Brazilian Journal of Medical and Biological Research (1997) 30: 315-323
ISSN 0100-879X

Calcium handling by vascular myocytes in hypertension

R.C.A. Tostes[3], D.W. Wilde[2], L.M. Bendhack[4] and R.C. Webb[1]

Departments of [1]Physiology and [2]Anesthesiology, University of Michigan, Ann Arbor, MI 48109-0622, USA
[3]Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, 05508-900 São Paulo, SP, Brasil
[4]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto, SP, Brasil

Abstract

Calcium ions (Ca^{2+}) trigger the contraction of vascular myocytes and the level of free intracellular Ca^{2+} within the myocyte is precisely regulated by sequestration and extrusion mechanisms. Extensive evidence indicates that a defect in the regulation of intracellular Ca^{2+} plays a role in the augmented vascular reactivity characteristic of clinical and experimental hypertension. For example, arteries from spontaneously hypertensive rats (SHR) have an increased contractile sensitivity to extracellular Ca^{2+} and intracellular Ca^{2+} levels are elevated in aortic smooth muscle cells of SHR. We hypothesize that these changes are due to an increase in membrane Ca^{2+} channel density and possibly function in vascular myocytes from hypertensive animals. Several observations using various experimental approaches support this hypothesis: 1) the contractile activity in response to depolarizing stimuli is increased in arteries from hypertensive animals demonstrating increased voltage-dependent Ca^{2+} channel activity in hypertension; 2) Ca^{2+} channel agonists such as Bay K 8644 produce contractions in isolated arterial segments from hypertensive rats and minimal contraction in those from normotensive rats; 3) intracellular Ca^{2+} concentration is abnormally increased in vascular myocytes from hypertensive animals following treatment with Ca^{2+} channel agonists and depolarizing interventions, and 4) using the voltage-clamp technique, the inward Ca^{2+} current in arterial myocytes from hypertensive rats is nearly twice as large as that from myocytes of normotensive rats. We suggest that an alteration in Ca^{2+} channel function and/or an increase in Ca^{2+} channel density, resulting from increased channel synthesis or reduced turnover, underlies the increased vascular reactivity characteristic of hypertension.

Correspondence
R.C.A. Tostes
Laboratório de Hipertensão
Departamento de Farmacologia
ICB I, USP
Av. Prof. Lineu Prestes, 1524
05508-900 São Paulo, SP
Brasil
Fax: 55 (011) 818-7433
E-mail: rtostes@usp.br

Presented at the XI Annual Meeting of the Federação de Sociedades de Biologia Experimental, Caxambu, MG, Brasil, August 21-24, 1996.

Research supported by the National Institutes of Health (No. HL18575; R.C. Webb), the American Heart Association-MI (No. 38G5945; D.W. Wilde) and CNPq (R.C.A. Tostes).
Publication supported by FAPESP.

Received November 27, 1996
Accepted January 6, 1997

Key words
- Vascular smooth muscle
- Hypertension
- Calcium
- Voltage-operated calcium channels
- Vascular reactivity
- Bay K 8644

Hypertension *vs* increased peripheral resistance *vs* abnormalities of vascular function

Hypertension is considered to be a multifactorial disease in which genetic and environmental factors, humoral and neural systems, and also intrinsic changes in the vasculature play a role. The disease in its established phase is characterized by a normal cardiac output and an elevated total peripheral resistance (1,2) which is the major indication that the vasculature plays an important role in maintaining high blood pressure.

Considering the possible mechanisms which could account for the elevated peripheral resistance, a large number of studies have paid special attention to structural and functional abnormalities of the vasculature. Increased vascular reactivity, i.e., increased sensitivity to vasoconstrictor agents and increased smooth muscle force generation ability, hypertrophy and remodeling, development of spontaneous tone, the presence of oscillatory contractile activity, and increased ionic permeability of the plasma membrane are some of the alterations observed in vessels from hypertensive animals (3-6). These vascular structural and functional changes are quite variable, and can be observed in some models of hypertension, but not in others, and in some vascular beds, when other regional beds in the same animal do not exhibit these abnormalities. Many factors may contribute to these particular vascular changes, including the primary cause for the hypertensive state, development and duration of the elevated blood pressure, the species, age and gender of the animals, the technique used to study the vascular change, the regional location of the vascular bed, and, in the case of vascular reactivity, the stimulus employed (5,6).

