PHARMACOLOGICAL CHARACTERISATION OF CALCIUM CHANNELS IN VASCULAR SMOOTH MUSCLE FROM HYPERTENSIVE AND NORMOTENSIVE ANIMALS

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PHARMACOLOGICAL CHARACTERISATION OF CALCIUM CHANNELS IN VASCULAR SMOOTH MUSCLE FROM HYPERTENSIVE AND NORMOTENSIVE ANIMALS.

BY

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ABSTRACT

A consistent feature of hypertension is an increase in peripheral vascular tone which is ultimately controlled by intracellular calcium levels. Over the past decade, evidence has emerged indicating an abnormality in ion handling by vascular smooth muscle as a possible cause of the elevated vascular tone. Various lines of evidence have led to the suggestion that there is an increased calcium influx in hypertension. The present work attempted to characterise calcium influx through receptor operated (ROC) and potential operated channels (POC) in tail artery from SHR and WKY rats, using nifedipine, a calcium antagonist, as a probe.

Potassium induced responses, were significantly more sensitive to nifedipine than norepinephrine responses in both SHR and WKY. However there was no difference in sensitivity to nifedipine, of either potassium or norepinephrine responses, between SHR and WKY at both ED_{100} or ED_{50} levels of stimulation. These results suggest a normal role for POC and ROC function in SHR animals.

The calcium sensitivity of the vessels was higher in SH rats when activated by ED_{50} levels of norepinephrine but not potassium, suggesting ROC alteration. The study also suggest that submaximal (ED_{50}) levels of agonists should be used, in addition to ED_{100} doses of agonists. Low concentrations of nifedipine (0.05n*M*) significantly reduced calcium sensitivity in potassium (ED_{100}) activated arteries from SH, but not WKY rats. Higher concentrations of nifedipine were required to significantly reduce calcium sensitivity in norepinephrine activated vessels from

both SHR and WKY. Maximal calcium responses in norepinephrine (ED_{100}) activated SHR vessels were more resistant to nifedipine than WKY vessels. These results suggest alterations in POCs and ROCs.

No differential sensitivity to nifedipine was found in young 'prehypertensive' (5 week old) animals. At the 10-12 week age group, SH rats were more sensitive, compared to WKY, at high doses of nifedipine. Wistar normotensive rats were more insensitive to nifedipine than WKY rats. Surprisingly the differential sensitivity, to nifedipine, between SHR and WKY was reduced at the 20 week age group. These results suggest that enhanced calcium influx may not precede development of high blood pressure. As blood pressure increases calcium influx may sustain the high vascular tone, although direct evidence is still lacking.

Collectively the results presented provide further indirect, evidence for altered calcium channel function in hypertension.

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LIST OF ABREVIATIONS

ATP	Adenosine 5'-triphosphate
B_{MAX}	Maximum binding capacity
°C	Degrees celsius (centrigrade)
CA-TS	Calcium antagonists
CNS	Central nervous system
cpm	Counts per minute
Ci	Curie
DBP	Diastolic blood pressure
DSS	Dahl salt-sensitive rat
DSR	Dahl salt-resistant rat
dpm	Disintegrations per minute
K_d	Apparent equilibrium dissociation constant
EGTA	Ethylene glycol bis (β-aminoethylether)-*N*-*N*' tetracetic acid.
fmoles	Femtomoles
g	Centrifugal force
i.p.	Intraperitoneal
i.v.	Intravenous
kg	Kilogram
MAP	Mean arterial pressure
mg	Milligram

ml	Millilitre
mm	Millimetre
m*M*	Millimolar
M	Molar
n*M*	Nanomolar
NE	-(-)Norepinephrine
5'ND	5' Nucleotidase
Pa	Pascals
PDE_I	Phosphodiesterase Type I
PNS	Postnuclear supernatant
PSS	Physiological salt solution
rpm	Revolutions per minute
SHR	Spontaneously hypertensive rat
SBP	Systolic blood pressure
6-OHDA	6-hydroxydopamine
TPVR	Total peripheral vascular resistance
TRIS	Tris(hydroxymethyl) amino methane
µl	Microlitre
VSM	Vascular smooth muscle
WKY	Wistar Kyoto Rat

Chapter 1

INTRODUCTION

1.1. General Introduction.

Despite diagnostic advances, 95 % of patients with hypertension are classified as suffering from essential hypertension (Bergland, Anderson & Wilhelmsen, 1976) and the etiology of this disease is unknown. Hypertension develops as the result of some pathophysiological disturbance of the blood pressure control system but no single cause has been identified. This control system consists of several dynamic, integrated and interacting mechanisms in equilibrium, striving to maintain a normal flow-resistance relationship. Thus hypertension may develop as the result of the alteration of one or more processes - the 'mosaic theory' of hypertension (Page & McCubin, 1965). Many investigators have stated that the disease is multifactorial (Paul, 1977) with hereditary factors playing a role in its aetiology (Pickering, 1961). A cardinal hemodynamic feature of the disease is a sustained elevation in total peripheral vascular resistance (TPVR) (Frohlich, Tarazi & Dunstan, 1969, Lund-Johansen, 1977), with cardiac output remaining normal.

As vascular resistance is mainly determined by 'basal' (myogenic) tone (Mellander& Johansson, 1968), it follows that, in hypertension, the increase in vascular resistance is the result of an increase in tone of vascular smooth muscle

(VSM) of primary resistance blood vessels. The tone of VSM is exquisitely regulated at the structural, neural, humoral and intrinsic (myogenic) level. Increased blood pressure, secondary to elevated VSM tone, may thus result from

1. Increase in neural vasoconstrictor influence
2. Increase in humoral vasoconstrictor influence
3. Structural alterations of the blood vessels
4. Intrinsic alterations in the regulation of contractile properties of the VSM effector cell.

By definition, essential hypertension cannot be produced experimentally by surgical or other interventions. A number of animal models of hypertension have been developed in order to enable the causes for increased tone to be investigated in the laboratory. Since Smirk's introduction of a laboratory animal model of genetically- determined hypertension (Smirk, 1949), a variety of susceptible rodent strains have become available: the Okamoto-Aoki strain from Japan (Okamoto & Aoki, 1963), otherwise known as the spontaneously hypertensive rat (SHR), and the Milan strain, introduced by Bianchi and coworkers (Bianchi, Fox & Imbasciati, 1973). The Dahl Salt-Sensitive (DSS) strain was developed at the Brookhaven National Laboratories in 1962 (Dahl, Heine & Tassinari, 1962). This strain is genetically predisposed to develop hypertension but only if maintained on a high salt (NaCl) diet. Of these strains, the SHR (Okamoto & Aoki, 1963) usually in comparision to its normotensive control, the Wistar-Kyoto strain (WKY) has been the most extensively studied strain.

1.2. Aetiology of Hypertension in SHR.

Okamoto & Aoki (1963) initially isolated a colony of SHR at the Faculty of Medicine, Kyoto University, by selective inbreeding of Wistar males having high blood pressure with Wistar females having slightly higher pressure than normal. A pure inbred strain of the SHR was obtained in 1969 (Tanase, Suzuki, Ooshima, Yamori & Okamoto, 1970).

Blood pressure in this srain is characterised by an initial high cardiac output and normal TPVR (5 - 12 wk) and a subsequent decrease in cardiac output to normal but an increase in TPVR in the established phase (Albretcht, 1974; Preutitt & Dowell, 1978). It has been shown that the development of high blood pressure in SHR is a multifactorially inherited condition caused by an autosomal additive inheritance with at least 3 - 5 major genes acting together (Tanase, Suzuki, Ooshima, Yamori & Okamoto, 1970) in concert with multiple minor genes (Yamori, Ooshima & Okamoto, 1970).

Expression of hypertension in the SHR is primarily controlled by genetic factors, with environmental factors having a minor, but important, role (Yamori, 1983). Tanase *et al.*, (1970) reported that the genetic factors account for a major part of the elevated blood pressure, while environmental factors tend to have an additive or subtractive effect on the development and extent of blood pressure elevation.

1.2.1. Influence of neurogenic mechanisms.

As the autonomic nervous system is intimately involved in the control of arterial pressure, heart rate, muscle tone and circulating adrenaline levels, it is likely that it may play some part in the initiation or maintenance of elevated arterial pressure in hypertension. Evidence for such a role is provided from studies which show either prevention or delay in the development of hypertension by interventions which interfere with the sympathetic nervous system (Abboud, 1984, Brody & Zimmerman, 1976, Brody, Haywood & Torvin, 1980). Additional evidence is provided by studies which show hyperreactivity of blood pressure to environmental stimuli (see below).

Many investigations have shown altered neurotransmitter levels and enzyme activities in the central nervous system (CNS) during various stages in the development of hypertension (Nagaoka & Lovenberg, 1977, Nakamura & Nakamura, 1978, Saavedra, Grobecker & Axelrod, 1978), although it must be emphasised that some of these studies are conflicting. For example, dopamine β-hydroxylase activity has been reported to be increased (Nakamura & Nakamura, 1978) and decreased (Nagaoka & Lovenberg, 1977) in the locus coerulus of the SHR compared to the WKY.

Further evidence for neurogenic participation is provided by studies of the effects of sympathetic denervation. Depletion of CNS stores of norepinephrine (NE) by intraventricular injections of 6-hydroxydopamine (6-OHDA), in young

(6wk) SHR delayed the onset of hypertension for 12 weeks whereas similar treatment in adult SHR caused only a slight fall in blood pressure (Kubo & Hashimoto, 1978). Prevention, by intraventricular injections of 6-OHDA, of development of hypertension has also been reported, in SH rats (Finch, Haeusler & Thoren, 1972). In another study, 6-OHDA, administered both intravenously and intraventricularily, reduced central and peripheral levels of norepinephrine, but there was no significant effect on the development of hypertension in the SHR - only a slight attenuation in the rise of blood pressure was evident (Yamori, Yamabe, De Jong, Lovenberg & Sjoerdsma, 1972). Furthermore chemical denervation of SHRs and WKYs at birth does not prevent the blood pressure rising to levels above those of denervated WKYs (Schomig, Dietz, Pascher, Luth, Mann, Schmidt & Weber, 1978). Similar results have been obtained with the New Zealand strain of genetically hypertensive rat (Clark, Jones, Phelan & Devine, 1978). The use of anti-nerve growth factor has also been reported to prevent the development of hypertension in the SHR (Provoost & De Jong, 1978). Interestingly, monotherapy with guanethidine, which produces a more permanent and complete sympathectomy than anti-nerve growth factor apparently does not prevent the development of hypertension (Johnsson & Macia, 1979), however intervention with a combination of anti-nerve growth factor and guanethidine produces a long lasting hypotensive effect in the SHR.

Although the above studies suggest the involvement of central catecholaminergic systems in the development of hypertension, there are difficulties with this

interpretation, since catecholamines have diverse effects upon blood pressure regulation with both pressor and depressor mechanisms being present in the CNS.

Direct recordings of sympathetic activity have provided some conflicting results about the participation of neural mechanisms in the development and maintenance of hypertension in the SHR. Initial indication for an increased central sympathetic drive in SHR was provided by studies showing an elevated sympathetic activity in the splanchnic nerve (Okamoto, Nosaka, Yamori & Matsumoto, 1967). Significant increases in discharge, compared to normotensive controls have been reported in renal and splanchnic nerves (Judy, Watanabe, Henry, Besch, Murphy & Hockel, 1976, Rickstein & Thoren, 1979). Sectioning of the splanchnic nerve produced a fall in blood pressure in SHR greater than in control WKY rat (Iriuchijima, 1973), suggesting an increased sympathetic nerve activity. Furthermore, a positive correlation was found between renal sympathetic nerve activity and mean arterial blood pressures in hybrid SHR/WKY rats (Judy, Watanabe, Murphy, Aprison & Yu, 1979). In contrast, at the level of lumbar sympathetic innervation, basal sympathetic nerve activity was essentially the same in SHR and WKY, despite a significantly higher systemic arterial pressure (Lais, Schaffer & Brody, 1974). The decrease in neural activity after hexamethonium was identical in both SHR and WKY, sugesting that the amount of nerve traffic originating in the CNS of the SHR is equivalent to that in the control rat. In the same study the vascular resistance of the SHR was significantly higher than that of the control, even after bilateral lumbar sympathectomy.

The evidence, suggestive of an increased neural activity directly causing the increased blood pressure, is compelling, however it must be mentioned that most of the studies were carried out in anesthetised rats. If indeed an increase in the activity of sympathetic nervous system results in an increase in TPVR, then the extent of this component should be detectable with the use of ganglionic blockers.

An investigation of SHR rats, chronically instrumented with miniaturised pulsed dopplerflow probes by Touw and co-workers (1980) showed that, in conscious SHR, maximal ganglionic blockade with hexamethonium, resulted in equivalent reductions in regional vascular resistance (renal, mesenteric and hindquarter) and arterial pressure. The changes were not significantly different between SHR and WKY. The study indicated that in both young and old SHR, vascular resistance and arterial pressure are sustained at elevated levels by some mechanism other than neurally derived vasoconstrictor tone. Similar decreases, in SHR and WKY, after ganglionic blockade have also been reported by Kubo (1979).

Therefore, although neurogenic mechanisms appear to be involved in the development of hypertension in the SHR, increased TPVR, also appears to be both initiated and maintained by non-neurogenic mechanisms. The role of neural factors should be considered along with other factors such as genetic, behaviourial and environmental factors. It has been suggested that as far as the nervous system is concerned, the difference between SHR and WKY may be that cardiovascular responses to environmental stress are exaggerated in the SHR (Brody, Faber, Mangiape & Porter, 1984).

1.2.2. Environmental influences.

Environmental changes have been shown to affect development of hypertension in the SHR. Deprivation of sensory stimuli (Lais, Bhatnagar & Brody, 1974), by placing newborn SHR in a quiet dark room as well as social isolation (Hallback, 1975) retards the development of hypertension.

Conversely, immobilization (Kwetnacky, McCarthy, Thoa, Lake & Kopin, 1979), chronic stress as well as combined-visual stimuli, provoke a greater increase in the arterial pressure of SHR than normotensive WKY (Yamori, Matsumoto, Yanabe & Okamoto, 1969). The effect of increased thermal stress is a greater increase in the blood pressure of SHR, compared to WKY (Nakamura & Nakamura, 1978). Recent studies provide evidence for exaggerated sympathetic discharge in response to environmental stress in SH rats. Basal sympathetic discharge was only slightly higher in SHR than in WKY, however, a major difference observed was the increase in discharge seen in response to a blast of air on the face of the animal (Lundin & Thoren, 1982). In addition renal sympathetic nerve discharge was significantly increased in the SHR.

In summary, whilst an abnormality in the central and peripheral nervous system is suggested by the studies, these do not account fully for the rise in TPVR and vascular reactivity.

1.2.3. Structural adaptations.

The role of structural modifications in the development of hypertension has recently been reviewed (Webb, 1981, Folkow, 1978).

To account for the increased vascular resistance and reactivity, Folkow (1956) and coworkers (Folkow, Hallback, Lundgren, Sivertsson & Weiss, 1973) proposed that the increase in reactivity occurs secondary to structural changes in the resistance vessels, and that these changes are the result of vascular smooth muscle (VSM) hypertrophy in response to high blood pressure. Folkow has also suggested that in some forms of essential hypertension, including SHR, the increased resting tone of blood vessels can be explained entirely by structural modifications of the vessels (Folkow, 1978) with enlargement of the media of resistance vessels occurring at the expense of lumen diameter. In his comprehensive review (Folkow, 1978), he summarises his view, that in essential hypertension, arterial structural adaptations occur to keep T constant as P rises in the relationship described in Laplace's modified law $T = P.r/w$ (where T= tension per unit wall tension, P= regional transmural pressure, r= internal radius of the artery, w= artery wall thickness). From the equation it is clear that decreases in r/w can be the result of an increase in w or a decrease in r or both. An alternate way of looking at this is by considering the relationship of resistance to flow ($R \propto 1/r^4$), where R= resistance and r= internal radius of the vessel. It is quite obvious that a minute decrease in r will affect R by an exponential factor.

Hemodynamic evidence for structural changes can be found in studies which show that vascular beds of hypertensives demonstrate higher flow resistance when maximally dilated (Conway, 1963), steeper slopes of dose response curves to vasoconstrictor agents (Sivertsson, 1970) and an increase in the magnitude of the maximal vasoconstrictor response (Mulvany, Hansen & Aalkajaer, 1978).

The change in the flow resistance relationship is thought to be due to the thickening of the arterial wall to such an extent that it encroaches into the lumen, even when fully relaxed. This concept is supported by work done by Mulvany *et al.*, (1978). They measured the morphologic properties of resistance vessels in the mesenteric beds of SHR and normotensive rats. They found that the SHR rats had a 14% smaller lumen and a 40% thicker media compared to normotensive vessels. Upon histological examination they found that the SHR vessels had an extra smooth muscle layer compared to the controls.

It is not clearly known whether the increase in thickness is due to hyperplasia or hypertrophy. Whilst there is some evidence for hyperplasia (Webb, 1981), hypertrophy can also occur in the same animal. For example Daniel and co-workers (1984) report that in the renal and mesenteric vasculature of SHR, the thickness of the artery wall is significantly different from WKY. Whereas this increase in thickness is due to hyperplasia in muscular arteries, it is due to hypertrophy (mesenteric bed) and hyperplasia (renal bed) in elastic arteries.

Another type of structural alteration which has been proposed to contribute to

increased vascular resistance is rarefaction of resistance vessels. Rarefaction has also been reported in the resistance vessels (12 to 25 microns) in the cremaster muscle of SHR, but not WKY (Hutchins & Darnell, 1979). In rats, subjected to antihypertensive therapy, which prevented hypertension development, there was no reduction in the number of small arterioles. Haack, Schaffer and Simpson (1980) studied cutaneous microvessels in SHR, WKY and normal Wistar rats. They found that both SHR and WKY had significantly fewer third order arterioles than Wistar rats. There was no significant difference between SHR and WKY at this level. At the level of the fourth order arterioles, not only did SHR have significantly fewer arterioles than WKY and Wistar rats, but the number of arterioles was also significantly lower in WKY compared to Wistar rats despite their pressures being equal. The rarefaction could therefore be coincidental and not causal to the development of hypertension.

Since the structural change appears to be caused by an increase in arterial pressure (Folkow, 1982), the change is considered to be secondary and not primary. Evidence for this is provided by studies in which regional hypotension, produced by aortic obstruction, was followed, 2 - 3 weeks later, by reversal of structural changes (Folkow, Gurevich, Hallback, Lundgren & Weiss, 1971). However recent studies have provided some evidence that some structural changes occur prior to to development of hypertension. An increase in the cross sectional area of the media, of small muscular arteries, was found in pre-hypertensive (3 - 5 week) SHR. This change occurred after birth but prior to the development of hypertension (Daniel, Kwan, Lee & Smeda, 1984).

Whilst changes in the media lumen ratios would explain increased reactivity in flow resistance (perfused) studies, they do not explain a number of observations in both perfused preparations and *in vitro* isolated strips or ring preparations of VSM.

For example, in the perfused hindquarter preparation from SHR, the change in pressure produced by serotonin (5-HT) was greater than that produced by either norepinephrine or potassium (K^+) (Cheng & Shibata, 1980). A differential sensitivity to norepinephrine, vasopressin and barium chloride ($BaCl_2$) in perfused mesenteric beds isolated from SHR has been observed (Lais & Brody, 1978). Other results which cannot be explained by structural changes include, differences in reactivity of a number of vascular smooth muscle preparations from pre-hypertensive SHR (Lais & Brody, 1978, Swamy & Triggle, 1980a, Mulavny, Aalkajaer & Christensen, 1980) , differences in sensitivity of vascular smooth muscle to different stimulants (Haeusler & Finch, 1972, Collis, De Mey & Vanhoutte, 1980, McGregor & Smirk, 1970), vascular smooth muscle responses to non-physiological cations (Goldberg & Triggle, 1977), vascular reactivity differences seen in vessels protected from (Hansen & Bohr, 1975) or not exposed to high blood pressure (Greenberg & Bohr, 1975) and sensitivity differences which occur in nonvascular smooth muscle (Corbett, Goldberg, Swamy, Triggle & Triggle, 1980). Some of these studies are briefly discussed.

1.2.4. Functional alterations.

A number of studies, the results of which cannot be accounted for by structural changes, point to an intrinsic defect of the smooth muscle cell itself.

In perfused mesenteric beds of both genetic and renal hypertensive animals, the mean response to serotonin (5-HT) was 420 - 730% of the control level, whereas the responses to both NE and angiotensin were no more than 50% greater than the control level (McGregor & Smirk, 1970). They suggested that the differential augmentation of resistance, induced by two agonists probably indicated a functional alteration of the smooth muscle. Cheng and Shibata (1980) reported that NE and 5-HT induced changes in perfusion pressure in the hindquarter preparation from SHR were quantitatively and qualitatively different. Similar results of differential sensitivity were also reported by Haeusler and Finch (1972). They suggested that an enhanced excitation-contraction coupling could account for the higher maximal response to 5-HT. Later on they (Finch & Haeusler, 1974) showed that while the response to NE was elevated in hypertension, the response to calcium in depolarised mesenteric artery preparations was not. They put forward the view that a specific alteration in the adrenergic (α) receptor was a primary abnormality in VSM from hypertensive rats. Studies done on young 'prehypertensive' SHR and WKY rats by Lais and Brody (1978) indicated a lower threshold for NE responses but not for $BaCl_2$. There was no difference in $BaCl_2$ sensitivity in contrast to differences with NE responses. Such results suggest the possibility of a membrane alteration which resides in the linkage of the NE receptor occupation to excitation-contraction coupling.

