar-Kyoto (WKY) or spontaneously hypertensive (SHR) strains were excised and placed in CV3M, which consists of 4 mM L-glutamine, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.13 After a rinse in CV3M, the tissue was soaked for 10 minutes in KG solution, consisting of (mM) 140 K-glutamate, 20 μg/ml gentamicin, 20 mM HEPES buffer at pH 7.3, and 16 mM NaHCO3 dissolved in 85% modified Eagle’s medium-Earles salts and 15% horse serum.
were incubated at 37° C with collagenase (30 mg in 10 ml KG solution) for 30 minutes. The collagenase supernatant was discarded, and the tissue was exposed to three or four 15-minute incubations in 15 ml of 1 mg/ml trypsin in KG solution. After each incubation, the supernatant fractions were then centrifuged for 15 minutes at 200g, washed by suspension in CV3M, and centrifuged at 200g for 5 minutes. The cell pellets were resuspended in 10 ml of CV3M plus 5 μM 5-bromo-2'-deoxyuridine (BrdU) and sedimented in a 15 ml tissue culture flask. The muscle cells were resuspended in CV3M + 5 μM BrdU, diluted to a density of 70,000 cells/ml, and plated on poly-L-lysine-coated glass coverslips. Cells were grown in a 5% CO2 incubator at 95% humidity and 37° C and were used after 3–7 days in culture.

Measurement of Whole-Cell Calcium Currents

A coverslip of cells was placed in a perfusion chamber which contained an external solution consisting of (mM) CaCl2 20, tetraethylammonium (TEA) chloride 135, MgCl2 1, glucose 10, HEPES 10 (pH adjusted to 7.4 with TEA OH, 22° C). The pipette solution consisted of (mM) cesium glutamate 150, EGTA 0.1, MgCl2 0.1, HEPES 10 (pH adjusted to 7.4 with CsOH). Patch pipettes (2–8 MΩ) filled with cesium glutamate solution to eliminate K+ current were used to voltage clamp single vascular muscle cells. Whole-cell currents were stimulated by 300 msec depolarizations at intervals of 10–15 seconds. As previously detailed, different holding potentials were used to separate the T and L calcium channel currents. T current was calculated by digital subtraction of current obtained during a test pulse from −30 mV to 0 mV from that obtained during a test pulse from −80 mV to 0 mV. Depolarization from −80 mV to 0 mV elicits peak T current and activates a fraction of the total L current. The L component is deduced by subtracting the calcium current resulting from a −30 mV to 0 mV test pulse. Peak L current was elicited by depolarizing from −30 mV to +30 mV. The peak magnitudes of these two current types were then added together to obtain the total current. The voltage-clamp currents were amplified by a List EPC-7 (List-Electronic, Darmstadt-Eberstadt, West Germany) with 0.5 Gohm feedback resistor and filtered at 500 Hz. All data were digitized (sampling rate, 5,000/sec) and stored on floppy disks to permit analysis at a later time. Leak and capacitative currents were subtracted for each record by summation of currents during depolarizing pulses with linearly scaled current obtained during 10 mV hyperpolarizing pulses. Figures were traced from corrected currents printed from digitized data.

Statistics

Comparisons between WKY and SHR cells were made by the unpaired t test, while comparisons of currents within the WKY or SHR sample population were analyzed by the paired t test. A value of p < 0.05 was accepted as significant. Unless stated otherwise, all analyses were performed on 30 WKY and 30 SHR cells obtained from nine (WKY) and 13 (SHR) different primary cultures.

Results

Figure 1 shows T and L calcium currents recorded from a WKY and SHR cell. T currents were recorded during depolarizing pulses from −80 mV to −30 mV, while L currents were elicited by depolarizing from −30 mV to +30 mV. In the WKY cell (upper tracings), the peak magnitude of the T calcium current was larger than the L current. However, in the SHR cell (lower tracings), the L current was the predominant current type. The exact breakdown of total cell current in WKY and SHR cells into its T and L components is found in Table 1. Cells from WKY and SHR had the same amount of total whole-cell current, with 30 WKY and 30 SHR cells averaging 167±20 pA and 139±15 pA, respectively. However, the relative contribution of the T and L currents to total cell calcium current was different between WKY and SHR venous cells. The T current accounted for 67% of the total calcium current in WKY cells, and its magnitude (100±14 pA) was significantly greater than the L current (67±8 pA) in the same cells. The opposite was seen in SHR venous cells, where the L current represented 62% of the total calcium current and its magnitude (87±12
Table 1: Separation of Total Calcium Current into L and T Components