Evidence of vascular structure abnormalities in hypertension and their possible contribution to increased vascular reactivity and, consequently, to the elevated peripheral resistance has been extensively discussed (for reviews, see 5-7). According to Folkow (8,9), the increased peripheral resistance in hypertension is associated with structural changes, and the increased vascular reactivity could be explained as resulting from narrowing of the vasculature due to increased media thickness which reduces the luminal diameter of the resistance vessels. However, some observations indicate that other factors are involved in the vascular changes observed in hypertension. Abnormalities of vascular function, i.e., altered excitation-contraction coupling, are considered to be an alternative mechanism which could explain the increased vascular reactivity in hypertension. For example, an increase in the threshold sensitivity of the vascular smooth muscle to vasoconstrictor agents cannot be explained by structural alterations and more likely reflects changes in the function properties of vascular smooth muscle (5). Furthermore, vessels from hypertensive animals exhibit differences in relative sensitivity when stimulated with different agonists (10), a finding that does not fit Folkow's hypothesis and supports the idea that vascular functional abnormalities are involved in the increased vascular reactivity observed in hypertension.

Abnormalities of vascular function *vs* Ca^{2+} handling

Since a rise in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) is the principal process that initiates contraction in vascular myocytes (11), the maintenance of the steady-state $[Ca^{2+}]_i$ is critically important to vascular smooth muscle cells (VSMCs) (12). The sources of activator Ca^{2+} are both extracellular (ion channels in the plasma membrane) and intracellular (ion channels in the sarcoplasmic reticulum) and their relative contribution to force development varies between different arteries (13). Ca^{2+} channels can be

subdivided into those whose opening probability is modulated by changes in the membrane potential, the voltage-operated Ca^{2+} channels (VOCs), and those whose opening probability is primarily controlled by agonist-induced receptor activation (for reviews, see 14,15). However, the actions of many vasoactive agonists involve modulation of the VOCs and in almost all myocytes the L-type (long lasting) VOCs appear to be the major route for Ca^{2+} entry (14).

Extensive evidence indicates that a defect in the regulation of $[Ca^{2+}]_i$ plays a role in the increased vascular reactivity in hypertension (16). The cellular mechanisms involved in abnormal Ca^{2+} handling in VSMCs are numerous and include increased Ca^{2+} entry, decreased storage in subcellular fractions or decreased Ca^{2+} extrusion (16,17). We will not discuss all the possible mechanisms involved in abnormal Ca^{2+} handling in hypertension, but the reader will be mostly referred to excellent overviews. Our specific aim in this mini-review is to present evidence that changes in membrane Ca^{2+} channel density and function exist in VSMCs from hypertensive animals. The major observations supporting this hypothesis have been divided into four sections.

Contractile activity in response to depolarizing stimuli is increased in arteries from hypertensive animals demonstrating altered voltage dependence of the Ca^{2+} channel in hypertension

Noon et al. (18) and Winquist and Bohr (19) observed that arteries isolated from hypertensive rats maintain a spontaneous active tone in the resting state, which is abolished by the removal of external Ca^{2+} and by Ca^{2+} channel blockers (20). Similarly, studies performed in aortic strips from DOCA-salt hypertensive rats have shown that when these arteries are depleted of intracellular Ca^{2+} stores, they contract when placed in Ca^{2+}-containing solution whereas normotensive arteries do not. Under similar conditions, VSMCs isolated from the thoracic aorta of DOCA-salt rats exhibit an increase in $[Ca^{2+}]_i$ while no changes are observed in normotensive cells. Contractions and increases in $[Ca^{2+}]_i$ do not occur in the presence of the Ca^{2+} channel antagonist, nifedipine (21). Other investigators have demonstrated that high concentrations of Ca^{2+} induce contractions in aortic segments from spontaneously hypertensive rats (SHR) but do not alter tone in those from normotensive rats (3). On the other hand, Ca^{2+} channel blockers are more effective in reducing blood pressure and vasoconstrictor responses in smooth muscle from hypertensive subjects than in normotensive ones (22-24). From these observations it has been suggested that the Ca^{2+} permeability of the plasma membrane is increased in hypertension and that the VOCs are involved in this abnormal Ca^{2+} influx.

Supporting this idea, an increased reactivity to KCl has been reported in vessels from hypertensive rats (25-26). Soltis and Field (27) observed that femoral arteries from DOCA rats exhibit an increased reactivity to KCl and to norepinephrine only in the presence of normal extracellular Ca^{2+} concentration, but not in the presence of low extracellular Ca^{2+}. These authors suggested that the increased reactivity to KCl in arteries from hypertensive rats is related to an increased sensitivity to extracellular Ca^{2+}. This increased contractile response to KCl may be explained by changes in the membrane potential or in the voltage dependence of the Ca^{2+} channels in VSMCs from hypertensive animals.