Changes in sensitivity have been reported in tissues protected from hypertension. Hansen and Bohr (1975) studied vascular sensitivity in femoral artery strips which were protected from hypertension by chronic obstruction of the external iliac artery. The contralateral artery was untouched. They reported that changes in sensitivity to vasoactive agents were not altered in strips protected from hypertension. The threshold doses of agonists required to produce constrictor responses remained significantly lower in hypertensive animals. Goldberg and Triggle (1980) found that elevated vascular sensitivity to $LaCl_3$ persisted despite antihypertensive therapy prior to birth. The question arises as to the nature of the abnormality which gives rise to the observed sensitivity changes.

Recent research has provided evidence that a fundamental abnormality of the VSM, in hypertensives, resides in its cellular membrane (see review by Postnov & Orlowe, 1984). The membrane is the cell structure that is in the best position to sense environmental changes for which altered cellular activity may be neccessary. From a mechanistic view point, the environmental change can most readily induce a physical or chemical change in the membrane with which it is in contact. In view of the extensive functional variations amongst VSM and the factors that contribute to this variation (Bell, Webb & Bohr, 1984), it is not surprising that most of the factors that act on the membrane have been implicated in hypertension (Webb & Bohr, 1981). These include,

1. alterations in the membrane potential of VSM (Harder, Contney, Willems & Stekiel, 1981, Hermsmeyer, 1980, Harder & Hermsmeyer, 1983, Cheung, 1984),

2. alterations in the electrogenic pump (Webb & Bohr, 1979),

3. altered membrane permeability to ions (Noon, Rice & Baldessarini, 1978, Jones & Hart, 1975, Jones, 1982),

4. increased vascular sodium membrane permeability (Friedman, 1982),

5. decreased membrane stability, possibly due to abnormal Ca^{2+} binding (Jones, 1974, Zsoter, 1977, Holloway & Bohr, 1973).

Irrespective of the precise pathogenetic factors implicated in hypertension (neural, humoral, myogenic or any combination thereof), the common denominator at the cellular level is an alteration in cytosolic Ca^{2+} levels in VSM. Since Ca^{2+} plays a vital role in the contractile function of smooth muscles from tissues of varying structure and function, a dysfunction of its regulation may conceivably lead to an altered contractile state. Pharmacological, biochemical, both direct and indirect, and clinical studies have provided evidence for an altered Ca^{2+} regulation in hypertension (for reviews see Robinson, 1984, McCarron, 1985, Kwan, 1985b, Lau & Eby, 1985). An alternate theory is based on the concept of altered sodium homeostatis (Blaustein, 1977, Blaustein, 1984, MacGregor, 1985a). In the next section altered Ca^{2+} regulation is discussed.

1.3. Calcium And Smooth Muscle Function.

Vascular smooth muscle (VSM) is dependent on (Ca^{2+}) for activating the contractile apparatus (Filo, Bohr & Ruegg, 1965, Kuriyama, Yushi, Suzuki, Kitamura & Itoh, 1982, Prosser, 1974, Bohr, 1973, Van Breemen, 1977), just as other types of muscles are (Fuchs, 1974, Fleckenstein, 1977). It has been shown that the threshold for mechanical activation of the contractile proteins is of the order of $10^{-7}M$, and that full activation occurs at a free intracellular Ca^{2+} concentration (Ca^{2+}_{INT}) of about $10^{-5}M$ (Filo *et al.*, 1965). Since the free Ca^{2+}_{INT} content of VSM has been recorded to be approximately $10^{-7}M$ (DeFeo & Morgan, 1985), and the free extracellular calcium (Ca^{2+}_{EXT}) content about 1.5 X $10^{-3}M$, a large inward electrochemical gradient is present (Van Breemen & Loutzenhiser, 1980). This gradient of approximately 9300 cal/mol is poised to provide a ready source of activator Ca^{2+}. However the ions do not rush into the cells because the membrane permeability to Ca^{2+} is very low (Casteels & Van Breemen, 1975). Only during excitation contraction coupling can Ca^{2+} enter the cell to have any effect.

1.3.1. Calcium entry routes.

In electrically active smooth muscle, depolarisation of the plasma membrane towards threshold will initiate action potentials, which generate submaximal contractions. The concept thus evolved that there was a Ca^{2+} entry pathway that is activated by a decrease in membrane potential. Observations that exogenously applied agonists or transmural stimulation could produce contraction

of VSM without depolarising the muscle membrane brought about the concept of pharmacomechanical coupling (Somlyo & Somlyo, 1968a, Su, Bevan & Ursillo, 1964, Droogmans, Raeymakers & Casteels, 1977). Subsequently two groups (Bolton, 1979, Meisheri, Hwang & van Breemen, 1980) independently suggested that separate receptor operated (ROC) and potential operated (POC) Ca^{2+} influx pathways (channels) exist in smooth muscle cells. Evidence for their existence is based on the following points,

1. It has been shown that Ca^{2+} influx stimulated by 80m*M* K^+ and 10^{-6}*M* NE are additive when both modes of activation are applied simultaneously to rabbit aorta (Meisheri, Hwang & van Breemen, 1980).

2. The Ca^{2+} antagonists, D600, diltiazem and nifedipine, selectively inhibited Ca^{2+} influx, stimulated by high K^+ with a lesser effect on NE (10^{-5}*M*) induced influx (Cauvin, Cameron, Meisheri, Yamamoto & van Breemen, 1984b).

3. The Ca^{2+}channel agonist,Bay K 8644 (Schramm, Thomas, Towart & Franckowiak, 1983a, 1983b), stimulated aditional Ca^{2+} entry when added to rabbit aorta previously exposed to 10^{-5}*M* NE but not when added to those previously exposed to 80 m*M* K^+ (Cauvin *et al.*, 1984).

4. Finally a key piece of evidence is provided by the observation that agonists can produce Ca^{2+} entry into VSM without membrane depolarisation (Droogmans, Raeymakers & Casteels, 1977, Cauvin, 1985).

A third route of entry for Ca^{2+} is the 'leak pathway'. Whether this intrinsic Ca^{2+} leak plays a role in the activation of smooth muscle has not been fully investigated. Normally under relaxed conditions this leak is countered by effiçient Ca^{2+} sequestering and extruding processes which maintain Ca^{2+} levels below

below threshold. Under conditions of impaired Ca^{2+} homeostatic mechanisms the leak pathway may play a role in activation (Loutzenhiser & Van Breemen, 1983).

1.3.1.1. Receptor operated (ROC) and potential operated (POC) channels.

POCs can be activated upon depolarisation of the membrane by exposure to a high K^+ solution or subsequent to receptor occupation by an agonist. The latter may occur in tissues where agonists cause both contraction and membrane depolarisation as in the rabbit mesenteric vessels (Kubo & Hashimoto, 1978). It is generally accepeted that K^+-induced contractions depend more on an influx of Ca^{2+} (Frohlich, Tarazi & Dunstan, 1969, Meisheri, Hwang & van Breemen, 1980) than do NE responses, with the latter exhibiting varying dependencies.

In contrast to POC-mediated processes, agonist-induced responses may depend on two sources of Ca^{2+}, one internal, the other external: It has been shown that the initial (phasic) response to NE, in aorta, is less dependent on Ca^{2+}_{EXT} (Lipe & Moulds, 1983), than the tonic response. The slower washout of NE responses, in a 0 Ca^{2+} EDTA buffer, compared to K responses (Hudgins & Weiss, 1968), and the persistence of a response in presence of La^{3+}, all indicate that the agonist causes the release of an intracellular bound Ca^{2+} store which is limited in size (Van Breemen, Farinas, Gerba & McNaughton, 1972). The tonic response, which follows the phasic, has been attributed to the influx of Ca^{2+} from the extracellular space and presumably enters the cell via the ROC. The dependence of contraction of blood vessels from different regions upon extra or intracellular Ca^{2+} is variable,

with vessels of smaller diameter being more dependent on extracellular Ca^{2+} than larger ones like the aorta (Sutter, Hallback, Jones & Folkow, 1977, Mulvany & Nyborg, 1980, Cauvin, Saida & van Breemen,1984a, Loutzenhiser, 1985).

To summarise, Ca^{2+} may enter the cell through either POCs, ROCs, (or both) and by passive diffusion (see Figure. 1-1). The Na^{2+}/Ca^{2+} exchange system is not shown in Figure. 1-1 as its role is not clearly defined (see below).

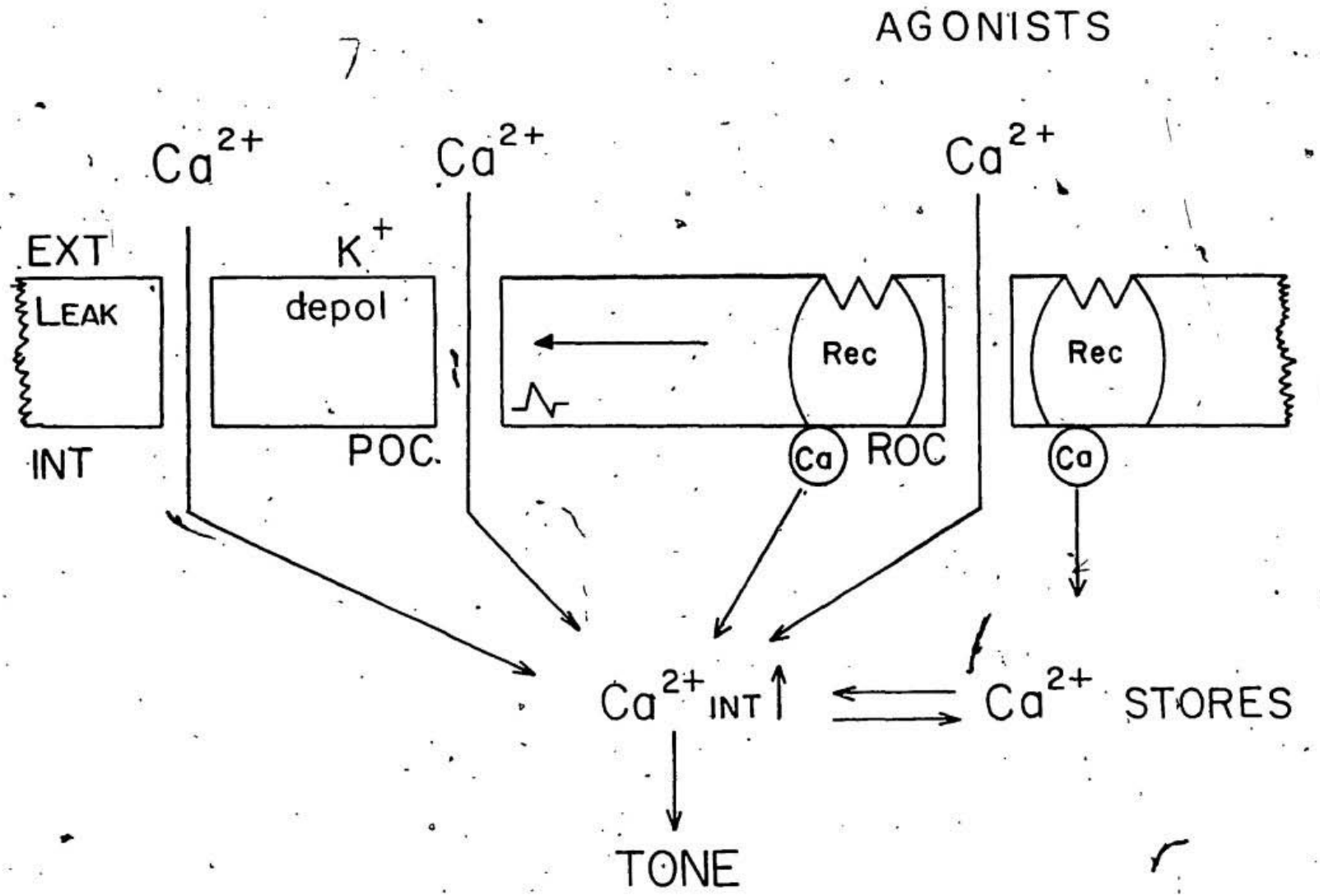
1.3.1.2. Calcium homeostatis: extrusion and intracellular stores.

In order to maintain Ca^{2+}_{INT} levels below threshold, there exist mechanisms which either 'pump' out Ca^{2+} or bind Ca^{2+} to intracellular organelles.

Two mechanisms have been suggested to exist for extrusion of Ca^{2+}. The first is an ATP-dependent active Ca^{2+} transport mediated by a calmodulin- regulated Ca^{2+}-ATPase (Morel, Wibo & Godfraind, 1981, Casteels, 1980, Grover, Kwan, Crankshaw, Crankshaw, Garfield & Daniel, 1980). The other is a Na^{2+}/Ca^{2+} exchange that derives its energy from the inwardly directed transmembrane gradient (Van Breemen, Aaronson & Loutzenhiser, 1979, Grover, Kwan & Daniel, 1981). The relative contribution of the two systems is not yet clearly known. The Ca^{2+}-ATP-dependent pump is the one that has been best characterised and is thought to be the major extrusion system. The role of the Na^{2+}/Ca^{2+} system cannot be clearly defined due to its low activity, compared to the Ca^{2+}-ATP-dependent pump, which present problems in its characterisation (Daniel, Grover & Kwan, 1982). For recent reviews see (Van Breemen, Aaronson

Figure. 1-1.

Digramatic representation of vascular smooth muscle membrane showing various Ca^{2+} entry routes.


AGONISTS
Ca2+
Ca2+
Ca2+
EXT
K+
LEAK
depol
Rec
Rec
INT
POC
Ca
ROC
Ca
Ca2+ INT
Ca2+ STORES
TONE

& Loutzenhiser, 1979, Daniel, 1985, Jones, 1980, Somlyo & Somlyo, 1983, Grover, 1985).

The operation of an ATP-dependent Ca^{2+} pump is essential for maintenance of steady state Ca^{2+} levels, given the influx of Ca^{2+} during a normal stimulus, however it is unlikely that the moment to moment regulation of relaxation is due to Ca^{2+} efflux, since relaxation can take place in the presence of La^{3+} and without a net change in cell Ca^{2+} (Meuller & Van Breemen, 1979). This is due to sequestration of Ca^{2+} by intracellular organelles. The endoplasmic reticulum is thought to be a major physiological site for Ca^{2+}_{INT} sequestration. Calcium accumulation by the ER is an energy-dependent process supported by ATP (Raeymaekers & Casteels, 1981). The ER may be the intracellular store which releases Ca^{2+}, upon stimulation of VSM by an agonist, and contribute to the initial phasic response.

Additional sites for sequestration may include the mitochondria, where affinity for Ca^{2+} is low but the capacity for binding is high, as well as passive binding to the internal aspect of the plasma membrane. Whereas both of these sites may act as stores, their role in regulating, under physiological conditions, Ca^{2+}_{INT} is not fully known. The passive binding of Ca^{2+} has been thought to have regulatory properties as regards membrane permeability to Ca^{2+} and other ions (Haeusler, 1983). Details of cellular Ca^{2+} homeostatis have been recently reviewed by Cavero and Spedding (1983).

1.4. Altered Cellular Calcium Handling.

Several studies have been conducted, the results of which are indicative of an altered cellular Ca^{2+} handling.

Increased myogenic activity has consistently been observed in arteries from hypertensive animals. These include studies on large arteries (Somlyo & Somlyo, 1968b, Noon, Rice & Baldessarini, 1978, Fitzpatrick & Szentivanyi, 1980) and small muscular arteries (Brann, Root & Halpern, 1980).

Noon *et al.*, (1978), observed that aortic strips from SHR, not WKY, relaxed in Ca^{2+}-free Locke's medium. Subsequent restoration of Ca^{2+} to the bathing media elicited increases in the resting tension of aorta from SHR only. In addition the relaxation rate in a Ca^{2+} free Locke's medium was faster in the SHR strips than in WKY. They interpreted their findings as resulting from a membrane, which was *leaky* to Ca^{2+}. Mochizuki, Yamamoto, Kondo, Aoki, Mizuno and Hotta (1979) reported that addition of Ca^{2+} to aortae placed in normal buffer caused an increase in basal tension, without any stimulant present, whereas tissues from WKY did not develop tension. Additional evidence comes from studies in which tissues from hypertensive animals respond to non-physiological cations such as La^{3+} (Shibata, Kurahuchi & Kuchii, 1973, Bohr, 1974, Goldberg & Triggle, 1977). Goldberg & Triggle (1977) reported that La^{3+} induced responses in aorta from SHR but not from WKY. This response persisted in a '0' Ca^{2+} buffer and in the presence of D600, a Ca^{2+} antagonist. Similar responses were also observed in non

VSM from hypertensive animals (Corbett, Goldberg, Swamy, Triggle & Triggle, 1980).

These studies are supportive of the the notion that membrane of VSM from SHR is leaky to ions such as Ca^{2+}. The increased permeability has been linked to an unstable VSM membrane, which is dependent upon Ca^{2+} binding to the membrane (Bohr, 1963). Bohr (1963) showed that, in isolated blood vessels, responses induced by NE or K^+ increased with increasing concentrations of Ca^{2+} up to a Ca^{2+}_{EXT} concentration of 2.0m*M* : beyond this, Ca^{2+} had an inhibitory effect. Hansen and Bohr (1975) found that more Ca^{2+} was required to inhibit responses to K^+ in SHR femoral artery strips than in WKY strips. This was interpreted as due to deficient binding of Ca^{2+} to the membrane ie. more Ca^{2+} was neccessary to stabilise the membrane in SHR. The membrane is now thought to be leaky not only to Ca^{2+} but to other ions also (Jones, 1974, Jones & Hart, 1975, Jones, 1982).

Attempts to measure Ca^{2+} fluxes in a variety of tissues from hypertensive rats utilising $^{45}Ca^{2+}$ have had varying results. In SHR aortae, compared to WKY, $^{45}Ca^{2+}$ uptake has been reported to be increased (Webb & Bhalla, 1976) or decreased (Zsoter, 1977), whereas the La^{3+} resistant Ca^{2+} influx is either decreased (Shibata *et al.*, 1975), or unchanged (Zsoter *et al.*, 1977).

Defects in the Ca^{2+}ATP dependent pump would tend to raise Ca^{2+}_{INT} levels. Since this pump extrudes Ca^{2+} any defect in this would manifest as a decrease in

the relaxation rate, upon removal of stimulant, of contracted vessels (Field, Janis & Triggle, 1972, Cohen & Berkowitz, 1976). Decreases in the activity of the pump, reflected as reduced Ca^{2+} uptake in SHR aortic microsomes, have been reported by several authors (Aoki, Yamamitsha, Tazumi & Hotta, 1974, Webb & Bhalla, 1976). Similar functional defects have been reported in plasma membrane fractions in both aorta (Wei, Janis & Daniel, 1976) and mesenteric arteries (Kwan, Belbeck & Daniel, 1979) from SHR rats and two models of renovascular hypertension (Kwan, Belbeck & Daniel, 1980). This defect exists prior to development of hypertension (Kwan *et al.*, 1979) and is also found in nonvascular smooth muscle (Shibata, Kuchi & Taniguchi, 1975, Kwan, 1985a).

Some studies, however, report faster relaxation in tissues from SHR: for example, Swamy and Triggle (1980b) found that relaxation of maximal NE induced reponse was faster than that of K^+ induced response in carotid vascular strips from SHR. Similar results were obtained in iliac vascular strips (Swamy & Triggle, 1980a). Shibata *et al.*, (1973) reported that whereas aortic strips from SHR and WKY showed similar responses to the relaxant effects of nitroglycerine and papaverine, removal of Ca^{2+}_{EXT} resulted in a faster decay rate of NE- and K^+-induced responses in SHR tissues only. Pedersen, Mikkelsen and Anderson (1978) found that the relaxation rate of NE-induced responses, in thoracic aorta from SHR, was less than that of K^+ induced responses, following washout of the stimulants. However the rate of relaxation in a zero Ca^{2+} buffer was greater in SHR tissues activated by NE. Such results could be due to changes in excitation-contraction

contraction coupling such that the utilization of Ca^{2+} is different in the hypertensive state. Changes in the dependency of NE and K^+ induced responses, on Ca^{2+}_{EXT} have been reported in aortae from SHR (Pang & Sutter, 1981, Pedersen, Mikkelsen & Anderson, 1978) and are thought to reflect changes in excitation contraction coupling (Folkow, Hallback, Jones & Sutter, 1977). This change in coupling could take place at either the ROC or POC (or both) since influx occurs through these two pathways.

Mulvany & Nyborg (1980) reported an enhanced Ca^{2+} sensitivity in resistance vessels, from both young and old SHR, but not WKY rats, when stimulated by maximal doses of NE. The concentration of Ca^{2+} required to elicit a half maximal response, in the presence of NE, was lower than that required for K^+. Since the tonic component of the NE response depends on the influx of Ca^{2+} through the ROC, they suggested that there may be an alteration in the ROC of the hypertensive tissues but no differences in the POC. Furthermore this sensitivity was found not to be affected by chemical denervation, which lowered blood pressure in both SHR and WKY, suggesting that the increased sensitivity to Ca^{2+} was probably due to intrinsic differences in the VSM membrane and not affected by blood pressure or neurogenic influences. This may indeed be the case, however, studies with a group of compounds, which inhibit Ca^{2+} influx, appear to offer some evidence that a change in the POC should also be considered.

