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# Cellular Calcium Regulation in Hypertension

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**In vascular muscle cells, two distinct types of functionally important calcium ($Ca^{2+}$) channels, called transient (T) and sustained (L), are differentiated by dihydropyridine calcium antagonists (CaA). We studied the ratio of T/L $Ca^{2+}$ channels in isolated, spontaneously contracting azygous venous cells of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) by quantitating $Ca^{2+}$ currents and intracellular $Ca^{2+}$ release. While total transmembranous $Ca^{2+}$ current was not different between the two strains, the proportion of $Ca^{2+}$ currents carried by L-type channels was enhanced in vascular muscle cells from SHR. We have recently compared subcellular distribution of intracellular free $Ca^{2+}$ concentration in the same cells, at rest and during stimulation, by quantitation with a digital photon-counting camera. Fura-2 fluorescence intensity showed that $Ca^{2+}$ release was principally from sarcoplasmic reticulum and that cells from SHR had higher levels of $Ca^{2+}$ upon calcium channel stimulation, especially at the cell periphery. These findings suggest fundamental differences in SHR and WKY vascular muscle cells implicating the importance of changes in calcium channels, modulation of $Ca^{2+}$ release, and $Ca^{2+}$ uptake in SHR hypertension. Am J Hypertens 1989; 2:655–658**

KEY WORDS: **Vascular muscle, calcium channels, calcium concentration, fura-2, calcium release, SHR, WKY.**

Hypertension in its established phase is hemodynamically characterized by an increase in systemic vascular resistance.[1–3] This derangement is due to structural differences between normotensive and hypertensive subjects,[4] but also entails functional components.[5]

As the force-generating bridging between actin and myosin depends on changes of intracellular calcium activity (calcium free-ion concentration) and hence transmembranous calcium movements, modulations of transmembranous calcium influx and intracellular calcium activity, $(Ca^{2+})_i$, have a pivotal role in determining vasoconstriction. Therefore, mechanisms that lead to an increase in $(Ca^{2+})_i$ through these processes cause vasoconstriction.

Several lines of evidence suggest that abnormalities of calcium metabolism are involved in the pathogenesis of established human hypertension. In particular, low serum concentrations of ionized $Ca^{2+}$ have been observed in at least a fraction of patients with this disease.[6,7] Furthermore, there is an enhanced vasodilation[8,9] and antihypertensive effect[10,11] of calcium antagonists in patients with essential hypertension.

Studies using the platelet as a model of contractile systems indicate that alterations of $Ca^{2+}$ handling in human hypertensives may occur at the cellular level.[12,13] However, these studies do not determine whether similar alterations are operative in vascular muscle cells and, if so, whether they are causally implicated or a consequence of elevated blood pressure.

To define vascular muscle mechanisms which are altered in an animal model of hypertension prior to the development of elevated blood pressure, studies were

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carried out on isolated azygous vein cells from three-day-old spontaneously hypertensive rats (SHR) and genetically matched Wistar-Kyoto rats (WKY). The preparation and characterization of these cells from primary cultures were described in detail elsewhere.[14] These cells are spontaneously contracting and exhibit repeated contraction/relaxation cycles with a high shortening velocity, high pharmacological sensitivity, and electrophysiological integrity.[15,16]

## METHODS

Experiments were carried out on vascular muscle cells isolated from azygous veins of neonatal rats.[17,18] Whole cell recordings of $Ca^{2+}$ currents, isolated by replacement of $Na^+$ and $K^+$ in internal and external solutions allowed separation of L and T types of current, where L are longer lasting (sustained) and T are transient.[19]

In cells from similarly prepared cultures, intracellular $Ca^{2+}$ was quantitated by fura-2 fluorescence using the three wavelength protocols explained in detail elsewhere.[20] High resolution (0.5 $\mu$m) quantitation of $Ca^{2+}$ was carried out with a two-stage microchannel plate camera (Hamamatsu VIM). Excitation of fluorescence at 340, 360, and 380 nm with emission at 510 nm (three wavelength analysis) provides point-by-point quantitation of intracellular free $Ca^{2+}$ activity at each pixel.[20]