VSMCs from genetically hypertensive or experimentally induced hypertensive animals also exhibit greater sensitivity to the Ca^{2+} channel agonist Bay K 8644 (28-31) as will be discussed in the next section. These studies using an activator of VOCs also support the hypothesis that membrane permeability to Ca^{2+} is increased in hypertension.

Ca^{2+} channel agonists such as Bay K 8644 produce contractions in isolated arterial segments from hypertensive rats and minimal contraction in those from normotensive rats

The dihydropyridine Bay K 8644, a Ca^{2+} channel agonist, increases Ca^{2+} channel currents through L-type VOCs in a concentration-dependent manner probably due to an increase in the mean channel open time (28). One of the first lines of evidence supporting the hypothesis of changes in the function of VOCs in hypertension was obtained in studies evaluating the effects of Bay K 8644 on basal tension development in arteries from hypertensive and normotensive rats. Aoki and Asano (29) have shown that the addition of Bay K 8644 elicits a concentration-dependent contraction in SHR femoral artery in the absence of any contractile agent, which was not observed in arteries from normotensive rats. Similarly, Bruner and Webb (30) observed that Bay K 8644 produced tonic contractions in carotid artery strips from stroke-prone spontaneously hypertensive rats (SHRSP), but not in those from Wistar Kyoto (WKY) rats. The Bay K 8644-induced contractions were increased in the presence of 12 mM KCl, but were still higher in arteries from SHRSP.

The enhanced contractile responsiveness to the Ca^{2+} channel agonist Bay K 8644 has been documented in large conduit arteries and small muscular arteries from SHR/SHRSP and also in arteries from other types of hypertensive rats, including rats with coarctation-, mineralocorticoid- and N-nitro arginine-induced hypertension (31,32). However, the increased sensitivity to Bay K 8644 is not a general defect in arteries from hypertensive rats, since it was not observed in small arterioles from SHRSP (33).

A direct correlation of increased sensitivity to Bay K 8644 and high blood pressure has been demonstrated in these studies. For example, Bay K 8644 evoked contraction in aortic segments from coarctation-hypertensive rats above (thoracic), but not below (abdominal) the coarctation. In coarctation-induced hypertension these aortic segments are exposed to hypertensive and normotensive pressures, respectively (31). Other evidence supporting a role of elevated pressure in the abnormal responsiveness to Ca^{2+} channel agonists comes from experiments in which treatment with ramipril, an angiotensin-converting enzyme inhibitor, reduced both blood pressure and Bay K 8644-induced contraction in carotid arteries from SHRSP, but did not affect these variables in WKY rats (34). These studies support the idea that the increased contractile response to Bay K 8644 parallels or is dependent on the changes in blood pressure.

Intracellular Ca^{2+} concentration is abnormally increased in vascular myocytes from hypertensive animals following treatment with Ca^{2+} channel agonists and depolarizing interventions

The use of fluorescent indicators to quantify $[Ca^{2+}]_i$ has permitted a correlation between alterations in arterial tone and changes in $[Ca^{2+}]_i$. A number of investigators have found increased basal or agonist-induced levels of $[Ca^{2+}]_i$ in vascular myocytes from hypertensive animals. However, these findings have not been consistently obtained and the variability may be explained by the effects of cell dissociation procedures, the age of the animals studied, and phenotypic changes in cell culture systems. Increased basal and agonist-induced increases in $[Ca^{2+}]_i$ have been observed both in freshly isolated (21) and cultured aortic smooth muscle cells (35,36) as well as in aortic strips (37) from SHR and DOCA-salt rats compared to those in arterial cells/strips from normotensive rats.

With regard to the change in $[Ca^{2+}]_i$ stimulated by depolarizing agents, increased KCl-induced changes in $[Ca^{2+}]_i$ levels were observed in azygous vein cell culture from SHR compared to WKY rats (38), and in

both thoracic aorta dissociated cells (39) and strips (40) from coarctation-hypertensive rats compared to normotensive controls.

Sada et al. (37) observed that CGP-28392, a Ca^{2+} channel agonist, stimulated an increase in $[Ca^{2+}]_i$ in aortas from SHR but not in those from WKY rats. Similarly, Asano et al. (20) have recently shown that the addition of Bay K 8644 to strips of femoral arteries from WKY rats induces an increase in $[Ca^{2+}]_i$ which is accompanied by a moderate contraction. When the effects of Bay K 8644 were determined in the SHR arteries, both the elevation in $[Ca^{2+}]_i$ and the contraction were more evident than those in the WKY rats (20).