1.5. Calcium Antagonists.

The description of the activity of the calcium antagonists (CATs) (also known as Ca^{2+} channel antagonists, slow channel blockers or Ca^{2+} entry blockers) owes much to the pioneering work of Fleckenstein who recognised the ability of a number of heterogenous compounds, namely verapamil and prenylamine (and subsequently nifedipine and diltiazem) to mimick the effects of Ca^{2+} withdrawal in cardiac tissue (Fleckenstein, 1964). It is now known that the CATs achieve their negative inotropic and coronary vasodilatory effect by blocking the slow Ca^{2+} current (Fleckenstein, 1977, Fleckenstein, 1983). These compounds are also potent smooth muscle relaxants (Fleckenstein, 1977, Triggle, 1984a).

In addition to their structural diversity they also exhibit tissue selectivity. For example verapamil has a greater negative chronotropic action on cardiac tissue than does nifedipine (a 1,4 dihydropyridine); the latter is selective more towards peripheral VSM. Diltiazem affects both cardiac and smooth muscles and is somewhat more selective for smooth muscle (see review by Henry, 1980).

Despite such differences they achieve their main effect by inhibiting Ca^{2+} influx. It is generally believed that the compounds exert their relaxant effect by inhibiting Ca^{2+} mobilization through POC and ROC. The observed high sensitivity of K^+-induced responses is in contrast to the variable sensitivity exhibited by agonist-induced responses (Triggle & Swamy, 1980, Cauvin, Loutzenhiser & Van Breemen, 1983). The observation that K^+ responses are more sensitive to CATs is

consistent with the depolarisation activating POCs. The varying sensitivity observed with agonist-induced responses probably reflects different Ca^{2+} mobilization routes for the response, which vary from tissue to tissue and vessel to vessel (Cauvin, 1985, Cauvin & van Breemen, 1985) and can be predicted to reflect the varying utilization of POCs, ROCs and internal sources of Ca^{2+}.

The exact mode of interaction of the antagonist with the channel is not known. The action of verapamil, at least in cardiac muscle, is known to be "use - dependent" (i.e. the potency of CATs is increased if the muscle is depolarised), and probably does not involve simple plugging of calcium channels (Hess, Lansman & Tsien, 1984, Reuter, 1983). Their main site of action is the membrane of the VSM as these drugs lose their potency in skinned muscle preparations (Janis & Scriabine, 1983). A number of reviews have been published (Stone, Antman, Miller & Braunwald, 1980, Cauvin, Loutzenhiser & Van Breemen, 1983, Katz, Hager, Messino & Puppano, 1984, Bou, Llenas & Massingham, 1984, Triggle, 1984a, Katz, 1985), which cover in more detail the pharmacology, mechanism of action and clinical applications of CATs, which cannot, for obvious reasons, be covered in this thesis.

Apart from their clinical applications, CATs have been used as tools to examine calcium channels in nerves and muscle (Reuter, Porzia, Kokubun & Prod'hum, 1985). It is studies with CATs that suggest a fundamental disorder in hypertension may be related to one main site of action, that is, the POC.

Clinical investigations of the CATs, especially nifedipine, have indicated that they are effective antihypertensives (Aoki, Mochizuki, Yoshida, Kab, Kato & Takikawa, 1978, Aoki, Kawaguchi, Sato, Kondo & Yamamoto, 1982, Pedersen, Christensen & Ramsch, 1980). These studies indicate a contrasting hemodynamic effect in that the blood pressure in hypertensives is reduced significantly, whereas blood pressure is virtually unchanged in normotensives. In animal models of hypertension, a similar differential sensitivity has also been observed, both in SHR (Ishii, Itoh & Nose, 1980) and Dahl Salt Sensitive hypertensive and normotensive rats (Sharma, Fernandez, Triggle & Laher, 1984). It has also been demonstrated that there is an enhanced vasodilation during calcium channel blockade with verapamil in human essential hypertension (Hulthen, Bolli, Amann, Kiowski & Buhler, 1982). This vasodilatory effect is apparently related directly to the level of the blood pressure. If the blood pressure is higher then the antihypertensive effect is greater (Buhler, Hulthen, Muller, Kiowski & Bolli, 1982, MacGregor, Markandu, Rotellar, Smith & Sagnella, 1983). Such results suggest that there may be an altered function of calcium channels in providing the raised intracellular calcium levels necessary to maintain the increased tone of VSM in hypertensives.

The suggestion, based on an enhanced Ca^{2+} sensitivity of mesenteric resistance vessels stimulated with NE, that it is, probably, the ROC which may be altered (Mulvany & Nyborg, 1980) reflecting either an increase in population or differing affinities for calcium in SHR does not seem to be in congruency with the observed high sensitivity of the K^+ induced responses to CATs.

1.6. Objectives.

The objectives were to, determine the sensitivity of NE- and K^+-induced responses in the rat tail artery to nifedipine at both ED_{50} and ED_{100} doses of stimulation. Previous studies in assessing sensitivity of agonists to CATs have used either maximal or supramaximal concentrations and it is documented that at such doses the observed sensitivity does not reflect the true sensitivity (Cauvin *et al.*, 1983).

I also wished to see if an enhanced sensitivity to Ca^{2+} was evident in the rat tail artery when stimulated at both maximal and submaximal doses of NE and K^+.

Iriuchijima (1980), showed that nifedipine, administered i.p., at a dose which produced a marked fall in blood pressure, in SHR, had no effect in the normotensive controls. Similar differences were seen in Dahl hypertensive rats in studies where nifedipine was acutely (i.v.) administered (Sharma *et al.*, 1984). Therefore it is of interest to study the acute effects of nifedipine in anesthetised SHR, WKY and Wistar rats.

Nitrendipine, an analog of nifedipine, has been tritiated and since its availability has been widely used for radioligand binding studies. Binding studies have revealed that these compounds bind with high affinity to VSM plasma membrane fractions (Triggle, Agrawal, Bolger, Daniel, Kwan, Luchowski & Triggle, 1983). This is supported by good correlation with IC_{50} values obtained from functional studies and by antagonism by other calcium antagonists (Bolger, Gengo,

Klockowski, Siegel, Janis, Triggle & Triggle, 1983, Triggle, 1984b). Nitrendipine is thought to bind to a part of the Ca^{2+} channel (Triggle & Janis, 1984) and it was the intention to see if binding characteristics (K_d and B_{max}) were any different between tail artery obtained from SHR and WKY rats.

The tail artery was chosen as it has some properties of resistance vessels (Cheung, 1982) and a smaller diameter compared to the large conduit vessels (eg. aorta) used in the majority of studies where effects of CATs were assessed.

Chapter 2

METHODS AND MATERIALS

2.1. Animals.

Male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY), age 9 weeks, were purchased from Charles River Canada Inc (St, Constant, Quebec). These rats were derived from the original National Institutes of Health stock obtained from Kyoto University where the strain was developed (Okamoto & Aoki, 1963). The animals were used for the *in vitro*, *in vivo* and radioligand binding studies.

The rats were housed in the animal quarters of the Faculty of Medicine, Memorial University of Newfoundland, under minimal disease conditions, three rats to a cage. The rooms had a 12 hour light, 12 hour dark, light cycle with controlled humidity and temperature.

2.1.1. Rat food and water.

At all times, the animals had free access to food and water. The SHR and WKY rats were fed Purina Rat Chow (Charles River Inc, Montreal).

2.2. Blood Pressure Monitoring.

2.2.1. SHR and WKY rats.

Systolic and diastolic blood pressures of these rats were directly measured by femoral artery cannulation just prior to sacrifice, for *in vitro* studies and for continuous measurement (*in vivo* study).

Rats were anesthetised with sodium pentobarbital (Somnotol. 35mg kg^{-1}. i.p.). The rats were then placed on a heating pad (37°C) which was thermostatically controlled by a control module (Harvard Apparatus). The femoral artery was exposed and cannulated with polyethylene tubing (PE 50). After cannulation, 0.5ml of heparinised saline (1% v/v) was injected into the artery, to prevent clotting at the tip of the cannula. Blood pressure was recorded, continuously, via a pressure transducer (Statham P23AA or P23ID) on a Beckmann Dynograph recorder (Model IR4111) or a Gould (Brush 220) recorder. The arterial cannula and the transducers were filled with heparinised saline.

2.3. Experimental Procedures.

2.3.1. In vitro sensitivity to nifedipine.

Anesthetised rats were sacrificed by cervical dislocation. The dorsal tail artery was exposed, carefully removed and placed in warm (37°C) oxygenated physiological salt solution (PSS). Under a magnifying glass, connective tissue adhering to the artery was removed carefully, care being taken not to stretch the artery.

After cleaning the artery, 2-3 mm ring segments of the proximal end of the vessel were cut. These rings were suspended between two 'L' shaped hooks.

These isolated preparations were suspended, in pairs, in 25 ml double jacketed organ baths containing Krebs bicarbonate buffer maintained at 37°C and aerated with 95% O_2 and 5% CO_2 gas mixture. The pH of this buffer was between 7.35 and 7.4. The tissues were subjected to a preload tension of 0.5g . One hook was anchored to a fixed point in the bath whilst the other was connected to a force transducer (Grass FT 03C). Isometric tension was recorded on either a Beckmann R-611 dynograph or a Grass 7D polygraph.

2.3.2. Calcium sensitivity.

Tail artery ring segments were used in this study and were set up exactly as described above (see section 2.3.1.).

2.3.3. In vivo sensitivity to nifedipine.

Animals were anesthetised with sodium pentobarbital (35 mg kg^{-1} i.p.) and the femoral artery cannulated, for blood pressure measurement as described above (see section 2.2.1). The femoral vein was cannulated with PE 50 tubing for drug infusion. Rats with systolic pressures equal to or above 170mm Hg (1 mm Hg=133.322 Pa.) (SHR) and systolic pressures equal to or below 140 mm Hg (WKY and Wistar) were used in this study. After cannulation, the blood pressure was allowed to stabilise before any drug was infused. Any clotting at the cannula tip was cleared by rapidly forcing heparinised saline (0.1ml) into the arterial cannula.

2.3.4. Radioligand binding assay.

2.3.4.1. Tissue preparation.

Tail arteries from old (>16 wk), male and female, SHR and WKY rats were removed after sacrifice by cervical dislocation and placed in ice cold (4°C) Tris-HCl (50m*M*) buffer. When acute denervation was done, tissues were placed in warm PSS (see sect. 2.4.4.3). The arteries were then fastidiously cleared of adhering fat and connective tissue. The tissues were blotted on filter paper and the weight determined using a Mettler balance (Model H54). After weighing, the tissues were placed in 10 volumes/gm wet weight tissue of ice cold Tris-HCl buffer and finely minced with scissors before homogenisation.

2.3.4.2. Homogenisation.

A crude homogenate of the tissue was prepared by homogenising the tissues with a Polytron PT20 (Brinkman Instruments, Westbury. N.Y.) homogeniser, giving two bursts of 14 seconds duration at a rheostat setting of 7, with 10 up and down strokes.

2.3.4.3. Membrane preparation.

The homogenate was then subjected to a modified stepwise differential centrifugation procedure (Kwan, Belbeck & Daniel, 1979), schematically shown in Figure. 2-1.

The homogenate was centrifuged at 1500 X g for 10 minutes, to remove nuclei, cell debris and unbroken tissue. The pellet was discarded and the post nuclear supernatant (PNS) centrifuged at 9000 X g for 15 minutes in order to sediment

mitochondria. The mitochondrial pellet was discarded and the supernatant subjected to a 105,000 X g spin for a 45 minute period. The supernatant from this spin was discarded and the pellet, containing the plasma membrane enriched microsomes, resuspended in about 2.5ml ice cold Tris-HCl buffer and gently homogenised to give a protein density of 0.1 - 0.35 mg/ml. Aliqouts of the PNS and the plasma membrane enriched microsomes were stored, at 4°C, for marker enzyme assays on the same day.

2.3.4.4. Enzyme marker assays.

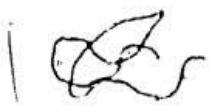
In order to assess the purity of the microsomes, enzyme marker assays were conducted on the PNS and the microsomes. 5'-Nucleotidase (5'ND) (Kwan, Belbeck & Daniel, 1979) and Phosphodiesterase type I (PDE_I) (Touster, Aronson, Dulaney & Hendrickson, 1970) are enzymes which mark for plasma membrane. 5'ND activity was assessed using a Sigma Diagnostics 5'ND reagent (Sigma Diagnostics Kit# 265-2). PDE_I activity was measured by determining the p-nitrophenol released from the ester substrate, thymidine 5'-monophosphate p-nitrophenyl ester.

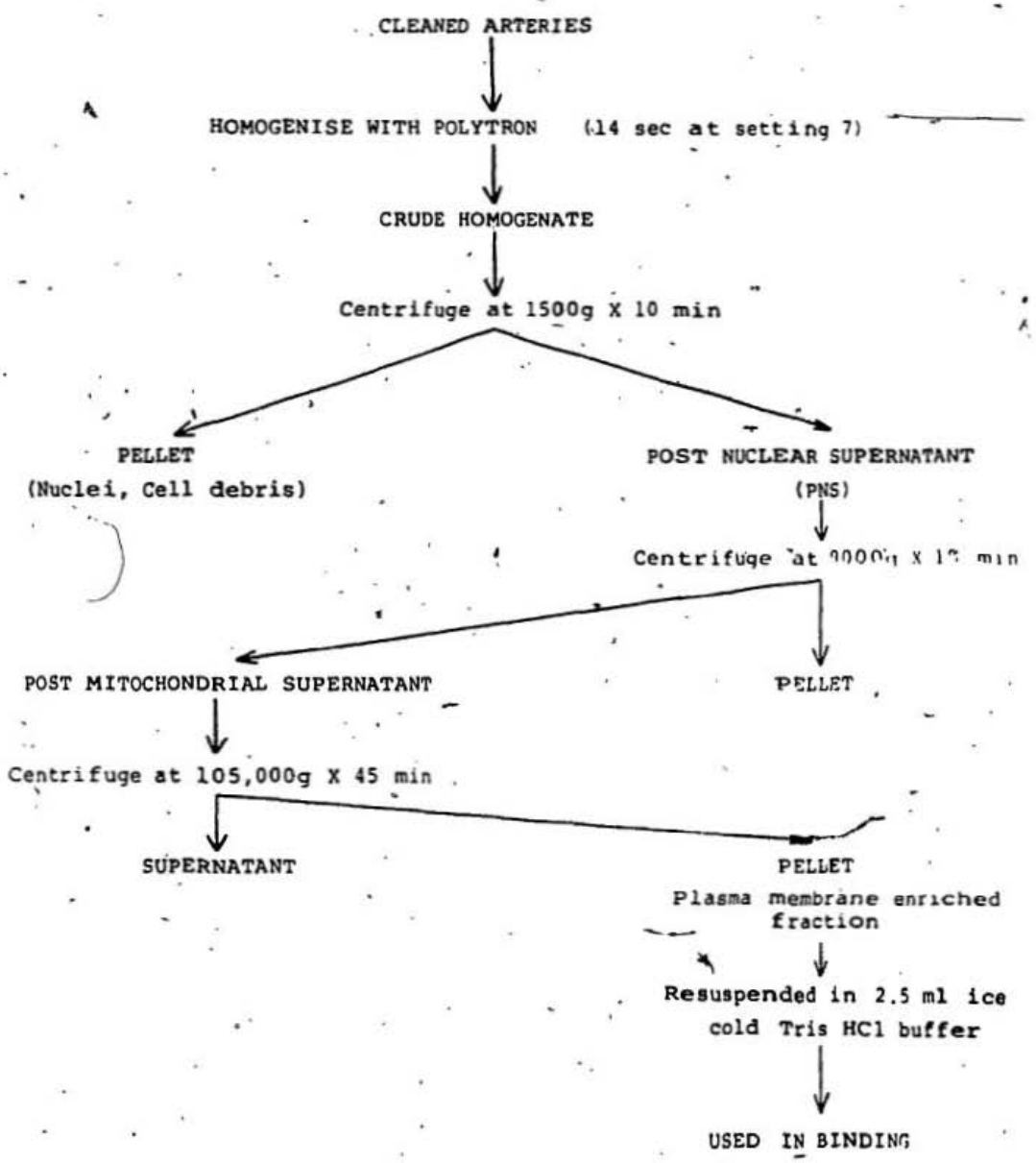
2.3.4.5. Protein determination.

The amount of protein in the plasma membrane enriched microsomes was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Figure. 21.

Schematic diagram of the differential centrifugation of rat tail arteries.




CLEANED ARTERIES
HOMOGENISE WITH POLYTRON (14 sec at setting 7)
CRUDE HOMOGENATE
Centrifuge at 1500g X 10 min
PELLET
(Nuclei, Cell debris)
POST NUCLEAR SUPERNATANT
(PNS)
Centrifuge at 9000g X 15 min
POST MITOCHONDRIAL SUPERNATANT
PELLET
Centrifuge at 105,000g X 45 min
SUPERNATANT
PELLET
Plasma membrane enriched fraction
Resuspended in 2.5 ml ice cold Tris HCl buffer
USED IN BINDING

2.4. Experimental Protocol.

2.4.1. In vitro sensitivity to nifedipine.

This study was performed with tissues from the following animals: 11-13 week old SHR (228 - 310g) and WKY (196 - 306g).

With the SHR and WKY rats only those animals which had systolic pressures above 160 mmHg (SHR) and below 130 mmHg (WKY) were used.

The initial protocol of this study was to equilibrate the tissues for a period of 90 minutes at a preload tension of 0.5g. During this period, the PSS was replaced every 15 minutes and the tissue adjusted such that it was subjected to a preload tension of 0.5g.

After the initial equilibration period a cumulative concentration-effect relationship (dose-response curve) to norepinephrine (NE) ($5 \times 10^{-9} M$ to $1 \times 10^{-4} M$) was determined. After allowing for a 60 minute recovery period, during which time the tissues were washed at least three times, the tissues were exposed to cocaine ($4 \times 10^{-5} M$) and propranolol ($1 \times 10^{-7} M$) for 20 minutes. These concentrations were previously shown to maximally inhibit, uptake I and β-receptor mediated relaxations (Triggle & Laher, 1985) in the rat tail artery. A second dose-response curve to NE was determined. From the second curve, ED_{50} (median effective concentration) and ED_{100} (maximal effective concentration) values of NE were extrapolated. After a further 60 minute recovery period (with

at least three washes) the same concentrations of cocaine and propranolol were added for 20 minutes and responses to either ED_{50} or ED_{100} obtained. The response was recorded for a period of 10 minutes, after which the tisuues were washed. The whole procedure was repeated until the responses were consistent (usually by the third response). These responses were taken as controls. Nifedipine (bath concentration in a range of $1 \times 10^{-10} M$ to $1 \times 10^{-7} M$) was added to the organ baths, for a period of 30 minutes. The baths were covered with aluminium foil and the experiment conducted in subdued light conditions. At the end of the 30 minute nifedipine incubation period, and in the presence of cocaine and propranolol, the tissues were stimulated with either their respective ED_{50} or ED_{100} levels of the agonists. The mechanical response was recorded for a period of 10 minutes. The tonic component of this response was measured, in mg tension, at the 10 minute period and related to the tonic response, after 10 minutes, in the control responses.

A similar protocol was used for responses to potassium (K^+). A dose- response curve was determined to K^+ by replacing the PSS with PSS containing 10 to 80 mM K^+. The depolarising buffers were all isotonic (by equimolar substitution of Na^+ by K^+). A second dose-response curve was obtained and the ED_{50} and the ED_{100} levels determined. After a 60 minute recovery period (with at least three washes), the tissues were stimulated with either ED_{50} or ED_{100} of K^+. This was done at 1 hour intervals until consistent responses were obtained. Nifedipine was added to the organ baths, one concentration per bath, for a period of 30 minutes

and the response to either ED_{50} or ED_{100} re-determined. The tonic response, 10 minutes after stimulation, was related to the response in absence of nifedipine. These studies were conducted in the presence of phentolamine ($1\mu M$), in order to block the effect of NE released from the nerve terminals by the high K^+ depolarising buffer.

2.4.2. Calcium Sensitivity

This study was performed on tissue from 11 - 13 week old male SHR (208 - 250g) and WKY(220 - 250g) rats. Only those animals with systolic pressures above or equal to 160mmHg (SHR) and below or equal to 130mmHg (WKY) were used.

Tail artery ring segments were set up as described above. The protocol employed was similar for determining the ED_{50} and ED_{100} values. After consistent responses were obtained the tissues were quickly washed twice with 'Ca^{2+}-free PSS' and then maintained in '0 Ca^{2+} EGTA PSS' containing phentolamine (K^+ responses) or cocaine and propranolol (NE responses) for a period of 20 minutes. The tissues were then stimulated with their respective agonists. NE was added to the bath in presence of '0 Ca^{2+} EGTA PSS' and the response, if any, recorded. K^+ induced responses were obtained by replacing the '0 Ca^{2+} EGTA PSS' with an isotonic depolarising PSS containing neither Ca^{2+} nor EGTA. The tissues were then washed 3 - 4 times with '0 Ca^{2+} EGTA PSS' to mop up Ca^{2+} being extruded from the cell.

To assess the calcium sensitivity, the tissues were then maintained in 'Ca^{2+}-free

PSS' for 10 minutes and again activated with NE or K^+ at ED_{50} or ED_{100}. After 5 minutes responses were obtained to cumulative addition of Ca^{2+} ($5 \times 10^{-5} M$ - $1 \times 10^{-2} M$). Responses were normalised to the maximum response.

The effect of nifedipine on calcium responses was assessed by a similar protocol. After control response curves were obtained, the tissues were exposed to varying concentrations of nifedipine, one concentration per bath, and the calcium responses redetermined. For this study, tissues were activated with maximal doses of either NE or K^+. The response to calcium in presence of nifedipine was related to the maximum response obtained in the control curve.