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<th>Total current</th>
<th>L Current</th>
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<td></td>
<td>pA</td>
<td>% of Total</td>
<td>pA</td>
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<tr>
<td>WKY</td>
<td>167±20</td>
<td>100</td>
<td>67±8</td>
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<td>(n=30)</td>
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<td>100±14</td>
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<tr>
<td>SHR</td>
<td>139±15</td>
<td>100</td>
<td>87±12</td>
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Separation of total whole-cell calcium current in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rat vascular muscle cells into its transient (T) and long-lasting (L) components. Although the total calcium current was the same in WKY and SHR cells (167±20 pA and 139±15 pA, respectively), the WKY cells had significantly more T than L current, while the SHR had predominantly L current. The sample size was n=30 for WKY and SHR.

As calcium currents were measured from more cells during this study, it became apparent that the proportion of T and L currents varied considerably from cell to cell within the WKY and SHR sample populations. This finding is illustrated in Figure 2, which shows a frequency-distribution of the ratio of L current/T current (L/T ratio) measured in each of the 30 WKY and 30 SHR cells studied. Of 30 WKY cells, only 10 (33%) had more L calcium current (L/T ratio > 1.0), while 20 (67%) had more T current (L/T ratio < 1.0). The mean value for the L/T ratio was 0.9 and the mode was 0.5 for the WKY sample population. In contrast, 24 of the 30 SHR cells (80%) had more L calcium current; the remaining six (20%) showed more T current. The mean value of the L/T ratio was 2.9 and the mode was 1.5 for the 30 SHR cells. Thus, the rightward shift of the SHR frequency-distribution graph is accounted for by a greater proportion of L calcium current in cells from the SHR.

After the previous data were collected to characterize the calcium channel types present in WKY and SHR cells, further experiments were performed to determine if voltage-dependent activation and inactivation of calcium channel currents would differ between representative WKY and SHR cells. To do this, current-voltage curves for activation and steady-state inactivation were performed on WKY and SHR cells, whose L/T ratios were near the mean or mode values for their respective sample populations. With this criterion, Figure 3 shows activation of calcium currents and the resulting current-voltage curves calculated from WKY and SHR cells with L/T ratios (0.8 and 3.0, respectively) representative of the 30 WKY and 30 SHR cells studied. Cells were depolarized from a holding potential of -80 mV to increasingly positive command potentials, which are indi-
 WKY  

SHR  

Figure 3. Whole-cell calcium currents recorded from a Wistar-Kyoto (WKY) (left tracing) and a spontaneously hypertensive rat (SHR) (right tracing) vascular muscle cell. Cells were depolarized from a holding potential (HP) of $-80 \text{ mV}$ to the command potentials indicated to the right of the resulting current traces. Calcium current in the WKY cell was activated at more negative command potentials than current in the SHR cell. The current-voltage relations resulting from these calcium currents are shown below. Compared with the WKY current-voltage curve, the current-voltage curve in the SHR is shifted to the right. Peak current occurred at $-20 \text{ mV}$ in the WKY and $+20 \text{ mV}$ in the SHR cell.

The clearly multiple nature of calcium currents has been demonstrated in both arterial and venous muscle cells$^{8-12}$ and in many other cell types.$^{13}$ In vascular muscle, most$^{8-10,12,18}$ but not all$^{11,19}$ reports indicate the presence of both the T and L calcium channel types. We have suggested that the L current contributes to contraction, while the T current is likely a pacemaker trigger.$^8$ The T calcium current is activated at membrane potentials positive to $-80 \text{ mV}$ and is completely inactivated by depolarizing to $-30 \text{ mV}$. In contrast, the L calcium current is activated at membrane potentials positive to $-30 \text{ mV}$ and is only partially inactivated at membrane potentials greater than $0 \text{ mV}$. Since T current activation and inactivation is shifted from L current by about $30 \text{ mV}$ in the hyperpolarizing direction, the T and L currents can be measured separately by using different holding potentials.$^8$