Experiments evaluating $^{45}Ca^{2+}$ uptake have also shown similar results. Shibata et al. (41) described greater $^{45}Ca^{2+}$ uptake by SHR aorta compared to that in normotensive aorta. High KCl concentrations increased $^{45}Ca^{2+}$ uptake in both SHR and control aorta, but the Ca^{2+} influx was greater in SHR aorta. In addition, the dihydropyridine-sensitive component of $^{45}Ca^{2+}$ influx is increased in aortic strips from SHR (42) as well as in quiescent cultured VSMCs from SHR aorta (17) compared to that in preparations from normotensive WKY rats. These observations demonstrate a close relationship between the increased effects of depolarizing interventions/Ca^{2+} channel agonists on changes in tone and alterations in $[Ca^{2+}]_i$ in arteries from hypertensive animals.

Considering the mechanisms involved in the higher Ca^{2+} influx via L-type VOCs in arteries from hypertensive animals, one can speculate that arteries from hypertensive rats are more depolarized in the resting state compared to those from normotensive rats. However, a series of studies have reported no differences between the membrane potential of arteries from SHR *vs* WKY (43), SHRSP *vs* WKY (44,45), and DOCA-salt *vs* control rats (46). On the other hand, changes in membrane potential in arteries from SHR have been reported by some authors (47,48).

Alternative explanations would be that 1) the L-type VOCs in VSMCs from hypertensive rats exhibit an altered voltage dependence in the resting state when compared to normotensive VSMCs, which is supported by the increased contractile activity to depolarizing interventions in arteries from hypertensive animals, and 2) an increased number of Ca^{2+} channels or an abnormal activation of these channels exists in hypertensive arteries. These mechanisms will be discussed in the next section. Figure 1 summarizes the possible mechanisms contributing to the increased Ca^{2+} influx in VSMCs from hypertensive animals.

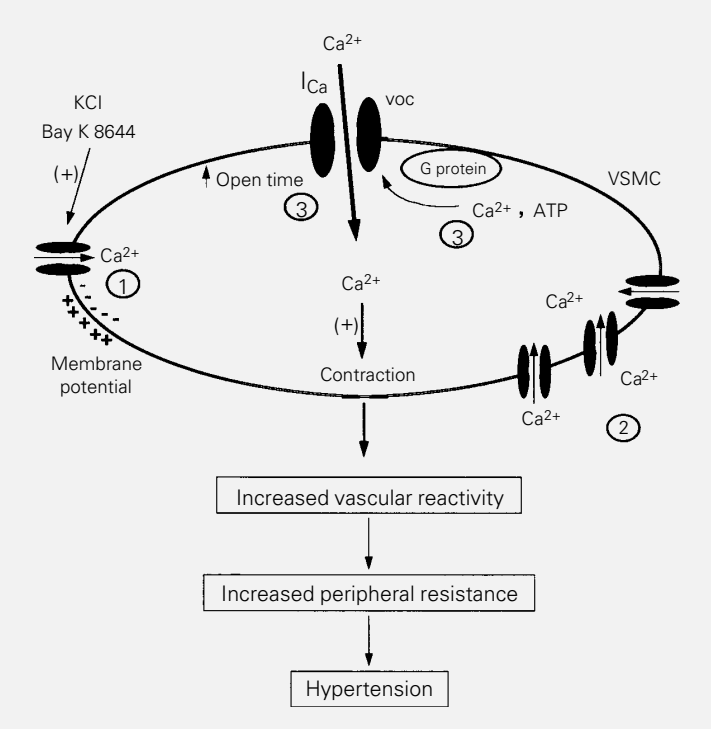

Figure 1 - Possible mechanisms contributing to the increased Ca^{2+} current in vascular myocytes from hypertensive animals. 1) L-type VOCs in VSMCs from hypertensive rats may exhibit an altered voltage dependence in the resting state when compared to normotensive VSMCs; 2) an increased number of Ca^{2+} channels may be expressed by these cells; 3) an abnormal function of these channels, represented by changes in the activation/inactivation parameters or by altered modulation of the channel activity by intracellular messengers, may exist in hypertensive arteries. VSMC, Vascular smooth muscle cell; VOC, voltage-operated Ca^{2+} channel; I_{Ca}, inward Ca^{2+} current.

Evidence from voltage- and patch-clamp techniques

Voltage- and patch-clamp techniques have proved to be extremely powerful tools for examining channel function and are considered the best evidence for alterations of Ca^{2+} channel activity in VSMCs in hypertension. However, like most techniques, they present some limitations since they involve disruption of the intracellular milieu and the preparation of single cells may also influence cell function and affect cell responses (49,50).