2.4.3. In vivo sensitivity to nifedipine

This study was performed in male SHR, WKY and Wistar rats at three age groups: 5 week old male SHR(69-91g) and WKY(75-83g), 11-13 week old SHR (332-370g), WKY (231-290g) and Wistar (332-370g) and in 20 week and older SHR (317-400g) and WKY (345-405g). Animals with systolic pressures above 160 mmHg (SHR) and below 130 mmHg (WKY and Wistar) (11-13 and >20 week old rats) were used in this study.

Prior to nifedipine infusion, control responses to the vehicle, which contained 30-35% ethanol (v/v), 5% glucose (v/v) and 1% Tween 80 (v/v), were obtained thrice, at 5 minute intervals. Attempts to dissolve nifedipine in a vehicle containing about 10% ethanol were unsuccessful. The 30% ethanol (v/v) was the minimum amount required, or the drug would precipitate out.

The effect of vehicle was to increase pressure, immediately after infusion, by less than 10% of the resting pressure (both systolic and diastolic).

Nifedipine was diluted from a stock solution (100% ethanol) to the required concentration with the vehicle prior to infusion. The drug was infused into the venous cannula in volumes of less than 0.10 ml, using a Hamilton Syringe, and cleared with saline (0.15 ml). The length of the cannula was such that the volume of the drug, in that length, would be about 0.10 ml, thus ensuring that all of the drug would be cleared from the cannula. The immediate fall in pressure was noted and related to the pre-infusion pressure. After 5 minutes the second dose of nifedipine was infused and the pressure change related to the pressure prior to the second dose. In this manner, nifedipine was infused, at 5 minute intervals at the following doses: 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 0.8 and 1.0 mg Kg^{-1}, and the pressure drop related to the pressure prior to each dose.

All the infusions were carried out in subdued light conditions and nifedipine was kept in aluminium foil covered vials.

2.4.4. Radioligand binding assay.

2.4.4.1. Binding assay.

Radioligand binding was done in the plasma membrane enriched microsomes. All assays were conducted in duplicate and were performed on the same day the microsome was prepared.

Membrane protein (15 - 42μg) was incubated in a total volume of 2ml incubation medium containing Tris-HCl (50 m*M*) buffer (pH 7.4) at 25°C and increasing concentrations of ^{3}H-nitrendipine (0.05 - 1.25 n*M*), for 45 minutes. ^{3}H-Nitrendipine was freshly diluted from stock solution with ice cold Tris-HCl buffer. The incubation was carried out in a gyratory shaker water bath (25°C).

The reaction was terminated by rapid filtration of the mixture under vaccum through Whatman GF/B filters positioned on a 12 hole cell harvester (Brandel Cell Harvester, Maryland.). The filters were washed twice, under vaccum, with 5 ml portions of ice cold Tris-HCl buffer. The filters were placed in scintillation vials and dried overnight at 37°C. 10 mls of aqeous scintillation cocktail (Formula 963. NEN Research Products, Boston) was added to the vials and left to equilibrate for 1 hour at room temperature. The filters were then counted in a Brinkman (LS 9000) liquid scintillation counter at an efficiency of 44% determined against external standards.

Nonspecific binding was determined by the addition of $2.5 \times 10^{-6} M$ unlabelled nifedipine to a duplicate set of tubes. Nonspecific binding was subtracted from total binding to tissues and filters to obtain specific binding.

Saturation radioligand binding curves were analysed by the method of Scatchard (1949), where the linear regression lines are drawn by the least square methods, on an Apple IIe computer using a standard Scatchard analysis package (Tallarida & Murray, 1981) From the above, the K_d(n*M*) of the radioligand and the B_{max}(fmol mg^{-1} protein) were determined.

2.4.4.2. Enzyme Marker Assays

5'ND activity was assessed by an enzyme kinetic method as described by Arkesteijn (1976) using a Sigma Diagnostics 5'ND Kit (# 265-2). The method detects the rate of formation of nicotinamide adenine dinucleotide (NAD), which produces a decrease in absorbance at 340nm. A 100μl microsome aliquot was added to equilibrated (at 30°C) 5'ND reagent (0.65 ml) and the decrease in absorbance at 340nm measured on a narrow bandwidth (dual recorder) spectrophotometer (Model. Beckmann. Model 25). PDE_I activity was measured in a total volume of 1.1ml containing 0.9ml of 0.1M glycine buffer (pH 9.02) with 10μl of 2m*M* ester substrate (thymidine. 5'-monophosphate p-nitrophenyl ester). To initiate the reaction, 100μl of PNS or microsome was added. The rate of increase in absorbance due to liberated p-nitrophenyl was determined on the same spectrophotometer as for the 5'ND activity but at 450nm and 37°C.

2.4.4.3. 6-Hydroxy Dopamine (6-OHDA) Treatment

Since ^{3}H-Nitrendipine is known to bind to neuronal tissue, we wished to know whether acute denervation of the nerve terminal would have any effect on binding. The arteries were denervated *in vitro*, using the method of Aprigliano and Hermsmeyer (1976a) . Cleaned tail arteries were exposed to a 300μg ml^{-1} concentration of 6-OHDA dissolved in an unbuffered electrolyte solution (PSS but $NaHCO_3$ and KH_2PO_4 omitted), containing 20μM glutathione, (which acts as an antioxidant), to bring the pH down to 4.9, for a period of 10 minutes. The tissues were then allowed to recover in PSS (pH 7.4), bubbled with 95% O_2-5% CO_2, for a period of at least 2 hours, when they were used for binding. Pilot studies had confirmed that after such a treatment there was no response to either field stimulation or exogenous tyramine.

2.5. Composition of buffers.

The physiological salt solution (PSS) used in the *in vitro* studies was a Krebs bicarbonate buffer of the following composition (millimolar):

NaCl	118
KCl	4.7
$NaHCO_3$	12.5
$CaCl_2.2H_2O$	1.8
KH_2PO_4	1.2
$MgCl_2$	1.2
Dextrose	11.1

The 'Ca^{2+}-free PSS' used in the calcium sensitivity study was the same as above with $CaCl_2$ omitted. '0 Ca^{2+} EGTA PSS' was the same as above except that

tetracetic acid (EGTA) was included. 'Ca^{2+}-free-K-PSS' contained known amounts of K^+ equivalent to ED_{50} and ED_{100}.

The unbuffered electrolyte solution was the same as above except that $NaHCO_3$ and KH_2PO_4 were omitted.

Tris-HCl (50mM) was made by dissolving 12.114 gm of Tris (Tris [hydroxymethyl] aminomethane) in 2 L of double distilled water and bringing the pH to 7.4 by the addition of concentrated HCl.

2.6. Drugs and Chemicals.

Nifedipine was generously donated by Bayer Pharmaceuticals, W. Germany and was dissolved in 100% ethanol, as stock solution and diluted as required. [^{3}H]-Nitrendipine, 2,6-dimethyl-3-carboethoxy-5-carbomethoxy(^{3}H)-4-3(3-nitrophenyl)-1, 4-dihydropyridine (specific activity, 74.4 Ci/mMol: 1 Ci = 3.7 x 10^{-10} Becquerels), was purchased from New England Nuclear (Boston, MA). It was stored, protected from light at -20°C, and used within 2 months of purchase.

l-Norepinephrine hydrochloride (-Arterenol), purchased from Sigma Chemical Co, St, Louis, was dissolved in 0.01N HCl as a $10^{-1}M$ stock solution. This stock was diluted as required in deionised water and kept on ice. All other drugs were purchased and dissolved in deionised water: Cocaine HCl (BDH Chemicals, Toronto), Phentolamine (Regitine HCl. Ciba Geigy Corp, NJ), Tyramine (Sigma, St, Louis) and 6-hydroxydopamine (Sigma, St, Louis).

Other reagents (and sources) were as follows:

Bovine Serum Albumin(BSA)	Sigma Chem Co. St. Louis. MO.
Calcium Chloride($CaCl_2 2H_2O$)	J.T.Baker Co. Phillipsburg. NJ.
Dextrose (D-Glucose)	BDH Chemicals. Toronto.
EGTA	Sigma Chem Co. St, Louis. MO
Glutathione (Reduced)	Sigma Chem Co. St. Louis. MO.
Heparin (Sodium Injection)	Allen & Hanbury's. Montreal.
Hydrochloric Acid	Fischer Scientific.
Magnesium Chloride($MgCl_2$)	J.T.Baker Co. Phillipsburg. NJ.
Potassium Phosphate(KH_2PO_4)	J.T.Baker Co. Phillipsburg. NJ.
Sodium Bicarbonate($NaHCO_3$)	J.T.Baker Co. Phillipsburg. NJ.
Sodium Chloride(NaCl)	J.T. Baker Co. Phillipsburg. NJ.
Sodium Pentobarbital (Somnotol)	M.T.C. Pharmaceuticals. Hamilton.
Tris-HCl	Sigma Chem Co. St, Louis. MO.
Tween 80	J.T. Baker Co. Phillipsburg. NJ.

2.7. Statistical Analysis.

Where indicated, results are expressed as the mean ± 1 standard error of the mean (S.E.M).

The IC_{50} values (concentration of nifedipine which inhibits the maximum response by 50%) for the *in vitro* nifedipine sensitivity study were determined by linear regression analysis as outlined by Sokal and Rohlf (1969).

The calcium sensitivity study was analysed by determining the ED_{50} values for the calcium dose-response curves by probit transformation. The means, here, are expressed as the geometric means. Tests of significance (Student's t test) were done on the log ED_{50} values and the geometric means rather than the arithmetic means (Fleming, Westfall, De la Lande & Jellet, 1972) using a statistical analysis package (Tallarida & Murray, 1981) on an Apple IIe computer.

The *in vivo* data were tested for differences by using the Student's t test and in the case of the 10 - 12 week age group, the means were compared, at each dose level by a oneway analysis of variance. Differences were considered to be significant at the $P < 0.05$ level (Scheffe's test).

Chapter 3

RESULTS

3.1. In Vitro Sensitivity to Nifedipine.

3.1.1. Blood pressures of rats.

The blood pressures (mm Hg) of male, 10 - 12 week old SHR and WKY rats are shown in Table. 3-1. The systolic (185.0 ± 4.8), diastolic (144.0 ± 4.1) and mean arterial pressure (MAP) (158.5 ± 3.8) of SHR were significantly different ($P < 0.05$) from corresponding values obtained in WKY control rats.

3.1.2. Effect of cocaine on NE dose response curve.

In tail artery ring preparations, from WKY, and in the absence of cocaine, the mean ED_{50} for NE ($7.6 \times 10^{-7} \pm 1.44M$) was not significantly different from the mean ED_{50} in SHR tissues ($1.41 \times 10^{-6} \pm 1.37M$). In both WKY and SHR, cocaine caused a leftward shift in the dose- response curve (Figure. 3-1) for NE. The ED_{50} values for NE in the presence of cocaine were not significantly different between SHR and WKY (Table. 3-2). These values were also not significantly different when compared to the pre-cocaine ED_{50} values. It should be mentioned that these studies were done on a limited number of tissues and all other tissues were subsequently exposed to $4 \times 10^{-5}M$ cocaine from the the very begining. The NE dose-response curve for all tissues are shown in Figure. 3-2. The potassium (K^+)

dose-response curve for all tissues are shown in Figure. 3-3. In both cases, the WKY tissues were more sensitive than the SHR tissues, but this sensitivity difference was not significant (Table. 3-3).

3.1.3. Characteristics of responses.

The responses to maximal (ED_{100}) stimulation by NE ($5 \times 10^{-5} M$) was characterised by a fast transient phasic component and a slow tonic component which was sustained for at least 10 minutes. The response to a submaximal (ED_{50}) concentration of NE was smaller in magnitude, characterised by a phasic component which took longer to reach maximum than the corresponding response in the ED_{100} stimulated tissues. The tonic component of this response was also sustained for 10 minutes although it tended to diminish (fade) somewhat and was not as well maintained as the tonic component in the ED_{100} stimulated tissues (Figure. 3-4). Exposure of rat tail arteries, in the presence of the α-adrenoceptor blocker, phentolamine, to an isotonic 60 mM K^+ (ED_{100}) solution induced a biphasic contraction, consisting of an initial fast, transient component and an ensuing slow, tonic component, which in some tissues had a tendency to diminish somewhat over the ten minute period. The response in tissues stimulated at the ED_{50} (K^+) level was less in magnitude and a slower development of the phasic component was evident. The tonic component was, like the ED_{50} response to NE, less well maintained.

3.1.4. Effect of nifedipine on NE and K^+ induced responses.

Nifedipine inhibited the responses of the tail artery when stimulated by either NE or K^+. Sample tracings of the effect of nifedipine on responses, elicited by NE and K^+ are shown in Figure. 3-5. From these it is apparent that the sensitivity of vessels stimulated by K^+ is greater than that of vessels activated by NE.

The $IC_{50}M$ values are shown in Table. 3-4. From the IC_{50} values it is apparent that the inhibitory effect of nifedipine was more pronounced in the vessels which were stimulated by K^+. The sensitivity of the vessels activated by NE were significantly ($P < 0.05$) lower (at least one log unit) than K^+ activated vessels. K^+ activated vessels ($IC_{50} = 3.9 \times 10^{-10} M$) from SHR animals appeared to be even more sensitive(74 times more) to nifedipine than NE activated vessels ($IC_{50} = 2.9 \times 10^{-9} M$) at ED_{100} levels.

For vessels activated by NE there was no significant difference between the sensitivities to nifedipine when stimulated at ED_{100} or ED_{50} levels.

In SHR vessels activated with K^+, the nifedipine sensitivity was higher (about 3 fold) at ED_{100} ($IC_{50} = 3.9 \times 10^{-10} M$), than at ED_{50} ($IC_{50} = 1.1 \times 10^{-9} M$) $\times 10^{-10}$), however this difference was not significant. Vessels from WKY animals exhibited almost similar IC_{50} values.

There were no significant differences in nifedipine sensitivity between SHR and WKY tail artery activated with either NE or K^+.

Table 3-1: Systolic, Diastolic and Mean Arterial Pressure of 10 - 12 week old male SH and WKY rats.

	SYSTOLIC mm Hg	DIASTOLIC mm Hg	MAP mm Hg
WKY (n=17)	116.2 ± 3.6	86.0 ± 4.8	96.0 ± 4.0
SHR (n=17)	185.0 ± 4.8[a]	144.0 ± 4.1[a]	158.5 ± 3.8[a]

MAP = (Systolic pressure + 2 Diastolic Pressure)/3

a = Significant difference ($P < 0.05$) (Students t test) between WKY and SHR.

Figure. 3-1.

Effect of cocaine on norepinephrine dose-response curves in 10-12 wk old male SH and WKY rats. Tissues were exposed to cocaine (40μM) for a period of 20 minutes. Points are the mean (± S.E.M.) of n=7 tissues in both strains.

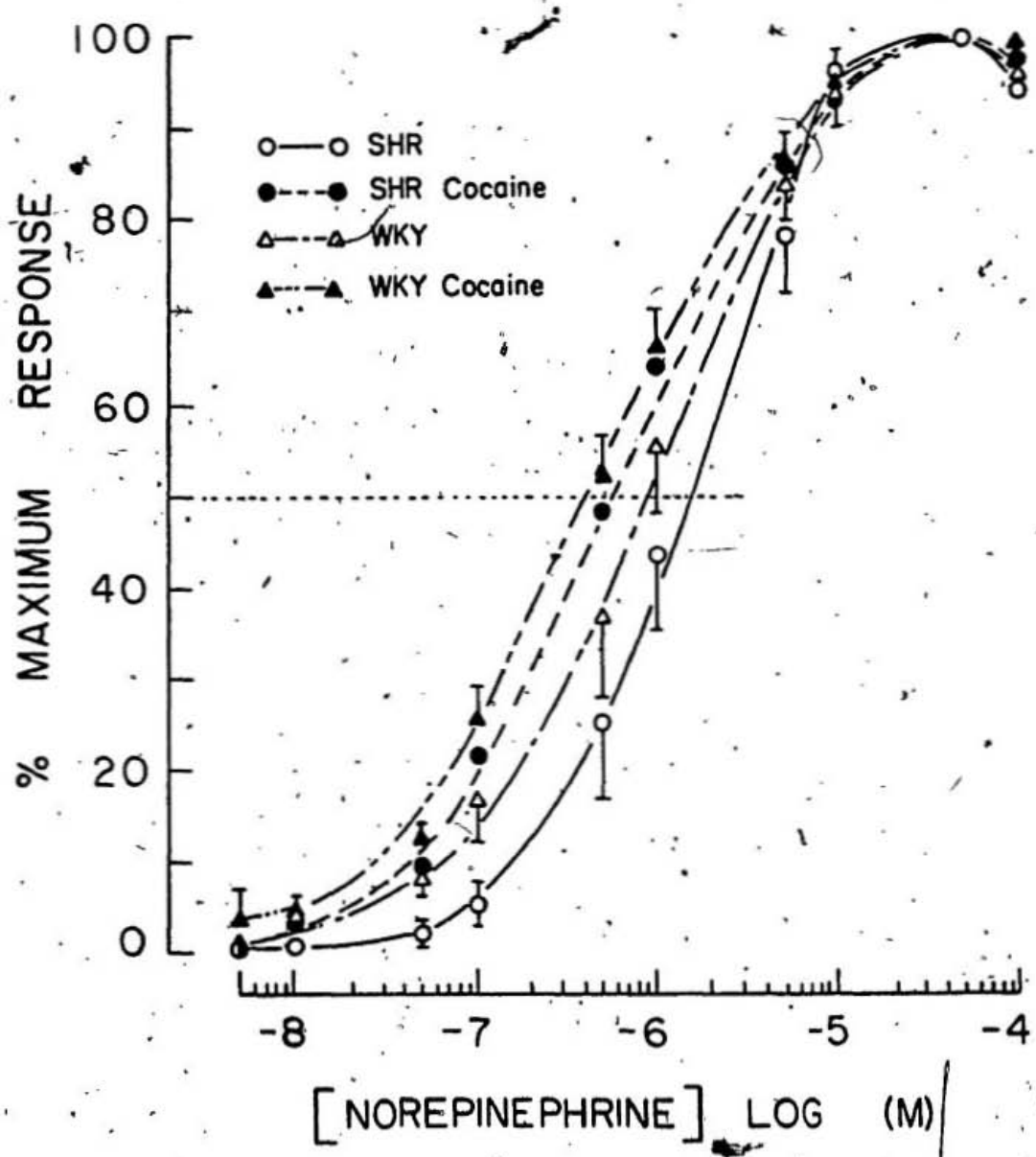

100
80
60
40
20
0
% MAXIMUM RESPONSE
SHR
SHR Cocaine
WKY
WKY Cocaine
-8
-7
-6
-5
-4
[NOREPINEPHRINE] LOG (M)

Table 3-2: Effect of cocaine on norepinephrine ED_{50} values in SH and WKY rats.

	NE ED_{50} (μM)	
	PRE COCAINE	POST COCAINE
WKY (n=7)	0.75 ± 0.14	0.52 ± 0.12
SHR (n=7)	1.41 ± 1.37	0.67 ± 0.15

ED_{50} values are Geometric Means (± S.E.M.).

Figure. 3-2

Dose-response curves to norepinephrine in 10-12 wk old male SH and WKY rats. These were obtained in tissues exposed to cocaine (40μM) for a period of 20 minutes. Points are the means ($\pm$ S.E.M.) of at least n=30 tissue in both strains.

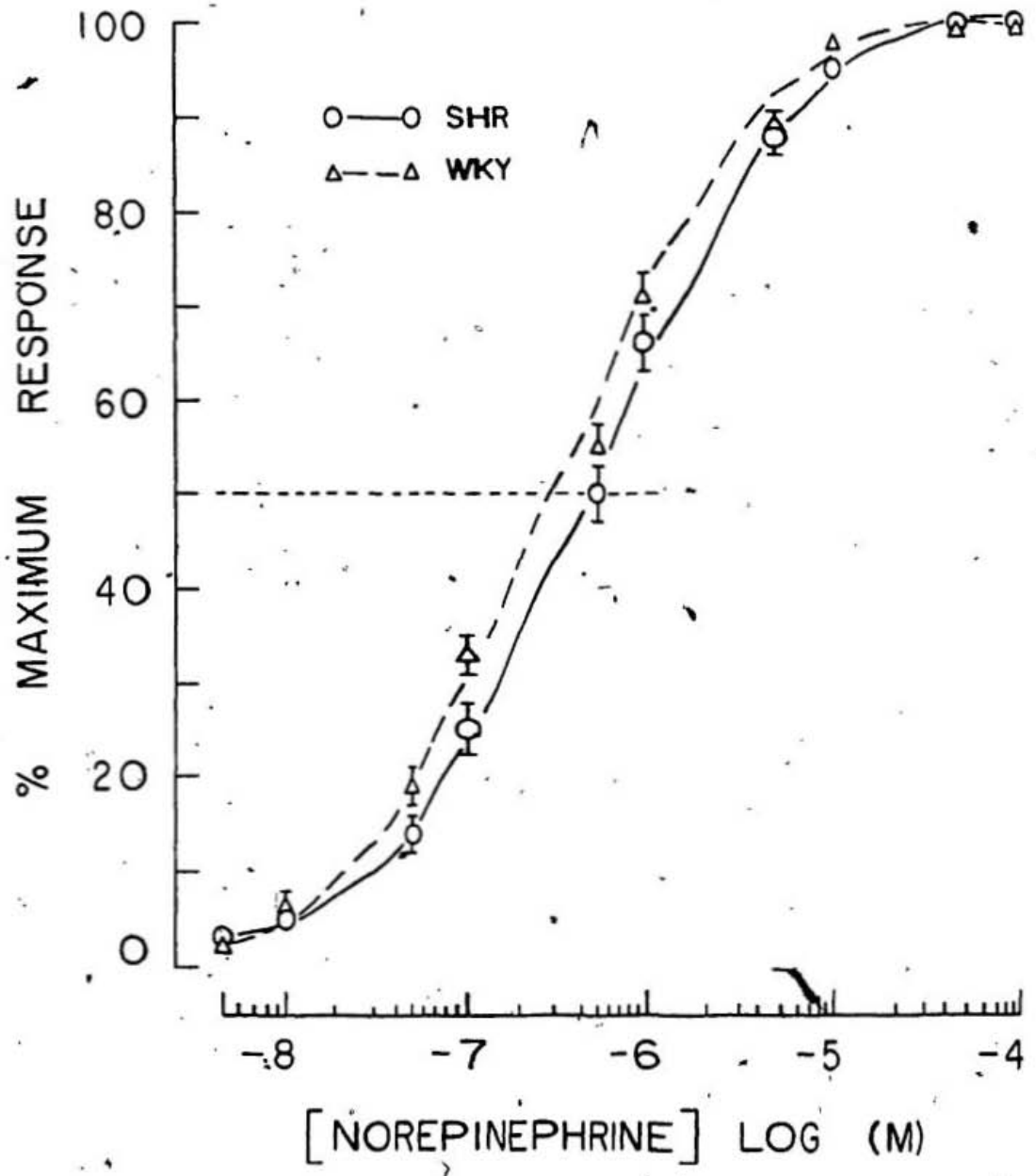

100
80
60
40
20
0
% MAXIMUM RESPONSE
SHR
WKY
-8
-7
-6
-5
-4
[NOREPINEPHRINE] LOG (M)

Figure. 3-3.