## RESULTS

**Altered Calcium Currents in Vascular Muscle Cells of SHR** The rationale to study $Ca^{2+}$ currents is based on the observation that alterations in $Ca^{2+}$ entry or exit might well lead to changes in tension without intervention by other ions.[21] Calcium which triggers excitation-contraction coupling presumably enters vascular muscle cells through voltage-dependent $Ca^{2+}$ channels. These precise $Ca^{2+}$ currents were recorded using tight-seal pipettes by patch-clamp techniques.[17,18,22] These techniques allowed the identification and characterization of two types of $Ca^{2+}$ channel currents. The low threshold, transient channel (T) is activated with small depolarizations and inactivates rapidly. The high threshold, long-lasting channel (L, sustained) is activated at more positive membrane potentials, inactivates slowly, and is sensitive to dihydropyridine calcium antagonists.[18] Based on these characteristics, it has been suggested that the T-channel participates in the generation of $Ca^{2+}$-dependent electrical spiking and spontaneous activity in vascular muscle cells, whereas the L-channel is involved in the triggering and maintenance of vascular muscle contraction.

Peak amplitudes of T, L, and total (sum of T and L) $Ca^{2+}$ currents were measured in 30 cells from WKY and 30 cells from SHR.[19] While no difference in total peak amplitude of $Ca^{2+}$ current was observed in these cells from neonatal rats, $Ca^{2+}$ current carried by L (sustained) channels contributed significantly more (62 ± 5%) to total peak current in SHR than in WKY (42 ± 7%) based on 60 experiments ($P < 0.05$). When ratios (L/T) of $Ca^{2+}$ current were calculated, the mean value for 30 WKY was 0.9 and 2.9 for 30 SHR cells (Figure 1). These results demonstrate that even at the prehypertensive stage, L channels are more prominent in SHR than WKY, suggesting a genetic membrane channel defect with the proper timing to be a cause of increased $Ca^{2+}$ in vascular muscle cells.

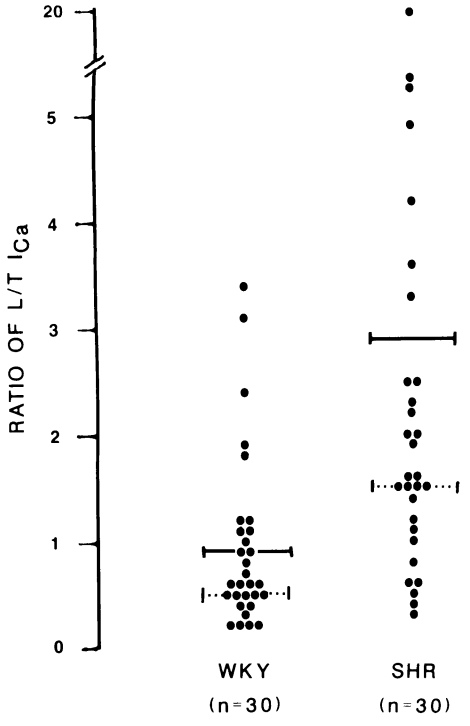


**Figure 1.** *Ratios of sustained (L) to transient (T) calcium current were greater in SHR than WKY isolated vascular muscle cells measured under whole cell voltage-clamp conditions. Each dot represents one cell from which the ratios were measured in 30 SHR and 30 WKY, with means indicated by solid lines (significantly different) and modes by dotted horizontal lines.*[19]

**Elevated Free Calcium Concentrations at Subsarcolemmal Sites** To study if these $Ca^{2+}$ currents associated with L channels are linked to altered intracellular $Ca^{2+}$ handling, the distribution of intracellular free $Ca^{2+}$ activities, $(Ca^{2+})_i$, was measured in isolated vascular muscle cells with fura-2. Incorporation of the fluorescent dye, quantitation of fluorescent intensities, and $(Ca^{2+})_i$ determination after correction for inhomogeneous dye distribution are described in detail elsewhere.[20,23]

In a study quantitating the distribution of intracellular free $Ca^{2+}$ in neonatal vascular muscle cells from SHR and WKY,[24] we have measured the average intracellular dye concentration, and quantitated $(Ca^{2+})_i$ of the whole cytoplasm, as well as in defined regions. We divided the cell into a peripheral boundary, 0.5 $\mu$m distant from the

cell edges, and central areas, defined as all that remains. Quantitation of $Ca^{2+}$ was according to the following equations which were applied to each of the 250,000 image elements

$$(Ca^{2+})_i = K_d\,(cR - 1.36)/(3.48 - cR),$$

where $K_d$ is the dissociation constant established by calibration procedures (239 nM at 37°C), R is the ratio of 510 nm fluorescence at 340/380 nm wavelength excitation, and c corrects for the inhomogeneous dye distribution according to

$$c = 0.92 + 2.24ae^{-0.0028dF},$$

where F is the fura-2 fluorescence intensity count at 360 nm excitation wavelength, a corrects for day-to-day variability of the optical system as calibrated by phosphor beads, and d adjusts for accumulation times. We have performed these measurements in cells perfused with isotonic medium and following administration of 100 mM potassium and 1 μM SDZ 202-791 (+S), a stereoselective dihydropyridine with calcium entry stimulating properties.