The Ca^{2+} channels, which are multisubunit proteins, flicker open and closed within milliseconds to permit influx of small amounts of Ca^{2+} in the resting state. These channels can be rapidly gated by voltage to increase, by several hundred-fold, the rate of Ca^{2+} entry. In voltage- and patch-clamp studies the important parameters are 1) the magnitude of the Ca^{2+} current, which usually is normalized against cell capacitance in order to eliminate differences in cell surface area, and 2) the voltage dependence of current activation and inactivation. Window currents are steady-state currents that arise from the opening of voltage-dependent Ca^{2+} channels over the narrow voltage range where steady-state activation and inactivation curves overlap. In this voltage range, channels cycle between closed, opened and inactivated states leading to small but stable currents (15).

Wilde et al. (51) observed no differences in the T (transient)-type Ca^{2+} current amplitude between cerebral artery VSMCs from SHRSP and WKY rats. However, increased maximal T-type Ca^{2+} inward current was observed in VSMCs isolated from the SHRSP azygous vein compared to cells from the normotensive outbred-panel-of-strains National Institutes of Health (N/nih) rats (52). These differences can be explained by the expression of T-type Ca^{2+} channels which have been primarily found in VSMCs from embryonic or neonatal rats.

In cultured VSMCs from neonatal azygous vein, the total inward Ca^{2+} current (I_{Ca}) arising from L-type channel activity is increased in cells from SHR, even though total inward current magnitude was similar for VSMCs from both SHR and WKY rats (53). Similarly, Cox and Lozinskaya (54) have reported larger L-type Ca^{2+} currents in mesenteric artery VSMCs from adult SHR compared to those in myocytes from WKY rats. However, studies from Ohya et al. (55) indicate that L-type Ca^{2+} channel activity is increased in young SHR, but the authors did not observe differences in the I_{Ca} or in the voltage dependence of channel inactivation between cells from adult SHR and WKY rats.

Wilde et al. (51) have reported that the inward L-type Ca^{2+} channel current in adult SHRSP cerebral artery VSMCs is approximately twice as large as that from WKY cells and the differences are not related to alterations in cell surface area. The authors have not found differences in the voltage dependence of current activation/inactivation between SHRSP and WKY cells, suggesting that the increased amplitude of Ca^{2+} channel current observed in the SHRSP VSMCs is due in part to increased channel density. On the other hand, analysis of the voltage dependence of Ca^{2+} channels in neonatal azygous vein VSMCs have shown changes for both activation and inactivation parameters in SHRSP cells (52). Paradoxically, a slower recovery from inactivation, which would cause a decreased Ca^{2+} current density, was observed in this study and, again, an increase in the number of Ca^{2+} channels was suggested as the mechanism responsible for the increased Ca^{2+} entry in VSMCs from hypertensive rats.

Ca^{2+} channel differences may have been hidden in some of these studies due to the following factors: 1) the different specialized regional arteries used; 2) the use of freshly isolated or cultured cells; 3) the experimental conditions such as the inclusion

in the pipette of substances that regulate Ca^{2+} channel activity (ATP, Mg^{2+}, GTP); 4) the choice of the charge carrier: Ba^{2+} *vs* Ca^{2+} (low and high concentrations), and 5) the selection of the hypertensive strain (SHRSP *vs* SHR; WKY *vs* N/nih). These considerations might also explain some of the differences between the findings reported above.

Regarding the possibility of an increased number of Ca^{2+} channels in hypertensive VSMCs, Ikeda et al. (56) have not observed differences in the binding density or in the dissociation constant of [^{3}H](+)-PN200-110, a dihydropyridine Ca^{2+} channel antagonist, in aortic membranes from SHR and WKY rats. Hermsmeyer et al. (57) have recently compared Ca^{2+} channel dihydropyridine binding in VSMCs from SHRSP and N/nih rats by using a fluorescent dihydropyridine compound closely related to nitrendipine. Interestingly, these authors observed that, although binding occurs with the same affinity in aortic and azygous vein VSMCs from neonatal SHRSP and N/nih rats, both aortic and azygous vein myocytes from SHRSP have fewer binding sites for the dihydropyridine probe compared to those from N/nih vessels. These results suggest that changes in Ca^{2+} channel function, and not an increase in the number of channels, at least in azygous veins, are responsible for the greater Ca^{2+} influx seen in vessels from hypertensive rats.