We have reported previously that T is the predominant type of calcium current in cultured azygos venous cells from WKY.$^8$ This study concurs with the predominance of the T current in these WKY cells and shows that in similar vascular cells from genetically matched SHR, the L current is three times greater than the T current. Since all cells studied were obtained from neonatal rats (1–3 days old) and blood vessels are not effectively innervated until at least 1–3 weeks of age,$^{20}$ it is unlikely that a neurogenic influence in the blood vessel wall or exposure of the vascular muscle cell to norepinephrine accounted for the greater proportion of L channel current in SHR cells. It is also unlikely that a structural alteration of the blood vessel wall secondary to a greater distending pressure was involved. Although some studies report that mean arterial pressure is minimally elevated in neonatal SHR,$^{21,22}$ other studies find no discernible hypertension until after 4 weeks of age.$^{23,24}$ Furthermore, the vascular cells in this study were taken from the
venous side of the circulation. Rather, our data complement experiments by other investigators, who find changes in agonist sensitivity and contractile responses in vascular muscle from SHR before the onset of hypertension. Mulvany et al.\textsuperscript{25} have reported an increased calcium permeability induced by norepinephrine in mesenteric arteries from SHR at 4 weeks of age but not in similar arteries from renal hypertensive rats, suggesting that the increased calcium sensitivity is genetically determined in the SHR blood vessels.

Voltage-dependent activation and inactivation of L calcium current occurs at more positive membrane potentials than activation and inactivation of T calcium current.\textsuperscript{8} Thus, in SHR cells showing predominantly L current, the current-voltage curves for activation and inactivation occurred at more positive potentials than similar curves from WKY cells. Calcium current in the WKY cells activated and inactivated with lesser amounts of depolarization in keeping with the predominance of T current in these cells. Thus, calcium current in WKY vascular muscle is transiently activated at more negative membrane potentials for only a fraction of a second, while calcium influx in SHR vascular muscle appears during greater depolarization and is sustained for seconds to minutes.

In our experiments, the T current completely decayed during maintained (300 msec) depolarizing pulses, whereas the L current still averaged 10% of its original magnitude at the end of depolarization. This represents a faster decay of the L current than reported earlier in the same preparation from this lab and from investigators using other vascular muscle types.\textsuperscript{9} The faster decay of the L current in our experiments likely was caused by the lower EGTA concentration in our pipette solution (0.1 mM compared to 10 mM in earlier studies), the use of calcium rather than barium as the charge carrier, and the use of a positive command potential (+30 mV). These factors have been noted recently by others to increase the rate of calcium current inactivation during maintained depolarization,\textsuperscript{12} most likely by promoting calcium-dependent inactivation of the L calcium channel.

It is not certain how this altered voltage-dependency of calcium current would affect vasoconstrictor tone in the SHR. The final concentration of cytoplasmic calcium available to bind the contractile proteins will be determined simultaneously by not only the pattern of calcium influx, but also intracellular calcium binding, release, reuptake, and active extrusion by the sarcolemma (for review, see Kwan\textsuperscript{1}). The influx of calcium also will activate other ion channels, such as the calcium-activated K\textsuperscript{+} channels,\textsuperscript{26-29} whose function also could be altered in the hypertensive state.\textsuperscript{30} The major role for L calcium channels in initiating and maintaining vascular tone is likely but still speculative. The T current probably plays the major role in initiating spontaneous contraction, because T current is activated at relatively negative membrane potentials. However, the L current, which is activated at more positive membrane potentials, is the only calcium
current yet identified that could help to maintain vascular muscle tone by providing a continual supply of external calcium to sustain contraction and replete the stores of intracellular calcium.8,9,31

In venous cells from young animals, changes in calcium currents could fit into the pathophysiology of genetic hypertension, since a reduced venous compliance has been implicated in the early stages of the disease (for review, see Safar and London32). In view of our finding that the calcium channel current is altered in venous cells from young SHR, it will be important to compare these results with similar data collected on venous and arterial cells from adult SHR, and on neonatal and adult vascular cells from animals with other models of hypertension. In addition, it would be relevant to characterize calcium current in other cell types from SHR. If systemic hypertension in the SHR represents the manifestation of a universal membrane defect as has been suggested earlier, then nonvascular cells may show altered calcium channel properties as well.

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References


Key Words • calcium channel • hypertension • azygos vein • membrane alteration • calcium influx
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