Dose-response curve to potassium in 10 - 12 wk old male SH and WKY rats. Responses were conducted in the presence of phentolamine (1μM). Points are the mean (± S.E.M.) of at least n=26 tissues in both strains.

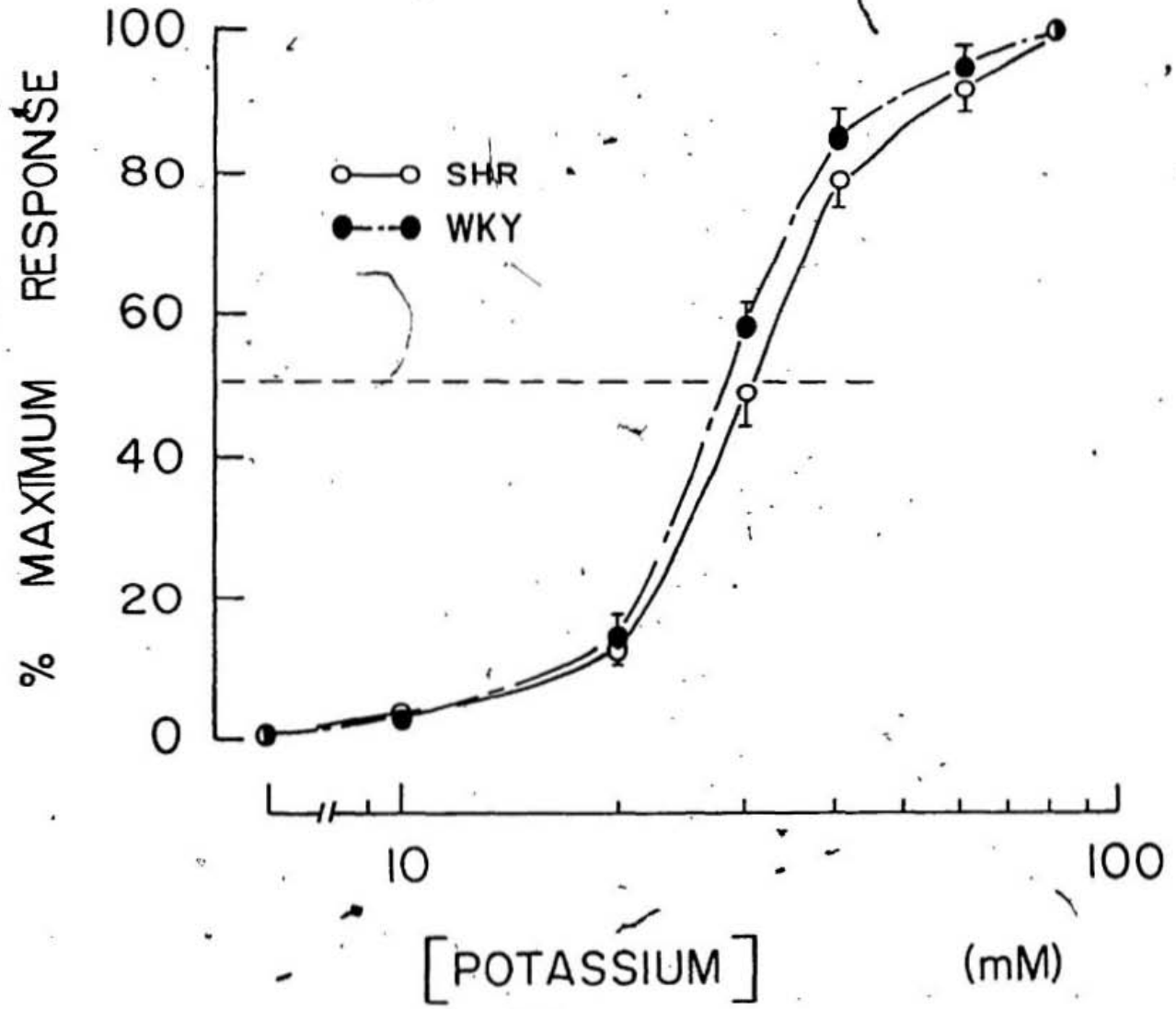

100
80
60
40
20
0
% MAXIMUM RESPONSE
SHR
WKY
10
100
[POTASSIUM]
(mM)

Table 3-3: ED_{50} values of norepinephrine and potassium in SH and WKY rats.

	ED_{50}	
	NE(μM)	K^+ (mM)
WKY	0.47 ± 0.08	29.4 ± 1.1
SHR	0.79 ± 0.15	30.4 ± 1.0

ED_{50} values are Geometric Means (± S.E.M.) calculated by probit analysis for a minimum of n=30 tissues in both NE and K^+ responses.

Figure. 3-4.

Typical responses of rat tail artery at ED_{100} of either norepinephrine ($50\mu M$) or potassium (60 mM) and ED_{50} of norepinephrine ($0.70 \pm 0.15\mu M$) or potassium (30.6 ± 0.95mM). **P** = Phasic component and **T** = Tonic component of the response.

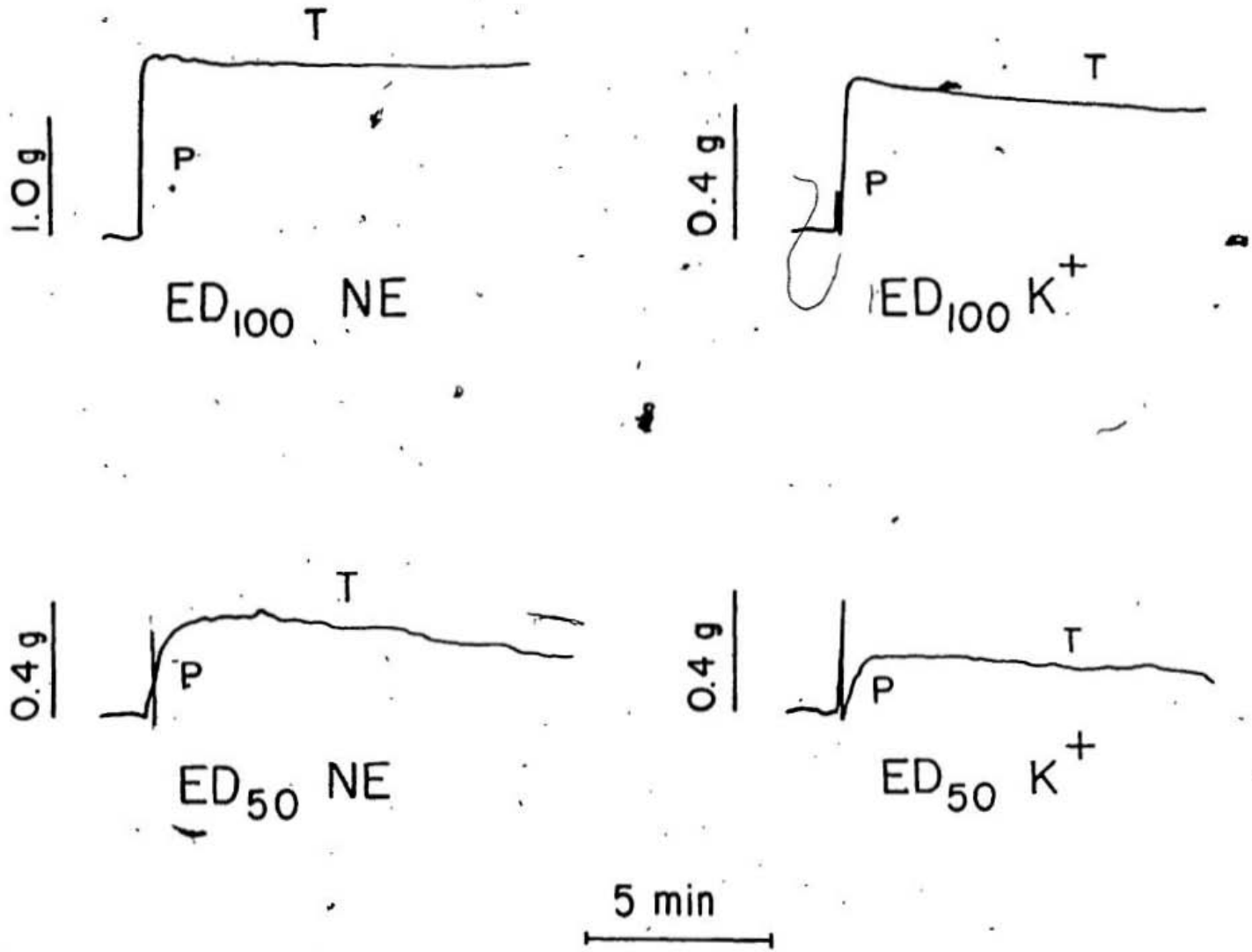

T
P
1.0 g
ED100 NE
T
P
0.4 g
ED100 K+
T
P
0.4 g
ED50 NE
T
P
0.4 g
ED50 K+
5 min

Figure. 3-5.

Sample traces of the inhibitory effect of nifedipine (30 min) on potassium (60 mM) and norepinephrine (10μM) activated rat tail artery. Superimposed on the control, are responses in the presence of nifedipine. Concentration of nifedipine is in log M.(*Please note that in the K^+ traces, the effect of nifedipine on the initial part of the phasic component is not that apparent. The maximum of this component is, however clearly reduced.*)

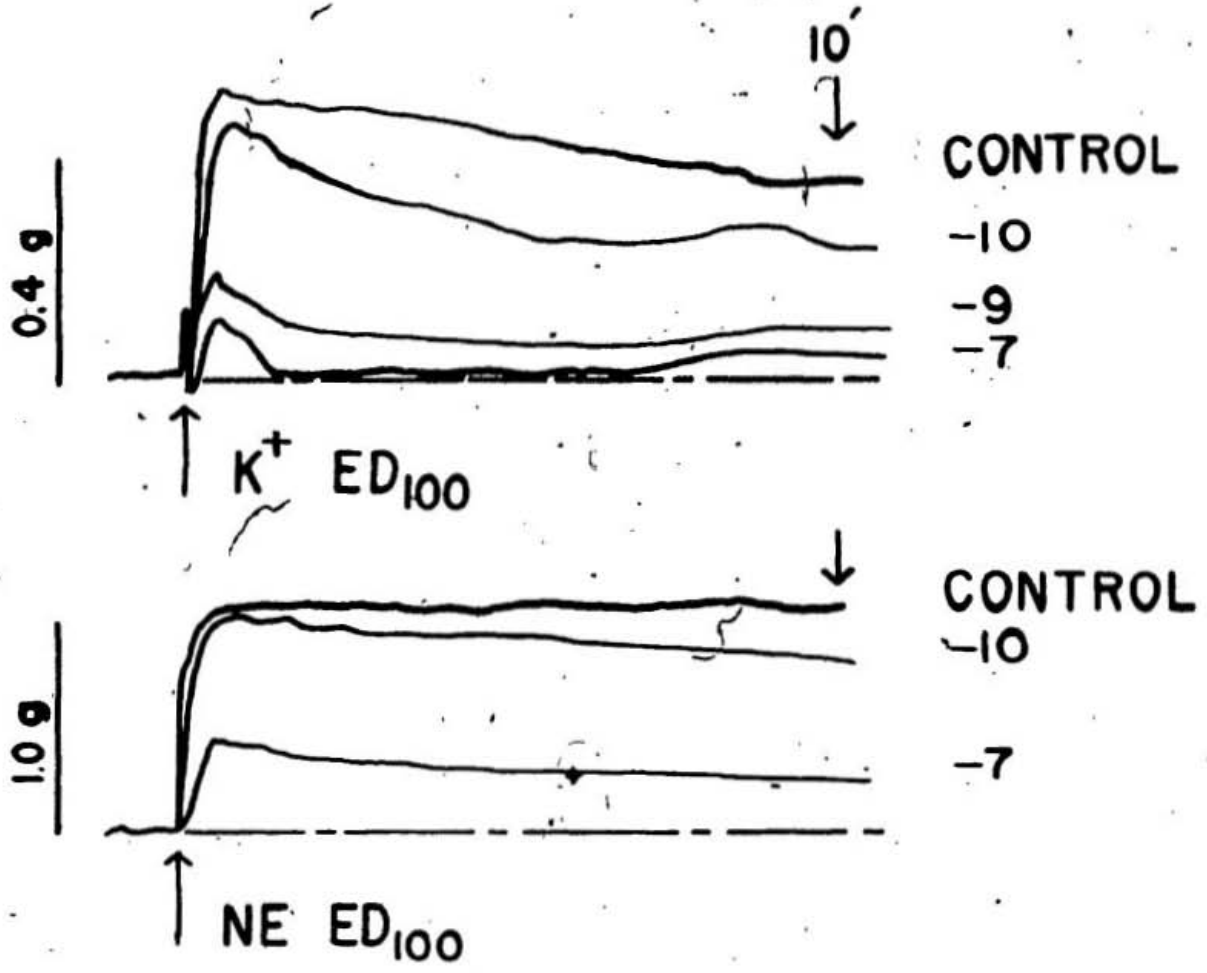

10'
CONTROL
-10
-9
-7
0.4 g
K+ ED100
CONTROL
-10
-7
1.0 g
NE ED100
5 MIN

Table 3-4: Nifedipine $IC_{50}(M)$ values in tail artery ring preparations stimulated with either K^+ or NE.

	NIFEDIPINE $IC_{50}(M)$			
	SHR		WKY	
	ED_{100}	ED_{50}	ED_{100}	ED_{50}
NE	2.9×10^{-8}	3.2×10^{-8}	2.6×10^{-8}	1.3×10^{-8}
	(n=22)	(n=17)	(n=11)	(n=18)
K^+	3.9×10^{-10} [a]	1.1×10^{-9} [a]	1.3×10^{-9} [a]	2.1×10^{-9} [a]
	(n=17)	(n=15)	(n=16)	(n=18)

IC_{50} values were calculated by linear regression analysis.

[a] = Significant difference between NE and K^+ $IC_{50}M$.

n = number of observations from which IC_{50} values were calculated.

3.2. Calcium Sensitivity Study.

3.2.1. Blood pressures of rats.

The blood pressures (mm Hg) of 10 - 12 week old male SH and WKY rats used in this study are shown in Table. 3-5. Systolic (SBP), diastolic (DBP) and mean arterial pressures (MAP) were all significantly (Unpaired Student's t test, $P < 0.05$) higher in SHR, compared with the WKY rats.

3.2.2. Calcium sensitivity.

The calcium sensitivity of tail arteries from SHR and WKY in response to maximal and submaximal activation by NE and K^+ was determined after depleting both extracellular and intracellular sources of Ca^{2+}. After Ca^{2+}_{EXT} depletion, in tissues stimulated with NE there was a phasic response and a severely reduced (in most cases by upto 90%) tonic component. The tissues were then washed (3-4 times) with the '0 Ca^{2+}, EGTA PSS' to mop up Ca^{2+} being extruded from the cell. The tissues were then exposed to a 'Ca free PSS' (no EGTA) and exposed again to NE. Five minutes later a cumulative Ca^{2+} dose-response curve was determined. Here there was no distinguishable phasic response with stimulation at both levels, indicating that intracellular activator Ca^{2+} was indeed depleted (Figure. 3-6.). Keatinge (1972) has reported a similar phenomenon in sheep carotid arteries. Arteries responded to NE after a 30 minute exposure to "simple Ca^{2+}-free saline". However after exposure to EDTA, which resulted in depletion of extracellular Ca+(2+) to below threshold levels, a response still persisted. Keatinge (1972) suggested that the source of Ca^{2+} for this response

was intracellular. There was no response at all when the tissues were exposed to a K^+ depolarising Ca^{2+} free PSS (Figure. 3-7). As with the NE stimulation, Ca^{2+} was added to the organ bath 5 minutes after activation. In both NE and K^+ activation, a higher concentration of Ca^{2+} was added only after the prior response had reached a plateau.

The same figures show that the responses to Ca^{2+} in the presence of ED_{50} levels of either NE or K^+, attain maxima at a bath concentration of 5.0 m*M* whereas responses in vessels stimulated at ED_{100} reached a maximum response at 10.0 m*M*.

The Ca^{2+} dose response characteristics are shown in Figures. 3-8., 3-9., 3-10 and 3-11 for vessels activated with NE (ED_{100} and ED_{50}) and K^+ (ED_{100} and ED_{50}) respectively. The corresponding Ca^{2+} sensitivities (expressed as Ca-pD_2 values) are shown in Table. 3-6. In general the Ca^{2+} sensitivities of SHR vessels were higher than those of WKY, the difference being greater when vessels were activated with NE. However the difference in Ca^{2+} sensitivity was significant ($P < 0.05$) between SHR (Ca-$pD_2 = 3.46 \pm 0.04$) and WKY (Ca-$pD_2 = 3.26 \pm 0.06$) only when the vessels were activated at the ED_{50} level. At maximal stimulation there was no significant difference ($P > 0.05$) in Ca^{2+} sensitivity between SHR and WKY.

Table 3-5: Systolic, Diastolic and Mean Arterial Pressure of 10 - 12 week old male SH and WKY rats. Calcium sensitivity study.

	SYSTOLIC mm Hg	DIASTOLIC mm Hg	MAP mm Hg
WKY (n=9)	106.5 ± 7.0	80.2 ± 6.0	89.0 ± 6.0
SHR (n=9)	190.5 ± 8.0[a]	153.3 ± 6.0[a]	165.6 ± 1.9[a]

MAP = (Systolic pressure + 2 Diastolic Pressure)/3

a = Significant difference (Unpaired Student's t test, $P < 0.05$) between WKY and SHR.

Figure. 3-6.

Calcium dose-response records in tail arteries challenged with norepinephrine (NE) at ED_{50} and ED_{100}. Initial response is the control response in normal physiological salt solution (PSS). At *A* the tissues were depleted of intracellular calcium. The tissues were then stimulated (*B*) with norepinephrine in a Ca^{2+} free PSS (no EGTA). Five minutes later calcium was added cumulatively to the bath. Calcium concentrations are final bath concentrations. All experiments were carried out in the presence of cocaine ($40\mu M$) and propranolol ($0.1\mu M$.)