The altered properties of the Ca^{2+} channels may be explained by a prolonged mean channel open time, which could result from gene mutation, post-translational changes in the channel subunits or from alterations in the systems involved in the control of VOC activity in VSMCs (14,17). Ca^{2+} channel activity may be modulated by intracellular Ca^{2+} and Mg^{2+}, pH, G proteins, ATP, IP_3, the cyclic nucleotides cAMP and cGMP, protein kinase C, phosphatases, tyrosine kinases and calmodulin (15). For example, the L-type Ca^{2+} channel currents in smooth muscle cells are reversibly inhibited by changes in $[Ca^{2+}]_i$. It is possible that in VSMCs from hypertensive animals, which exhibit higher $[Ca^{2+}]_i$ levels, this inhibitory Ca^{2+}-dependent inactivation process is defective. The mechanism of inhibition of Ca^{2+} current by changes in $[Ca^{2+}]_i$ is not known and a change in this feedback mechanism in hypertension is speculative.

Finally, comparing the Ca^{2+} channel maximum current density in VSMCs from the azygous veins of neonatal SHRSP, SHR, WKY and N/nih rats it has been observed that the increased Ca^{2+} current density appears to be proportional to the increase in maternal blood pressure (52). From these findings we may conclude that, independent of the mechanism causing the increased Ca^{2+} influx in hypertensive VSMCs, increased number of channels or altered function, there is a clear association between increased Ca^{2+} current, altered vascular reactivity and hypertension. Further studies will help to elucidate the mechanisms underlying the increased Ca^{2+} current in vascular myocytes from hypertensive subjects and lead to the development of more specific therapeutics for the management of hypertension.

