Calcium Channel Alterations in Genetic Hypertension

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We proposed earlier that voltage-dependent calcium (Ca\(^{2+}\)) current is altered in single azygos venous cells from Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). In this study, the effects of different intracellular concentrations of ethylene glycol-bis-N,N',N',N'\(^{-}\)-tetraacetic acid (EGTA) on Ca\(^{2+}\) currents were investigated. Vascular muscle cells from SHR and WKY rats were equilibrated with pipette solution containing 0.1 mM or 10 mM EGTA. Increasing the EGTA concentration from 0.1 to 10 mM in SHR vascular cells significantly enhanced the peak amplitude of the longer lasting (L) current from 87 ±12 pA to 152 ±8 pA, while the transient (T) current amplitude was not significantly different (52 ±7 pA and 36±7 pA, respectively). In WKY rat vascular muscle cells, the amplitudes of the T and L currents were not significantly different with the same comparison of intracellular EGTA concentrations. These observations suggest that relatively low intracellular Ca\(^{2+}\) concentrations can more strongly modulate Ca\(^{2+}\) current through the L channel in SHR than WKY rat vascular muscle cells. (*Hypertension* 1989;14:453–456)

Studies to investigate mechanisms of hypertension have shown that regulation of calcium (Ca\(^{2+}\)) influx is altered in vascular muscle from spontaneously hypertensive rats (SHR). For example, an enhanced sensitivity to norepinephrine and external Ca\(^{2+}\) concentration,\(^1,2\) a reduced relaxation to vasodilators,\(^3,4\) and an increased incidence of spontaneous activity\(^5,6\) in blood vessels from SHR suggest that genetic hypertension is associated with changes in Ca\(^{2+}\) metabolism. However, the relatively few studies comparing Ca\(^{2+}\) influx across the sarcolemma in SHR vascular muscle have yielded contradictory results, with \(^{45}\)Ca influx in SHR vascular cells reported to be decreased, unchanged, or increased.\(^7,8\)

To compare more precisely Ca\(^{2+}\) influx in vascular muscle cells from SHR, we previously used the whole-cell, voltage-clamp method to measure two types of Ca\(^{2+}\) currents in azygos venous cells from SHR and Wistar-Kyoto (WKY) rats.\(^1\) We found that depolarization activated both transient (T) and longer lasting (L) Ca\(^{2+}\) channels\(^12\) in both SHR and WKY rat vascular muscle membranes, but that L channels predominated in the SHR vascular membranes.\(^11\) The difference in Ca\(^{2+}\) channel types between neonatal SHR and WKY rat venous muscle cells suggests that genetic factors, rather than age-related changes in circulating factors or perfusion pressure, likely underlie the altered Ca\(^{2+}\) channel composition in the vascular muscle cell membranes.

In this study, we increased the amount of the Ca\(^{2+}\) chelating agent ethylene glycol-bis-N,N',N',N'\(^{-}\)-tetraacetic acid (EGTA) in the pipette solution by 100-fold from that used previously. This resulted in a greater peak amplitude of L Ca\(^{2+}\) channel current in SHR vascular muscle cells from that seen previously, but no change in T Ca\(^{2+}\) current amplitude. Neither T nor L Ca\(^{2+}\) current amplitudes were changed significantly in cells from WKY rats. One implication of this finding is that the low concentration of Ca\(^{2+}\) remaining in 0.1 mM EGTA is an important modulator of Ca\(^{2+}\) influx through L Ca\(^{2+}\) channels in SHR vascular muscle cells.

**Materials and Methods**

Primary cultures of vascular muscle cells from azygos veins of 1-4-day-old rats were prepared as previously described.\(^11,12\) Neonatal rats were from the SHR or WKY rat strains. Cells were grown on poly-L-lysine-coated glass coverslips in a 5% CO\(_2\) incubator at 95% humidity at 37° C and used after 3-7 days in culture.
For measurement of whole-cell Ca\(^{2+}\) currents, cover slips of cells were placed in a perfusion chamber containing an external solution consisting of (mM): NaCl 140, KCl 10, CaCl\(_2\) 2, NaHCO\(_3\) 26, MgSO\(_4\) 1, glucose 10, HEPES 10 (pH adjusted to 7.4 with NaOH, 22\(^\circ\) C). Patch pipettes were filled with a cesium glutamate solution to eliminate outward K\(^+\) currents. The pipette solution was composed of (mM): cesium glutamate 150, EGTA 0.1, MgCl\(_2\) 1, HEPES 10 (pH adjusted to 7.4 with CsOH). In some experiments, the pipette solution contained 10.0 rather than 0.1 mM EGTA. Whole-cell Ca\(^{2+}\) currents were recorded after suction breakthrough into the cell and then measured during 300 msec depolarizing pulses. Different holding and test potentials were used to separate the T and L Ca\(^{2+}\) channel currents. Peak T current was recorded during a test pulse from a holding potential of -80 to -30 mV. Peak L current was elicited by depolarization from a holding potential of -30 to +30 mV. The voltage-clamp currents were amplified by a List EPC-7 (List-Electronic, Darmstadt-Eberstadt, FRG) patch-clamp amplifier with a 0.5 GΩ 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 from 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.

Statistical comparisons were made between vascular muscle cells from SHR and WKY rats by unpaired t tests. A p value of <0.05 was accepted as significant.