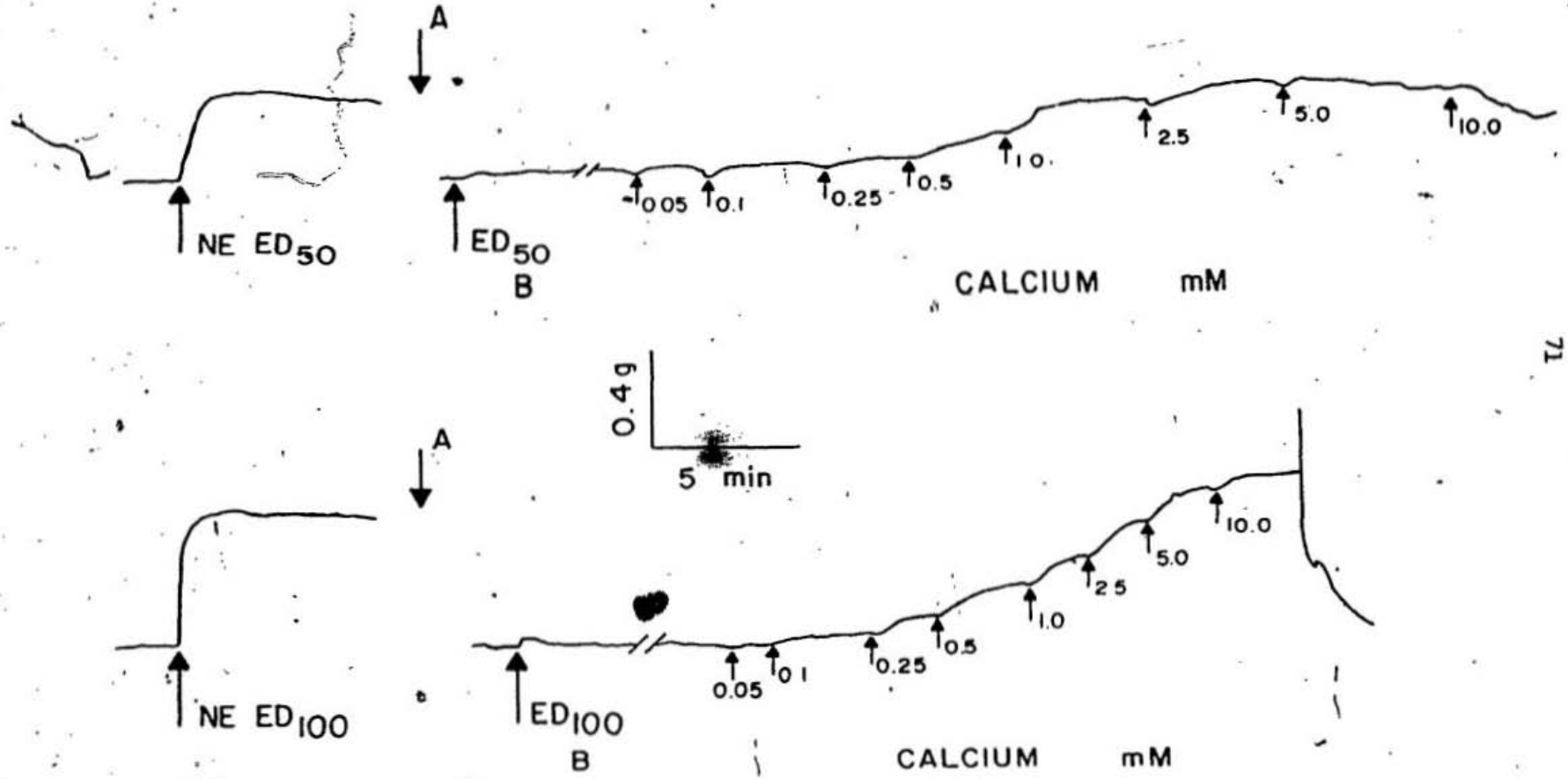

A
NE ED50
ED50
B
0.05
0.1
0.25
0.5
1.0
2.5
5.0
10.0
CALCIUM mM
0.4g
5 min
A
NE ED100
ED100
B
0.05
0.1
0.25
0.5
1.0
2.5
5.0
10.0
CALCIUM mM

Figure. 3-7.

Calcium dose-response records in tail arteries exposed to an isotonic K^+ depolarising PSS at ED_{50} and ED_{100}. Initial response is the control response in normal PSS. At *A* the tissues were depleted of extracellular calcium. At *B* the tissues were exposed to a Ca^{2+} free K^+ depolarising PSS. Five minutes later calcium was added cumulatively to the bath. Calcium concentrations are final bath concentrations. All experiments were carried out in the presence of phentolamine (1.0 μM).

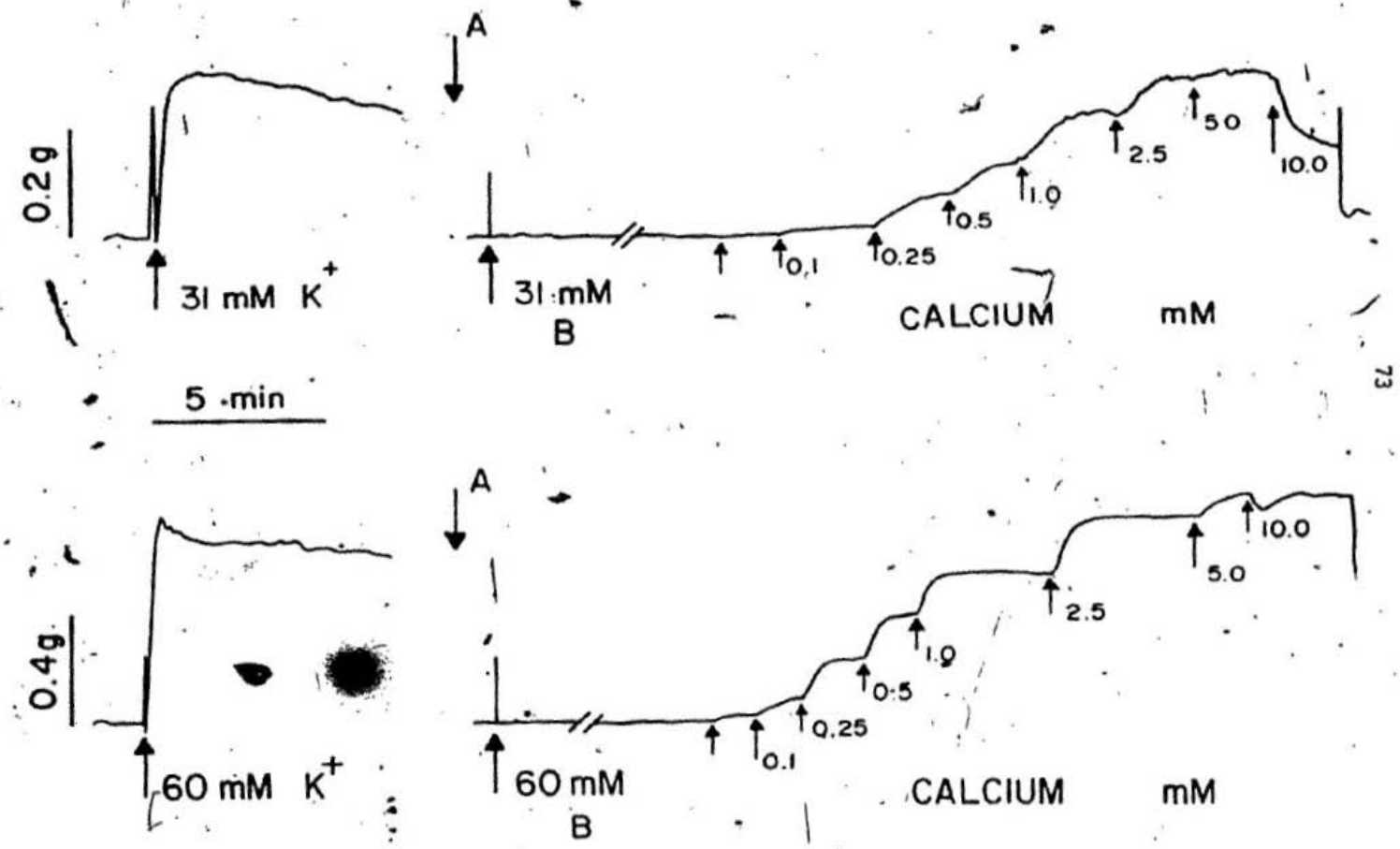

0.2g
31 mM K+
A
31 mM
B
0.1
0.25
0.5
1.0
2.5
5.0
10.0
CALCIUM mM
5 min
0.4g
60 mM K+
A
60 mM
B
0.1
0.25
0.5
1.0
2.5
5.0
10.0
CALCIUM mM

Figure. 3-8.

Calcium dose-response characteristics determined at ED_{100} level of activation with norepinephrine. These data were determined in tail arteries from SHR (open symbols) and WKY (closed symbols) rats. Responses are expressed as a % of response to 10.0 m*M* calcium. Each curve is the mean (± S.E.M) of at least n = 16 tissues. All experiments were carried out in the presence of cocaine (40 μM) and propranolol (0.1 μM).

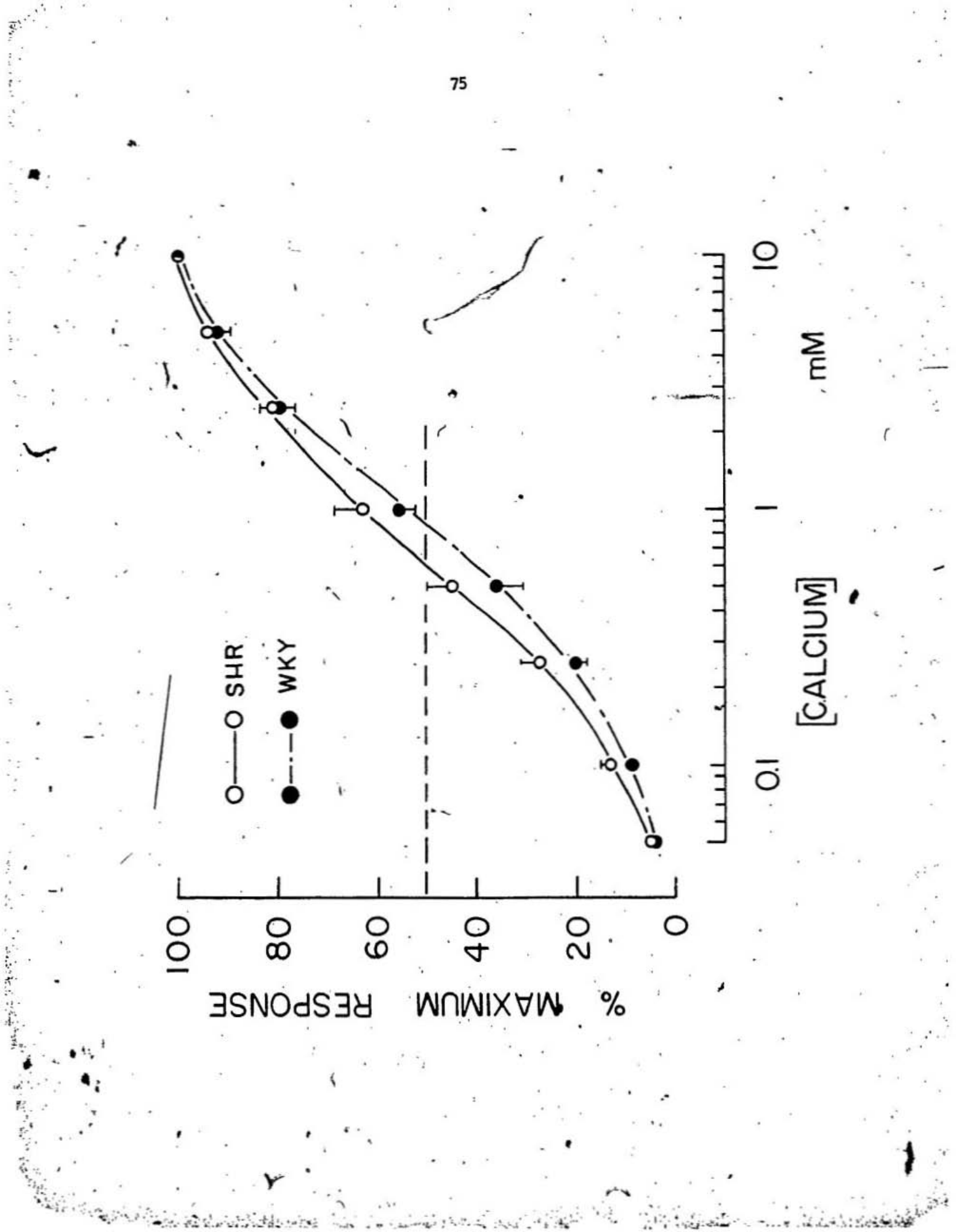

% MAXIMUM RESPONSE
100
80
60
40
20
0
SHR
WKY
0.1
1
10
mM
[CALCIUM]

Figure. 3-9.

Calcium dose-response characteristics determined at ED_{50} level of activation with norepinephrine. These data were determined in tail arteries from SHR (open symbols) and WKY (closed symbols) rats. Responses are expressed as a % of the maximum response. Each curve is the mean (± S.E.M) of at least $n = 8$ tissues. All experiments were carried out in the presence of cocaine (40 μM) and propranolol (0.1 μM).

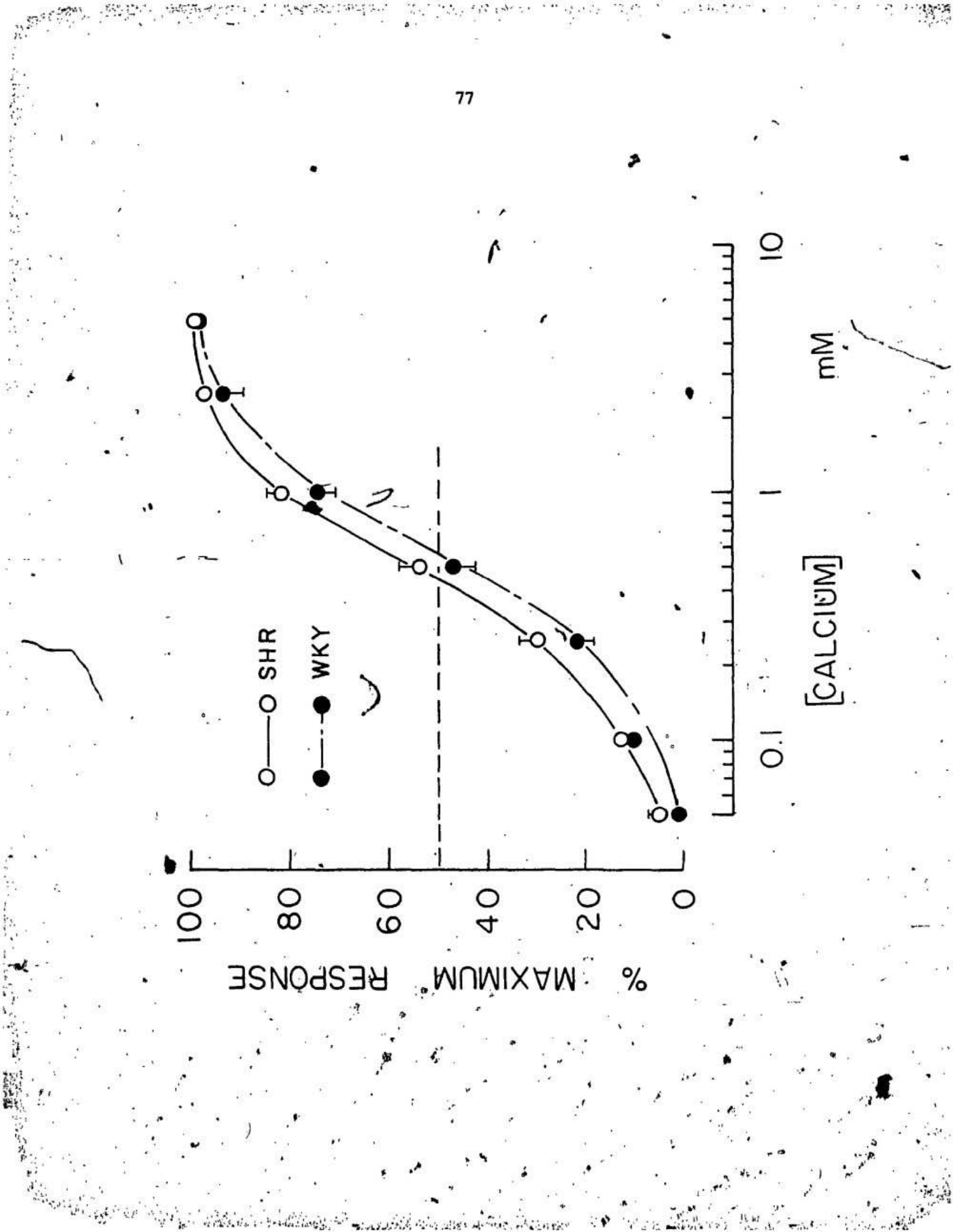

% MAXIMUM RESPONSE
100
80
60
40
20
0
SHR
WKY
0.1
1
10
mM
[CALCIUM]

Figure. 3-10.

Calcium dose-response characteristics determined at ED_{100} level of activation with K^+. These data were determined in tail arteries from SHR (open symbols) and WKY (closed symbols) rats. Responses are expressed as a % of response to 10.0 m*M* calcium. Each curve is the mean (± S.E.M) of at least n = 14 tissues. All experiments were carried out in the presence of phentolamine (10 μM).

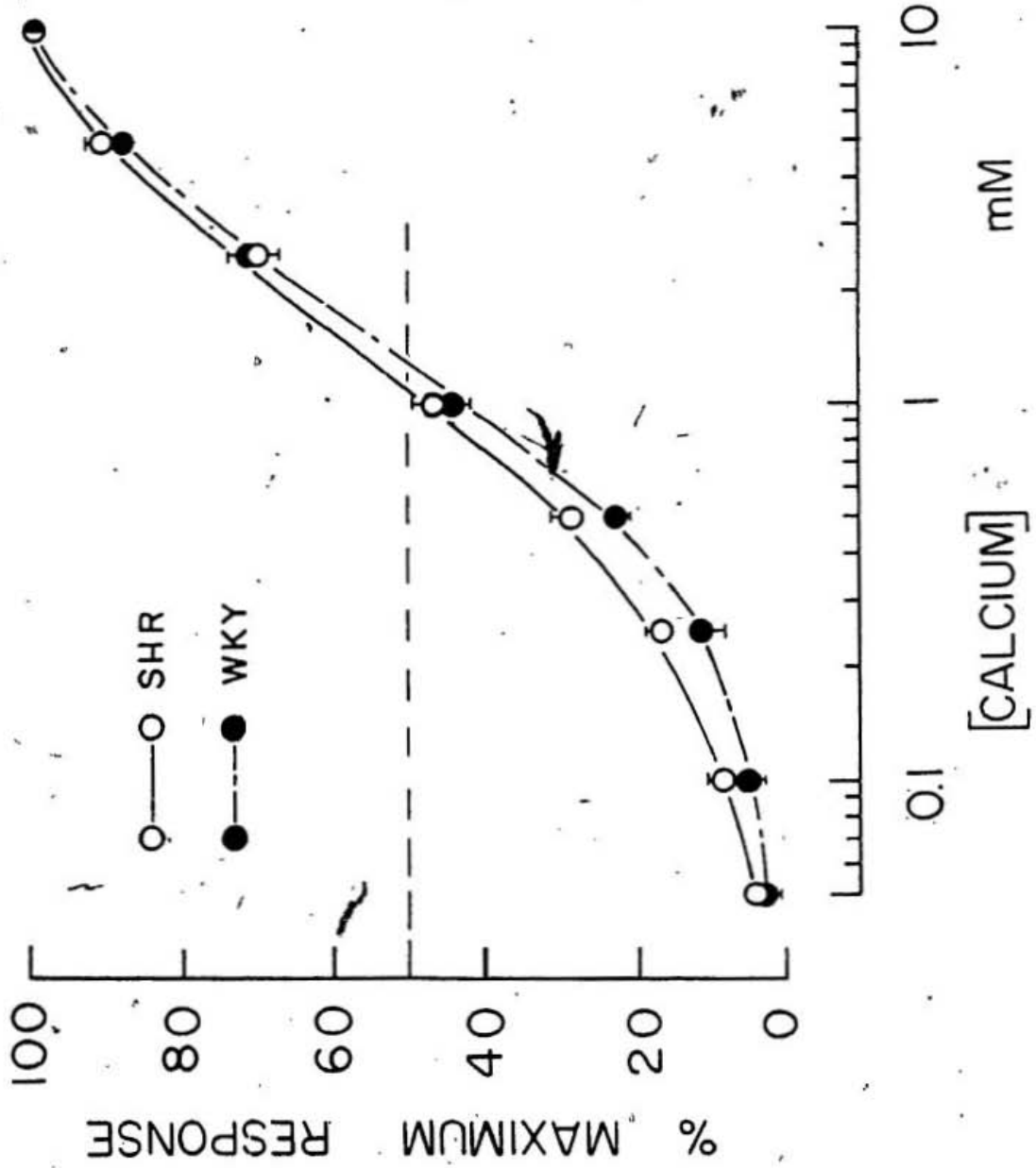

% MAXIMUM RESPONSE
100
80
60
40
20
0
SHR
WKY
0.1
1
10
[CALCIUM]
mM

Figure. 3-11.

Calcium dose-response characteristics, determined at ED_{50} level of activation with potassium. These data were determined in tail arteries from SHR (open symbols) and WKY (closed symbols) rats. Responses are expressed as a % of the maximum response. Each curve is the mean (± S.E.M) of at least n = 6 tissues. All experiments were carried out in the presence of phentolamine (1.0 μM).