References

1. Frolich ED, Tarazi RC & Dustan HP (1969). Reexamination of the hemodynamics of hypertension. *American Journal of Medical Sciences*, 257: 9-23.
2. Smith TL & Hutchins PM (1979). Central hemodynamics in the developmental stage of spontaneous hypertension in the unanesthetized rat. *Hypertension,* 1: 508-517.
3. Bohr DF & Webb RC (1984). Vascular smooth muscle function and its changes in hypertension. *American Journal of Medicine,* 77: 3-16.
4. Webb RC & Bohr DF (1981). Recent advances in the pathogenesis of hypertension: consideration of structural, functional, and metabolic vascular abnormalities resulting in elevated arterial resistance. *American Heart Journal,* 2: 251-264.
5. Triggle CR & Laher I (1985). A review of changes in vascular smooth muscle functions in hypertension: isolated tissue versus *in vivo* studies. *Canadian Journal of Physiology and Pharmacology,* 63: 355-365.
6. Lee RMKW & Smeda JS (1985). Primary versus secondary structural changes of the blood vessels in hypertension. *Canadian Journal of Physiology and Pharmacology*, 63: 392-401.
7. Mulvany MJ (1983). Do resistance vessel abnormalities contribute to the elevated blood pressure of spontaneously-hypertensive rats? *Blood Vessels,* 20: 1-22.
8. Folkow B, Hallbäck M, Lundgren Y & Weiss L (1970). Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats. *Acta Physiologica Scandinavica,* 80: 93-106.
9. Folkow B (1978). Cardiovascular structural adaptation: its role in the initiation and maintenance of primary hypertension. *Clinical Science and Molecular Medicine*, 55: 3S-22S.
10. Watts SW & Webb RC (1994). Mechanism of ergonovine-induced contraction in the mesenteric artery from deoxycorticosterone acetate-salt hypertensive rat. *Journal of Pharmacology and Experimental Therapeutics,* 269: 617-625.
11. Rembold CM & Murphy RA (1986). Myoplasmic calcium, myosin phosphorylation, and regulation of cross-bridge cycle in swine arterial smooth muscle. *Circulation Research*, 58: 803-815.
12. Himpens B, Missiaen L & Casteels R (1995). Ca^{2+} homeostasis in vascular smooth muscle. *Journal of Vascular Research*, 32: 207-219.
13. Cauvin C, Weir SW & Bühler FR (1988). Differences in Ca^{2+} handling along the arterial tree: an update including studies in human mesenteric resistance vessels. *Journal of Cardiovascular Pharmacology,* 12 (Suppl 6): S10-S15.
14. Kuriyama H, Kitamura K & Nabata H (1995). Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. *Pharmacological Reviews,* 47: 391-573.
15. Hughes AD (1995). Calcium channels in vascular smooth muscle cells. *Journal of Vascular Research,* 32: 353-370.
16. Kwan CI (1985). Dysfunction of calcium handling by smooth muscle in hypertension. *Canadian Journal of Physiology and Pharmacology*, 63: 366-374.
17. Orlov SN, Li JM, Tremblay J & Hamet P (1995). Genes of intracellular calcium metabolism and blood pressure control in primary hypertension. *Seminars in Nephrology*, 15: 569-592.
18. Noon JP, Rice PJ & Baldessarini RJ (1978). Calcium leakage as a cause of the high resting tension in vascular smooth muscle from the spontaneously hypertensive rat. *Proceedings of the National Academy of Sciences, USA*, 75: 1605-1607.
19. Winquist RJ & Bohr DF (1983). Structural and functional changes in cerebral arteries from spontaneously hypertensive rats. *Hypertension,* 5: 292-297.
20. Asano M, Nomura Y, Ito K, Uyama Y, Imaizumi Y & Watanabe M (1995). Increased function of voltage-dependent Ca^{2+} channels and Ca^{2+}-activated K^+ channels in resting state of femoral arteries from spontaneously hypertensive rats at prehypertensive stage. *Journal of Pharmacology and Experimental Therapeutics,* 275: 775-783.
21. Tostes RCA, Wilde DW, Bendhack LM & Webb RC (1997). The effects of cyclopiazonic acid on intracellular Ca^{2+} in aortic smooth muscle cells from DOCA hypertensive rats. *Brazilian Journal of Medical and Biological Research,* 30: 255-265.
22. Atkinson J, Sautel M, Sonnay M, Fluckiger JP, de Rivaz JC, Boillat N, Pitton MC, Porchet PA, Armstrong JM & Fouda AK (1988). Greater vasodepressor sensitivity to nicardipine in spontaneously hypertensive rats (SHR) compared to normotensive rats. *Naunyn Schmiedeberg's Archives of Pharmacology*, 337: 471-476.
23. Lederballe-Pedersen O, Mikkelsen E & Anderson KE (1978). Effects of extracellular calcium on potassium and noradrenaline-induced contractions in the aorta of spontaneously hypertensive rats. Increased sensitivity to nifedipine. *Acta Pharmacologica et Toxicologica*, 43: 137-144.
24. Cattaneo EA, Rinaldi GJ, Gende OA, Venosa RA & Cingolani HE (1986). Increased sensitivity to nifedipine of smooth muscle from hypertensive rats. *Journal of Cardiovascular Pharmacology,* 8: 915-920.
25. Hagen EC & Webb RC (1984). Coronary artery reactivity in deoxycorticosterone acetate hypertensive rats. *American Journal of Physiology*, 247: H409-H414.
26. Tostes RCA, Traub O, Bendhack LM & Webb RC (1995). Sarcoplasmic reticulum Ca^{2+} uptake is not decreased in aorta from DOCA hypertensive rats: functional assessment with cyclopiazonic acid. *Canadian Journal of Physiology and Pharmacology*, 73: 1536-1545.
27. Soltis EE & Field FP (1986). Extracellular calcium and altered vascular responsiveness in the deoxycorticosterone acetate-salt rat. *Hypertension,* 8: 526-532.
28. Hering S, Hughes AD, Timin EN & Bolton TB (1993). Modulation of calcium channels in arterial smooth muscle cells by dihydropyridine enantiomers. *Journal of General Physiology*, 101: 393-410.
29. Aoki K & Asano M (1986). Effect of Bay K 8644 and nifedipine on femoral arteries of spontaneously hypertensive rats. *British Journal of Pharmacology*, 88: 221-230.
30. Bruner CA & Webb RC (1990). Increased vascular reactivity to Bay K 8644 in genetic hypertension. *Pharmacology,* 41: 24-35.
31. Storm DS & Webb RC (1993). Contractile responses to Bay K 8644 in rats with coarctation-induced hypertension. *Proceedings of the Society for Experimental Biology and Medicine,* 203: 92-99.