**Results**

Two types of Ca\(^{2+}\) channels can be distinguished during whole-cell, voltage-clamp recording from azygos venous cells, as previously described.\(^{11,12}\) T Ca\(^{2+}\) current is activated and inactivated at negative membrane potentials and is measured during a depolarizing pulse from -80 to -30 mV. L Ca\(^{2+}\) current is activated and inactivated in a more positive range of membrane potential and is elicited during depolarization from -30 to +30 mV. This method was used to separate the two Ca\(^{2+}\) current types, and whole-cell T and L currents in azygos venous muscle cells from SHR and WKY rats are shown in Figure 1. In 30 SHR and 30 WKY rat azygos venous cells, we have found that the peak amplitude of the T type of Ca\(^{2+}\) current is greater than the L current amplitude in 65% of WKY rat cells. However, the L current amplitude predominates in 80% of SHR cells. In these experiments, 0.1 mM EGTA was present in the pipette solution.\(^{11}\)

In other SHR and WKY rat azygos venous cells, the pipette solution contained 10.0 mM EGTA. This maneuver would further reduce cytoplasmic Ca\(^{2+}\) ion concentration from that at lower EGTA concentration, and equally reduce Ca\(^{2+}\) immediately inside the cell membrane in both SHR and WKY rats. Figure 2 compares the peak magnitudes of T and L currents in SHR and WKY rat cells equilibrated with pipette solution containing either 0.1 or 10 mM EGTA.\(^{11}\) In seven WKY rat vascular cells containing 10 mM EGTA, the peak magnitudes of T and L Ca\(^{2+}\) currents were 123±49 pA and 66±8 pA. These values were not different from T and L current amplitudes (100±14 pA and 67±8 pA, respectively) measured in 30 other WKY rat cells containing 0.1 mM EGTA.\(^{11}\) In 10 SHR cells containing 10 mM EGTA, mean T current amplitude (36±7 pA) also was not significantly different from 52±7 pA, n=30 (t test). However, the peak L current amplitude in 10 mM EGTA was 152±8 pA, which was almost twice the amplitude (82±12 pA) of similar SHR cells containing 0.1 mM EGTA (significantly different by t test).

**Discussion**

Two types of Ca\(^{2+}\) currents have been measured in vascular muscle cells.\(^{12}\) The T current, which is activated at negative membrane potentials and inactivates rapidly, may play a role in pacemaker activity and initiating contraction. The L current, which activates at more positive membrane potentials and...
is sustained, likely provides extracellular Ca\textsuperscript{2+} to maintain contraction. We reported earlier that the proportion of T versus L Ca\textsuperscript{2+} current is changed in azygos venous cells from SHR. Vascular muscle cells from SHR showed a greater proportion of L current, whereas cells from WKY rats exhibited more T current. Since these vascular muscle cells were isolated from neonatal venous tissue, it is unlikely that neurogenic influences or structural changes in the blood vessel wall accounted for the alteration in membrane Ca\textsuperscript{2+} channel types.\textsuperscript{11} This suggests that voltage-dependent Ca\textsuperscript{2+} channels are altered in the vascular muscle membrane of SHR and concurs with earlier reports that genetic hypertension may be related to a basic membrane defect.\textsuperscript{1,13} However, factors other than Ca\textsuperscript{2+} channel type are likely important in regulating Ca\textsuperscript{2+} influx in SHR vascular muscle. Thus, increasing intracellular EGTA concentration from 0.1 mM to 10 mM in voltage-clamped vascular muscle cells enhanced the peak magnitude of the L current in SHR cells by almost twofold, but did not significantly affect L current size in WKY rat cells and had no effect on T current amplitude in vascular muscle cells from either rat strain. The results concur with recent reports from other laboratories that a rise in the intracellular Ca\textsuperscript{2+} concentration reduces the amplitude of the L current in vascular muscle, but does not modulate T current amplitude.\textsuperscript{14,15} We do not know why the amplitude of the L current in vascular muscle cells from WKY rats was not similarly affected by alteration of EGTA concentration in the pipettes, since L current in vascular muscle from the rat and rabbit portal vein\textsuperscript{15,16} and rabbit ear artery\textsuperscript{14} appears to be modulated by the cytoplasmic Ca\textsuperscript{2+} concentration. It is possible that the low or very low free Ca\textsuperscript{2+} concentration with EGTA present can modulate only SHR L channels. However, the range of Ca\textsuperscript{2+} approaching normal values cannot yet be confidently quantitated in patch-clamped cells. Regardless, the differences observed in these experiments suggest 1) the importance of intracellular Ca\textsuperscript{2+} in the modulation of L currents in SHR vascular muscle cells and 2) that small changes at even low intracellular Ca\textsuperscript{2+} concentrations in hypertensive animals could strongly modulate Ca\textsuperscript{2+} influx.

Studies in SHR vascular muscle cells have not uniformly revealed changes in the intracellular Ca\textsuperscript{2+} concentration (for reviews, see References 13 and 17). However, entry of external Ca\textsuperscript{2+} through the vascular muscle membrane would not necessarily be reflected in increased cytoplasmic Ca\textsuperscript{2+} levels, because Ca\textsuperscript{2+} may remain localized at or near the cytoplasmic membrane surface after entry. It is possible that these localized areas of Ca\textsuperscript{2+} near the cytoplasmic surface of the cell membrane are important in regulating Ca\textsuperscript{2+} influx through the L channel. Essential hypertension is likely a result of multiple interactive factors. Thus, it is not clear how altered Ca\textsuperscript{2+} channels would contribute to the increased arterial pressure characterizing systemic hypertension. However, at the cellular level, we suggest that there may be differences in the relative proportion of L to T channels in vascular muscle cells from SHR and that differences in inactivation of the L channel by intracellular free Ca\textsuperscript{2+} may play a regulatory role in determining changes, such as those that occur during aging, in long-term vascular muscle function.

References


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