Intracellular fura-2 concentrations were similar in cells of both SHR and WKY (62 and 85 μM, respectively, not significantly different). $(Ca^{2+})_i$ in central regions of nonstimulated cells perfused with isotonic medium was comparable in both strains (WKY: 119 *v* SHR: 126 nM). But $(Ca^{2+})_i$ in the peripheral boundary was slightly elevated in SHR (WKY: 100 *v* SHR: 130 nM, $P<0.05$). Upon stimulation of the cells by 100 mM potassium and SDZ 202-971, the average myoplasmic $Ca^{2+}$ activity in central regions of SHR tended to increase to a greater extent (WKY: 442 *v* SHR: 485 nM) accompanied by a more pronounced elevation of $(Ca^{2+})_i$ in the peripheral boundary (WKY: 539 *v* SHR: 866 nM, $P<0.05$). These results point to the important role intracellular $Ca^{2+}$ release from subsarcolemmal $Ca^{2+}$ pools may play in vascular muscle during the prehypertensive stage, suggesting a defect of SHR $Ca^{2+}$ uptake or release mechanisms in these cells.

## DISCUSSION/CONCLUSION

These studies were directed towards the characterization of cellular $Ca^{2+}$ handling abnormalities in SHR at a prehypertensive age. The results demonstrate that before development of elevated blood pressure in SHR, the sustained L $Ca^{2+}$ channel is more dominantly expressed,[19] and that $Ca^{2+}$ release or uptake mechanisms from subsarcolemmal stores are already altered. The underlying factors leading to changes in the membrane structure and intracellular organelles could well be linked. For example, $Ca^{2+}$ entering through L channels may have greater effects on the cytoplasmic sites which release intracellular $Ca^{2+}$. Or, $Ca^{2+}$ entry through L channels may have smaller effects on $Ca^{2+}$ uptake, as suggested by Table 1. Whichever is true, these results underline the importance of $Ca^{2+}$ regulation of submembranous $Ca^{2+}$ stores.

**TABLE 1. $Ca^{2+}$ ACTIVITY (FREE ION CONCENTRATION) IN AZYGOUS VEIN VASCULAR MUSCLE CELLS OF SHR *v* WKY (nmol/L)**

| | WKY | SHR |
|---|---|---|
| Peripheral rim | | |
| Resting | 100 ± 2 | 130 ± 2* |
| Stimulated | 530 ± 11 | 882 ± 33* |
| Central region | | |
| Resting | 119 ± 2 | 126 ± 5 |
| Stimulated | 442 ± 36 | 485 ± 31 |

*Resting condition was contractile cells between contractions in control solution. Stimulated condition was cells in 100 mM $K^+$ with 1 μM $Ca^{2+}$ agonist (SDZ 202-791S). The peripheral rim is defined as the 0.5 μm from the cell edge. Central region was all parts of the cell at least 1 μm from the cell edge, and actually includes the top and bottom peripheral edge contributions to that fluorescence (ie, optical sectioning of fluorescence was not practical in these thin cells).*

*Values are means ± SEM; n = 3 for WKY and 5 for SHR.*

* $P < 0.05$.

That total myoplasmic $Ca^{2+}$ activity in unstimulated cells from both strains is comparable tallies with the finding that elevated $(Ca^{2+})_i$ in platelets can only be observed in older SHR with elevated blood pressure.[25] Whether a similar elevation of peripheral myoplasmic $Ca^{2+}$ activity in vascular cells from adult SHR (with elevated blood pressure) develops is unknown. However, it could be postulated that the enhanced responsiveness of SHR to stimuli giving rise to the observed $Ca^{2+}$ increase could lead to a profound resetting of $Ca^{2+}$ uptake and extrusion mechanisms over time. To further characterize the responsible mechanisms, more studies of SHR are needed to investigate these processes in different ages and blood vessels.

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