32. Watts SW, Finta KM, Lloyd MC, Storm DS & Webb RC (1994). Enhanced vascular responsiveness to Bay K 8644 in mineralocorticoid- and N-nitro arginine-induced hypertension. *Blood Pressure,* 3: 340-348.
33. Storm DS, Stuenkel EL & Webb RC (1992). Calcium channel activation in arterioles from genetically hypertensive rats. *Hypertension,* 20: 380-388.
34. Traub O & Webb RC (1993). Angiotensin-converting enzyme inhibition during development alters calcium regulation in adult hypertensive rats. *Journal of Pharmacology and Experimental Therapeutics*, 267: 1503-1508.
35. Sugiyama T, Yoshizumi M, Takaku F & Yazaki Y (1990). Abnormal calcium handling in vascular smooth muscle cells of spontaneously hypertensive rats. *Journal of Hypertension,* 8: 369-375.
36. Bendhack LM, Sharma RV & Bhalla RC (1992). Altered signal transduction in vascular smooth muscle cells of spontaneously hypertensive rats. *Hypertension,* 19: II.142-II.148.
37. Sada T, Koike H, Ikeda M, Sato K, Ozaki H & Karaki H (1990). Cytosolic free calcium of aorta in hypertensive rats: chronic inhibition of angiotensin converting enzyme inhibitor. *Hypertension,* 16: 245-251.
38. Erne P & Hermsmeyer K (1989). Intracellular vascular muscle Ca^{2+} modulation in genetic hypertension. *Hypertension,* 14: 145-151.
39. Papageorgiou P & Morgan KG (1991). Intracellular free Ca^{2+} is elevated in hypertrophic aortic muscle from hypertensive rats. *American Journal of Physiology*, 260: H507-H517.
40. Papageorgiou P & Morgan KG (1991). Increased Ca^{2+} signaling after α-adrenoceptor activation in vascular hypertrophy. *Circulation Research*, 68: 1080-1084.
41. Shibata S, Kuchii M & Taniguchi T (1975). Calcium fluxes and binding in the aortic smooth muscle cells of spontaneously hypertensive rat. *Blood Vessels,* 12: 279-289.
42. Asano M, Masuzawa-Ito K, Matsuda T, Imaizumi Y, Watanabe M & Ito K (1993). Functional role of Ca^{2+}-activated K^+ channels in resting state of carotid arteries from SHR. *American Journal of Physiology*, 265: H843-H851.
43. Kuriyama H & Suzuki H (1987). Electrical property and chemical sensitivity of vascular smooth muscles in normotensive and spontaneously hypertensive rats. *Journal of Physiology,* 285: 409-424.
44. Hermsmeyer K & Harder D (1986). Membrane ATPase mechanisms of K^+-return relaxation in arterial muscles of stroke-prone SHR and WKY. *American Journal of Physiology*, 250: C557-C562.
45. Lamb FS & Webb RC (1989). Regenerative electrical activity and arterial contraction in hypertensive rats. *Hypertension,* 13: 70-76.
46. Longhurst PA, Rice PJ, Taylor DA & Fleming WW (1988). Sensitivity of caudal arteries and the mesenteric vascular bed to norepinephrine in DOCA-salt hypertension. *Hypertension,* 12: 133-142.
47. Cheung DW (1984). Membrane potential of vascular smooth muscle and hypertension in spontaneously hypertensive rats. *Canadian Journal of Physiology and Pharmacology*, 62: 957-960.
48. Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Tanaka Y & Fujishima M (1992). Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circulation Research*, 70: 660-669.
49. Sachs F & Auerbach A (1983). Single channel electrophysiology: Use of the patch clamp. *Methods in Enzymology*, 103: 147-176.
50. Tokuno H & Tomita T (1987). Collagenase eliminates the electrical responses of smooth muscle to catecholamines. *European Journal of Pharmacology*, 141: 131-133.
51. Wilde DW, Furspan PB & Szocik JF (1994). Calcium current in smooth muscle cells from normotensive and genetically hypertensive rats. *Hypertension,* 24: 739-746.
52. Self DA, Bian K, Mishra SK & Hermsmeyer K (1994). Stroke-prone SHR vascular muscle Ca^{2+} current amplitudes correlate with lethal increases in blood pressure. *Journal of Vascular Research*, 31: 359-366.
53. Hermsmeyer K, Sturek M, Marvin W, Mason R & Puga A (1989). Vascular muscle Ca^{2+} channel modulation in hypertension. *Journal of Cardiovascular Pharmacology,* 14 (Suppl 6): 45-48.
54. Cox RH & Lozinskaya IM (1995). Augmented calcium currents in mesenteric artery branches of the spontaneously hypertensive rat. *Hypertension,* 26: 1060-1064.
55. Ohya Y, Abe I, Fujii K, Takata Y & Fujishima M (1993). Voltage-dependent Ca^{2+} channels in resistance arteries from spontaneously hypertensive rats. *Circulation Research,* 73: 1090-1099.
56. Ikeda S, Amano Y, Adachi-Akahane S & Nagao T (1994). Binding of [^{3}H](+)-PN200-110 to aortic membrane from normotensive and spontaneously hypertensive rats. *European Journal of Pharmacology*, 264: 223-226.
57. Hermsmeyer K, White AC & Triggle DJ (1995). Decreased dihydropyridine receptor number in hypertensive rat vascular muscle cells. *Hypertension,* 25: 731-734.