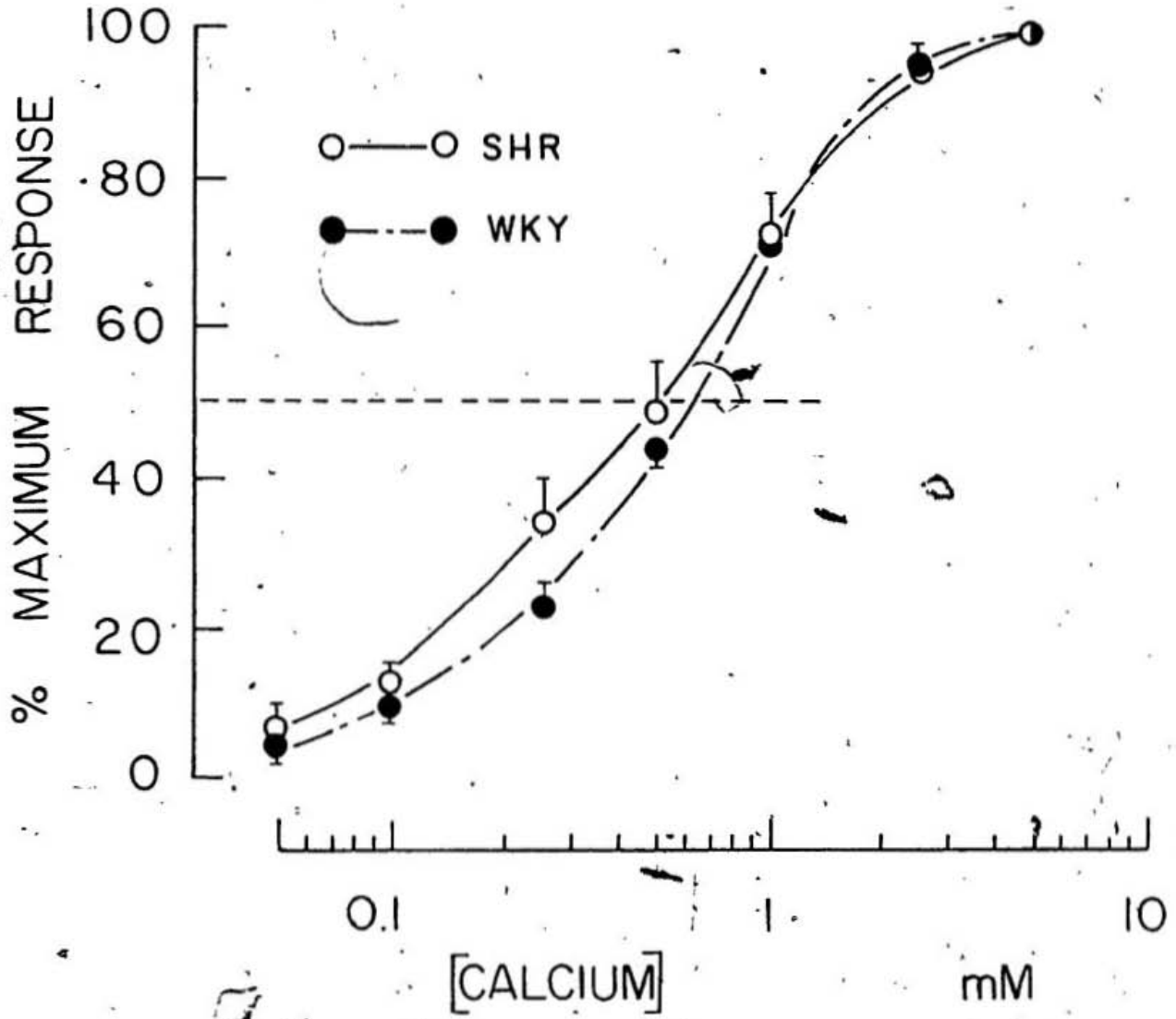

100
80
60
40
20
0
% MAXIMUM RESPONSE
SHR
WKY
0.1
1
10
[CALCIUM]
mM

Table 3-6: Calcium sensitivity of rat tail artery from SH and WKY rats activated at ED_{50} and ED_{100} levels of norepinephrine and potassium.

	CALCIUM pD_2			
	NOREPINEPHRINE		POTASSIUM	
	ED_{50}	ED_{100}	ED_{50}	ED_{100}
WKY	3.26± 0.06 (n=8)	3.18± 0.05 (n=16)	3.30± 0.06 (n=6)	2.97± 0.04[b] (n=14)
SHR	3.46± 0.04[a] (n=8)	3.27± 0.06[b] (n=16)	3.38± 0.08 (n=6)	3.06± 0.04[b] (n=14)

Sensitivity of vessels is expressed as pD_2 values, where $pD_2 = -\log[ED_{50}M]$. a = significant difference (Unpaired Student's t test, $P < 0.05$) between WKY and SHR. b. = significant difference between pD_2 values in ED_{50} and ED_{100} stimulated tissues, either with norepinephrine or potassium.

3.2.3. Effect of nifedipine on calcium sensitivity.

The effects of nifedipine, at three concentrations, on the calcium response characteristics of tail artery ring segments, are shown in Figure. 3-12 and Figure. 3-13 (SHR and WKY vessels, respectively, activated by NE) and Figure. 3-14 and Figure. 3-15 (SHR and WKY vessels, respectively, activated by K^+). In both cases the vessels were activated with maximal levels of NE and K^+. Corresponding Ca-pD_2 values are shown in Table. 3-7 for NE activated vessels and in Table. 3-8 for K^+ activated vessels. Nifedipine a concentration-dependent manner decreased the vessel sensitivity to Ca^{2+}. Low concentrations ($5 \times 10^{-11} M$) of nifedipine significantly ($P \leq 0.05$) reduced Ca^{2+} sensitivity of vessels from SHR, but not WKY, when activated by K^+ (Ca^{2+}-pD_2 3.14 ± 0.05 to 2.86 ± 0.09). At the higher concentrations, $1.0 \times 10^{-9} M$ and $1.0 \times 10^{-7} M$, the Ca^{2+} sensitivity was significantly reduced in both SHR and WKY vessels. In contrast, nifedipine, at concentrations of $5.0 \times 10^{-11} M$ and $1.0 \times 10^{-9} M$ had no significant effect on Ca^{2+} sensitivity of vessels activated by NE. At the higher concentration of $1 \times 10^{-7} M$ the Ca^{2+} sensitivity of vessels from both SHR and WKY was significantly different (Figure. 3-7). However the reduction in Ca^{2+} sensitivity was more significant ($P < 0.01$) in vessels from SHR than in vessels from WKY ($P < 0.05$). The maximum response, measured at 10.0 mM Ca^{2+}, was depressed more in WKY vessels (Figure. 3-13) than in in SHR vessels (Figure. 3-12). Also apparent in this study is the selective effect of nifedipine on K^+ activated responses. Nifedipine ($1 \times 10^{-7} M$) almost completely inhibited the response in K^+ activated vessels whereas in vessels activated by NE, responses were approximately 60% (SHR) and 30% (WKY) of the maximal NE activated Ca^{2+} response.

Figure. 3-12.

Effect of nifedipine on calcium dose-response characteristics of SHR tail artery ring preparations, maximally activated with norepinephrine ($10\mu M$). Responses in presence of nifedipine are expressed as % of the maximal response in the control condition. All experiments were carried out in the presence of cocaine ($40\mu M$) and propranolol ($1.0\mu M$). Points show means ± (S.E.M) of n = 4-3 observations.

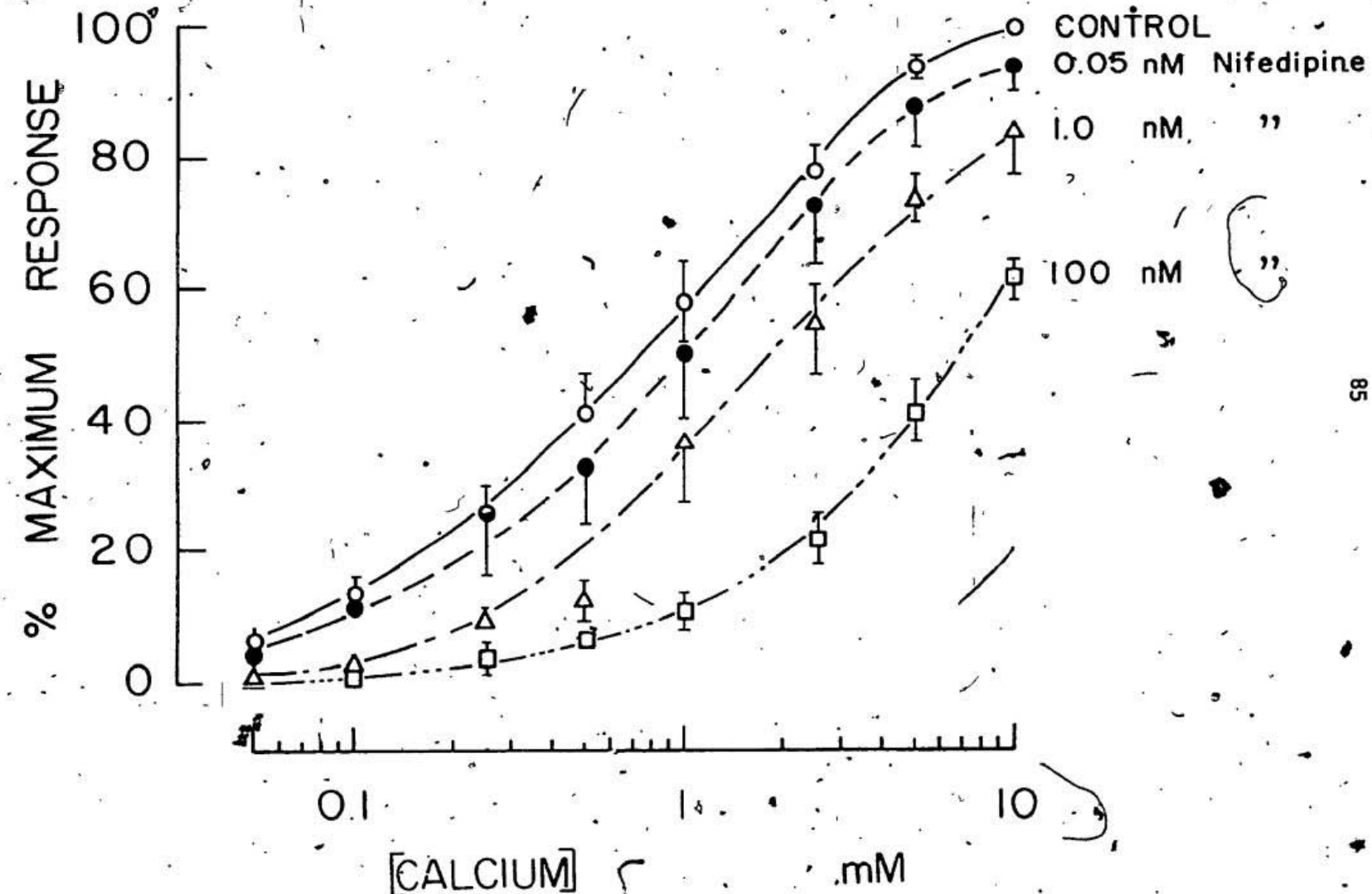

100
80
60
40
20
0
% MAXIMUM RESPONSE
CONTROL
0.05 nM Nifedipine
1.0 nM "
100 nM "
0.1
1
10
[CALCIUM]
mM

Figure. 3-13.

Effect of nifedipine on calcium dose-response characteristics of WKY tail artery ring preparations, maximally activated with norepinephrine ($10\mu M$). Responses in presence of nifedipine are expressed as % of the maximal response in the control condition. All experiments were carried out in the presence of cocaine ($40\mu M$) and propranolol ($0.1\mu M$). Points show means $\pm$ (S.E.M) of n = 4-3 observations.

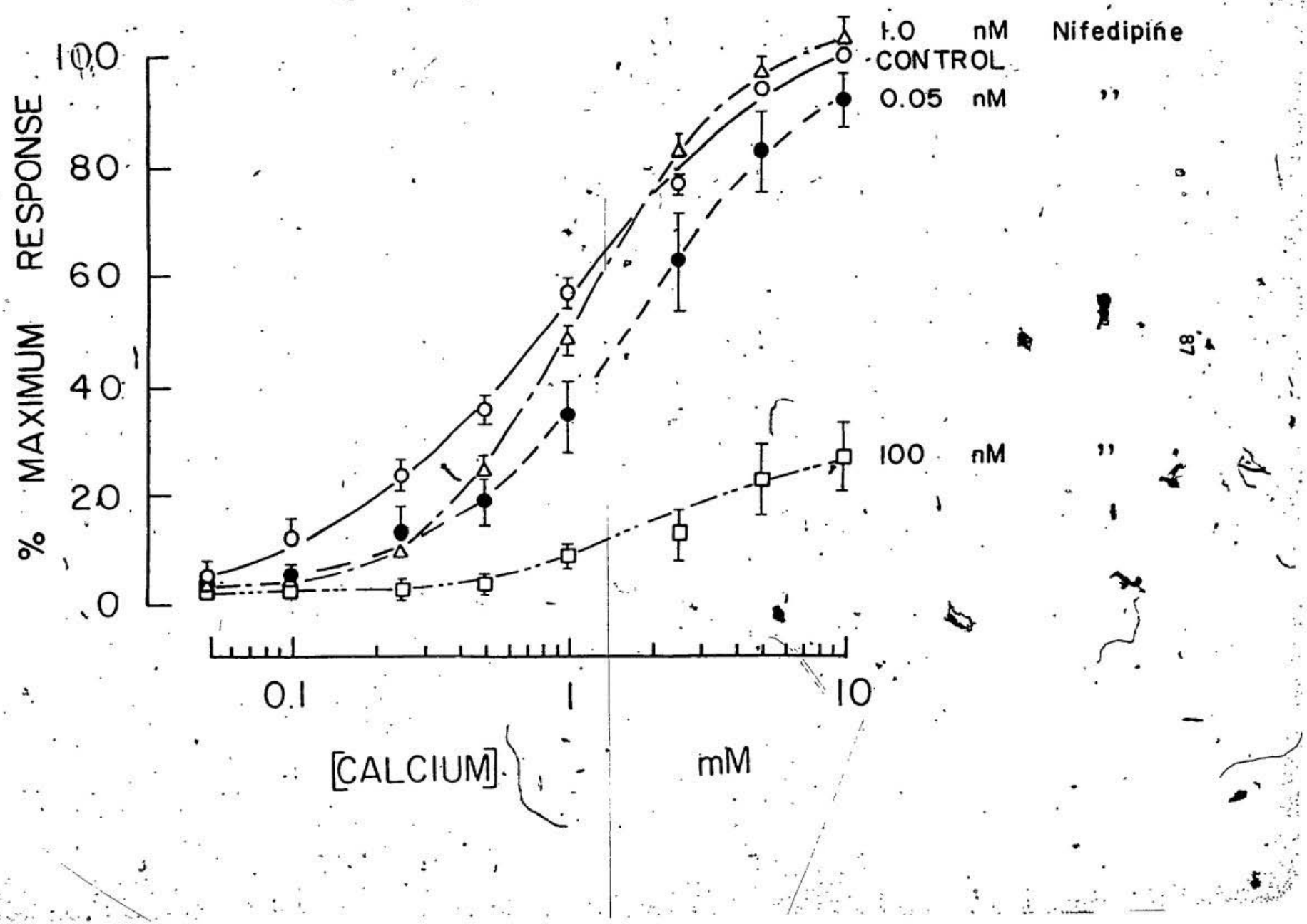

% MAXIMUM RESPONSE
100
80
60
40
20
0
0.1
1
10
[CALCIUM]
mM
1.0 nM Nifedipine
CONTROL
0.05 nM "
100 nM "

Table 3-7: Calcium sensitivity of rat tail artery from SH and WKY when activated at ED_{100} levels of norepinephrine. Effect of nifedipine.

	CALCIUM pD_2	
	NOREPINEPHRINE	
	SHR	WKY
CONTROL	3.30 ± 1.3(4)	3.12 ± 0.08(4)
Nifedipine(0.05 n*M*)	3.12 ± 0.12(4)	2.88 ± 0.11(4)
CONTROL	3.04 ± 0.008(4)	3.35 ± 0.12(4)
Nifedipine(1.0 n*M*)	2.77 ± 0.07(4)	3.03 ± 0.008(4)
CONTROL	3.27 ± 0.17(4)	3.06 ± 0.07(4)
Nifedipine(100.0 n*M*)	2.49 ± 0.06[b](4)	2.59 ± 0.06[a](4)

Sensitivity of vessels is expressed as pD_2 values, where $pD_2 = -\log[ED]_{50}]M$. Values are mean (± S.E.M)(n). Changes in sensitivity from control were analysed by unpaired Student's t test. [a] = significant ($P < 0.05$) difference from control. [b] = significant ($P < 0.01$) difference from control.

Figure. 3-14.

Effect of nifedipine on calcium dose-response characteristics of SHR tail artery ring preparations, maximally activated with potassium (60m*M*). Responses in presence of nifedipine are expressed as % of the maximal response in the control condition. All experiments were carried out in the presence of phentolamine (1.0μM). Points show means ± (S.E.M) of n = 4-3 observations.

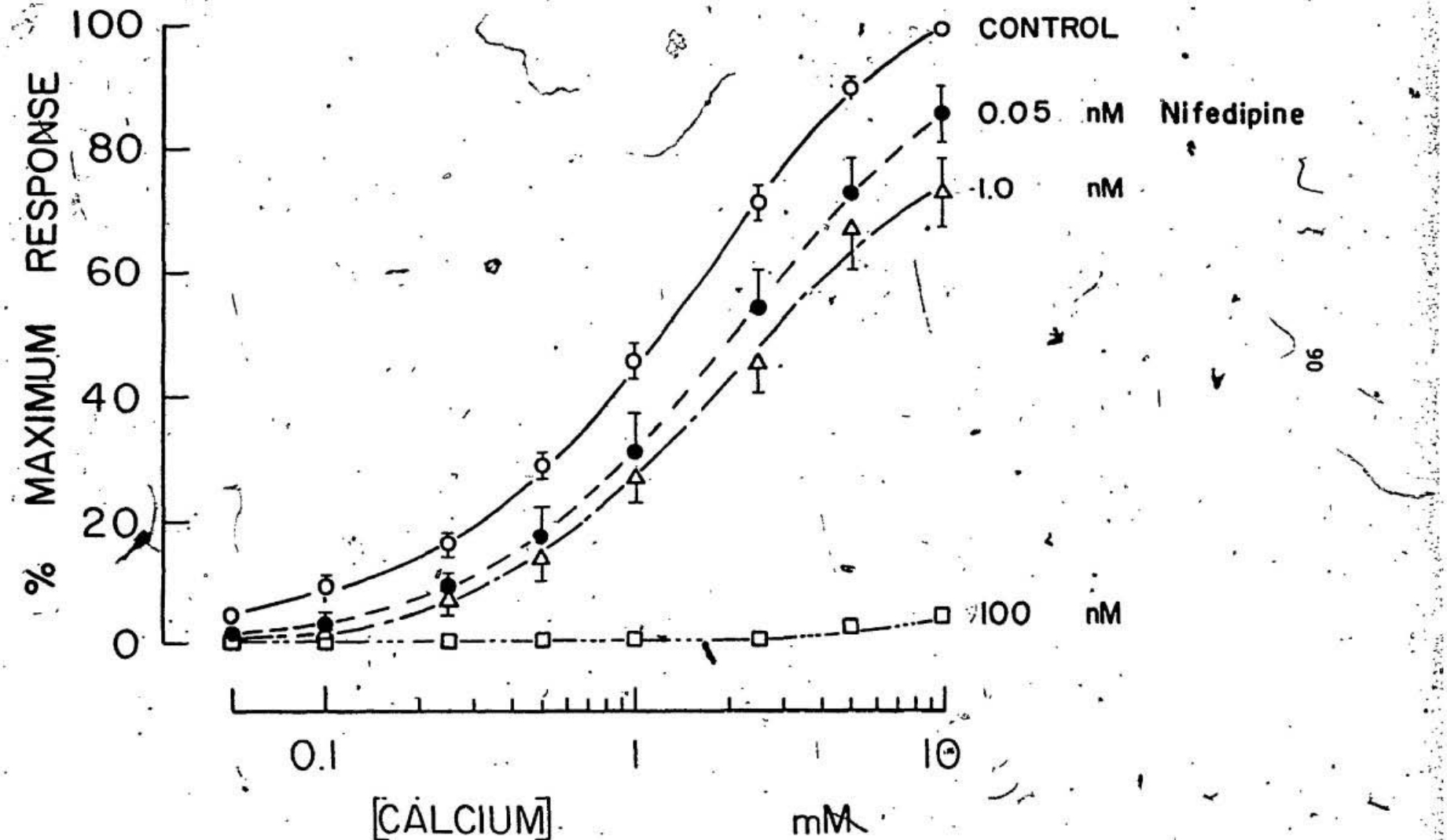

% MAXIMUM RESPONSE
100
80
60
40
20
0
CONTROL
0.05 nM Nifedipine
1.0 nM
100 nM
0.1
1
10
[CALCIUM]
mM

Figure. 3-15.

Effect of nifedipine on calcium dose-response characteristics of WKY tail artery ring preparations, maximally activated with potassium (60m*M*). Responses in presence of nifedipine are expressed as % of the maximal response in the control condition. All experiments were carried out in the presence of phentolamine (1.0μ*M*). Points show means ± (S.E.M) of n = 4-3 observations.

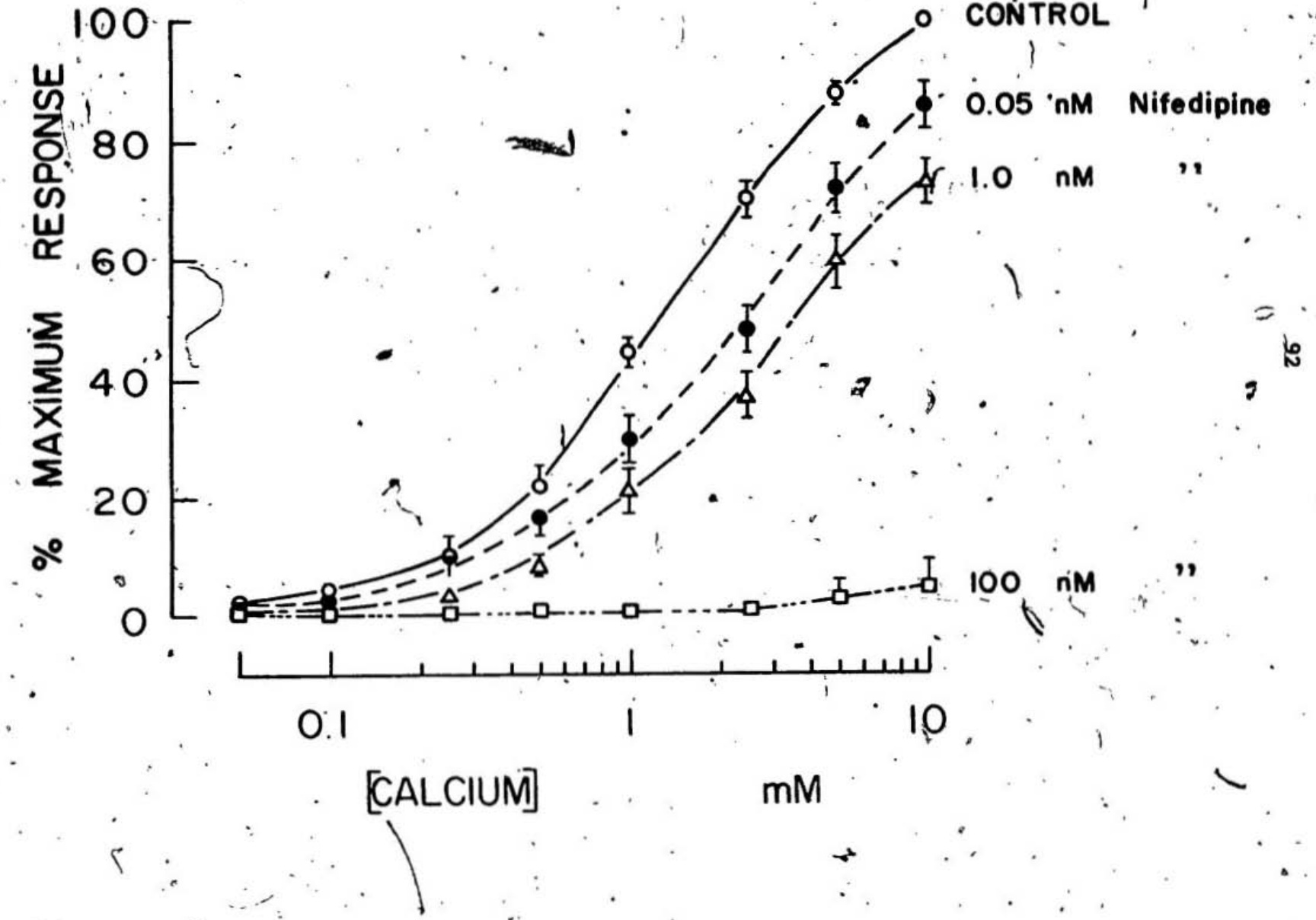

% MAXIMUM RESPONSE
100
80
60
40
20
0
CONTROL
0.05 nM Nifedipine
1.0 nM "
100 nM "
0.1
1
10
[CALCIUM]
mM

Table 3-8: Calcium sensitivity of rat tail artery from SH and WKY rats activated at ED_{100} levels of potassium. Effect of nifedipine.

	CALCIUM pD_2	
	POTASSIUM	
	SHR	WKY
CONTROL	3.14 ± 0.05(4)	2.85 ± 0.08(4)
Nifedipine(0.05 n*M*)	2.86 ± 0.09[a](4)	2.76 ± 0.05(4)
CONTROL	3.01 ± 0.07(4)	3.00 ± 0.08(4)
Nifedipine(1.0 n*M*)	2.78 ± 0.08[a](4)	2.60 ± 0.02[a](4)
CONTROL	3.04 ± 0.01(3)	3.07 ± 0.09(3)
Nifedipine(100.0 n*M*)	[a,1](3)	[a,1](3)

Sensitivity of vessels is expressed as pD_2 values, where $pD_2 = -\log[ED]_{50}]M$. Values are mean (± S.E.M)(n). Changes in sensitivity from control were analysed by paired Student's t test. [a] = significant ($P < 0.05$) difference from control. [1] = IC_{50} difficult to determine due to almost total inhibition of Ca^{2+} response.

3.3. In Vivo Sensitivity to Nifedipine.

3.3.1. Blood pressures of rats.

The blood pressures (mm Hg) of male SH and WKY rats, at the 5 week and > 20 week age group are shown in Table. 3-9. Blood pressures of 10 - 12 week old male SHR, WKY and Wistar rats are shown in Table. 3-10. The systolic (SBP), diastolic (DBP) and mean arterial pressures (MAP) of SHR rats were all significantly different (Student's t test, $P < 0.05$) when compared to the corresponsing pressures in the WKY rats. For the 10 -12 week age group, where three strains were used, the pressures were compared by oneway analysis of variance (ONEWAY). In this age group, the SBP, DBP and MAP of SHR rats were significantly higher (Scheffe's test, $P < 0.05$) than corresponding pressures in both WKY and Wistar rats. In addition, the DBP and MAP of Wistar rats were significantly higher than those of WKY rats (Table. 3-10).

3.3.2. Effect of nifedipine.

Nifedipine, at all doses, reduced the pressure in all strains. The pressure fell almost immediately after infusion and reached a minimum level in about 10 - 15 seconds at the 0.01, 0.02, 0.04, 0.1 and 0.2 mg Kg^{-1}) dose level, whereas at the higher doses the fall was even faster and reached a minimum low in about 5 seconds (Figure. 3-16). The pressure pulse width progressively decreased as the dose of nifedipine was increased. Invariably, at the the high doses (0.8 and 1.0 mg kg^{-1}), the pulse almost disappeared, at the gain used.

Except for some animals in the young 5 week age group, all animals survived the entire dose schedule.

The mean (± S.E.M.) percentage reduction in MAP, at each dose level, for the 5 week, 10 - 12 week and > 20 week age groups are shown in Tables. 3-11,3-12 and 3-13 respectively. The data are also presented in Figures. 3-17,3-18 and 3-19.

In the 5 week age group, there were no significant differences (Student's t test, $P > 0.05$), between the strains, in the mean percentage reductions in MAP. The effects of nifedipine on the three strains used in the 10 - 12 week age group are shown in Figure 3-18. Here significant differences (Scheffe's test, $P < 0.05$) between pairs of strains are shown. Significant differences between WKY and SH rats were seen when nifedipine was administered at the 0.1, 0.2 and 0.4 mg kg^{-1} dose levels. The Wistar rats were even more resistant to the hypotensive effect of nifedipine when compared to SH rats. Significant differences were observed at the 0.02, 0.1, 0.2, 0.4, 0.8 and 1.0 mg kg^{-1} dose levels (Table. 3-12 and Figure. 3-18). The responses at the 20 week or greater age group exhibit considerable variation over the entire dose range (Figure. 3-19). Here significant differences (Students's t test, $P < 0.05$) were only observed at two dose levels (0.2 and 1.0 mg kg^{-1}).

Table 3-9: Systolic, Diastolic and Mean Arterial Pressure of 5 week and 20 week old male SH and WKY rats.

	SYSTOLIC mmHg	DIASTOLIC mmHg	MAP mmHg
5 Week			
WKY (n=3)	92.5 ± 3.8	81.0 ± 5.8	85.0 ± 5.0
SHR (n=4)	141.2 ± 2.4[a]	126.2 ± 2.4[a]	131.2 ± 2.2[a]
20 Week			
WKY (n=7)	106.8 ± 3.0	76.0 ± 3.7	89.1 ± 3.7
SHR (n=10)	238.5 ± 9.0[a]	175.0 ± 6.5[a]	197.0 ± 6.7[a]

MAP = (Systolic pressure + 2 Diastolic Pressure)/3

[a] = Significant difference ($P < 0.05$) (Student's t test) between WKY and SHR.

Table 3-10: Systolic, Diastolic and Mean Arterial Pressure of 10 - 12 week old male SH and WKY rats.

	SYSTOLIC mmHg	DIASTOLIC mmHg	MAP mmHg
Wistar	119.0 ± 4.5[a]	104.0 + 4.0[a,b]	108.0 + 7.0[a,b]
WKY	114.0 ± 4.5[a]	78.5 ± 3.5[a]	86.0 ± 3.5[a]
SHR	207.0 ± 8.0	146.0 ± 5.0	169.0 ± 4.0

The values are the means (± S.E.M.) of n=7 rats in each group.

They were compared by analysis of variance. Differences were considered significant at the $P<0.05$ level (Scheffe's test).

[a] = significant difference compared to SHR and [b] = significant difference between WKY and Wistar.

Figure. 3-16.

Blood pressure recordings in response to increasing doses of nifedipine. Tracings are from a SHR. Values to the the left of the recordings are the systolic (superscript) and diastolic (subscript) pressures (mm Hg). Doses of nifedipine (mg kg^{-1}) are indicated to the right of the arrows.

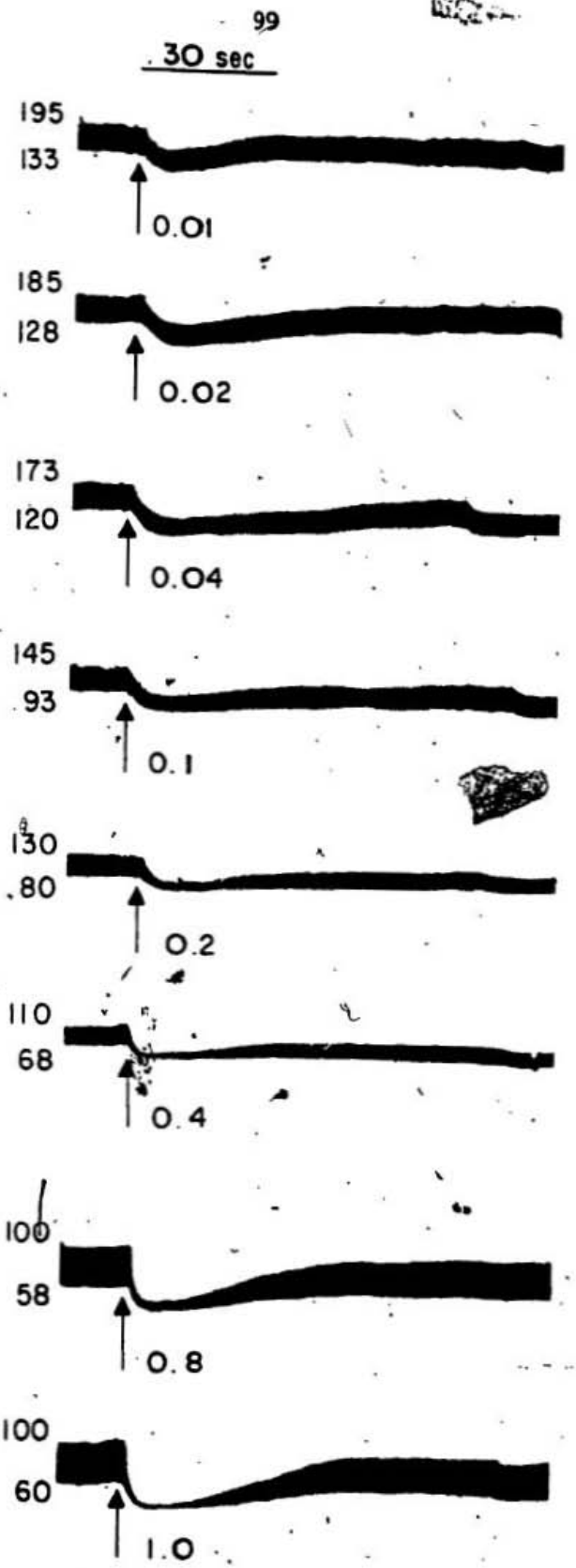

30 sec
195
133
0.01
185
128
0.02
173
120
0.04
145
93
0.1
130
80
0.2
110
68
0.4
100
58
0.8
100
60
1.0

Figure. 3-17.

Histogram of mean (± S.E.M.) percentage reduction in mean arterial pressure in 5 week old male SH (n=4) and WKY (n=3) rats.

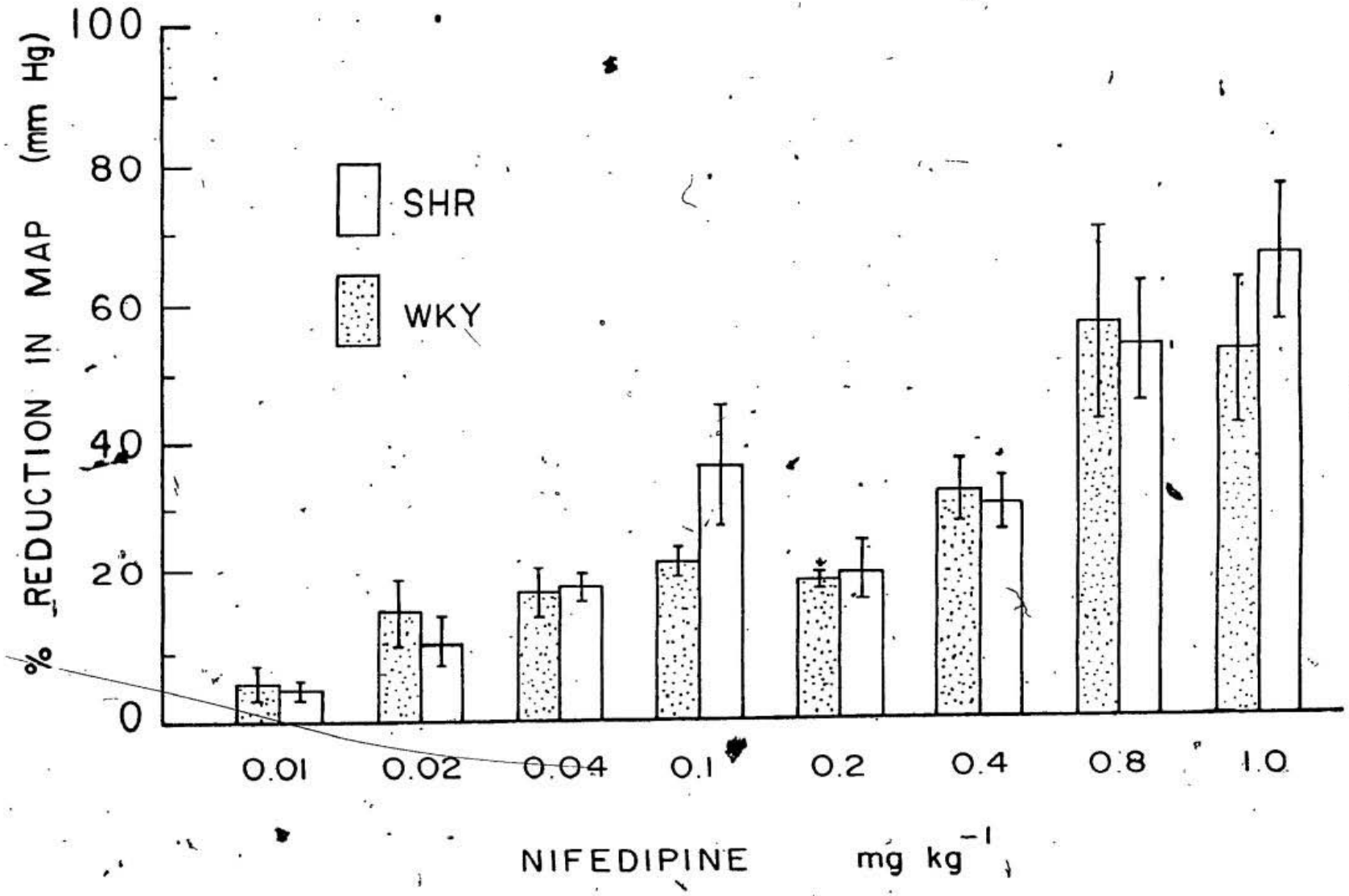

% REDUCTION IN MAP (mm Hg)
100
80
60
40
20
0
SHR
WKY
0.01
0.02
0.04
0.1
0.2
0.4
0.8
1.0
NIFEDIPINE mg kg⁻¹

Table 3-11: Mean percentage decrease in mean arterial pressure of 5 week old male SH and WKY rats induced by nifedipine.

DOSE mg kg^{-1}	MAP DECREASE (%) SHR	WKY
0.01	4.0 ± 1.3	5.0 ± 2.6
0.02	11.5 ± 2.5	15.0 ± 4.3
0.04	18.5 ± 2.5	18.0 ± 4.1
0.10	37.0 ± 9.0	23.2 ± 1.5
0.20	21.4 ± 4.0	20.3 ± 0.55
0.40	31.0 ± 4.0	33.3 ± 5.2
0.80	54.4 ± 8.0	57.5 ± 14.0
1.0	67.4 ± 9.5	52.3 ± 10.0

The values are the means (± S.E.M.) of n=4 (SHR) and n=3 (WKY) rats. They were compared by Unpaired Student's t test. Differences were considered significant at the $P < 0.05$ level.

Figure 3-18.

Histogram of mean (± S.E.M.) percentage reduction in mean arterial pressure in 10 - 12 week old male SH, WKY and Wistar rats (all n=7). Indicated are groups of animals between whom significant differences ($P < 0.05$) were found.

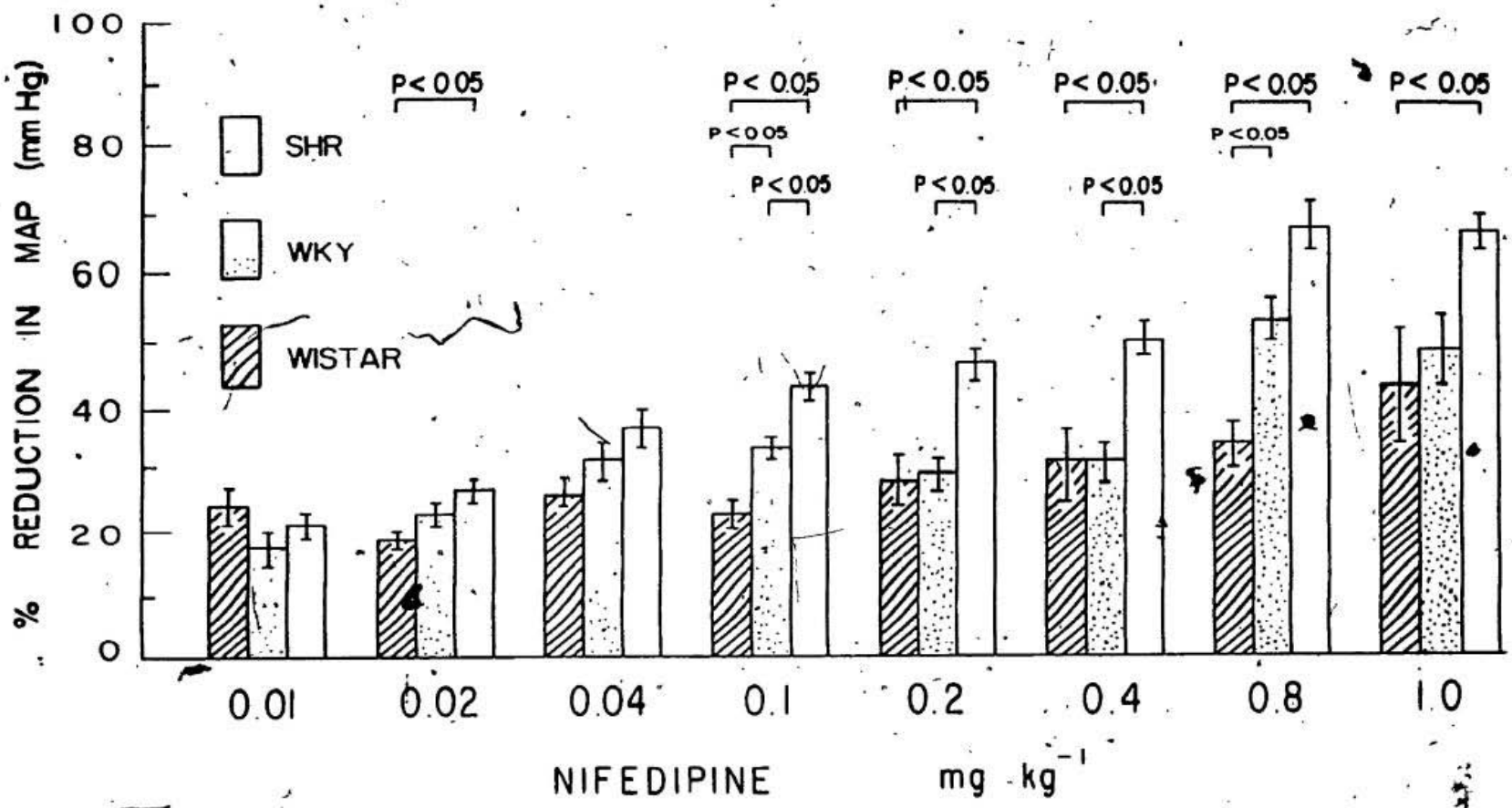

% REDUCTION IN MAP (mm Hg)
100
80
60
40
20
0
SHR
WKY
WISTAR
P< 0.05
0.01
0.02
0.04
0.1
0.2
0.4
0.8
1.0
NIFEDIPINE mg kg⁻¹
104

Table 3-12: Mean percentage decrease in mean arterial pressure of 10 - 12 week old male SH and WKY rats induced by nifedipine.

DOSE mg kg^{-1}	MAP DECREASE (%) SHR	WKY	WISTAR
0.01	21.1 ± 2.0	17.1 ± 3.3	24.7 ± 2.1
0.02	26.7 ± 1.7	22.8 ± 1.9	18.5 ± 1.5[a]
0.04	37.0 ± 3.5	31.6 ± 2.7	26.5 ± 1.9
0.10	43.3 ± 1.5	33.2 ± 1.8[b,c]	23.5 ± 2.7[a]
0.20	46.3 ± 2.5	29.1 ± 1.9[b]	27.9 ± 3.7[a]
0.40	51.0 ± 3.1	30.8 ± 2.7[b]	31.0 ± 5.8[a]
0.80	68.0 ± 4.3	53.7 ± 3.5[c]	34.1 ± 4.1[a]
1.0	67.4 ± 3.0	49.6 ± 5.8	43.4 ± 9.2[a]

The values are the means (± S.E.M.) of n=7 in all strains. Differences were considered significant (Scheffe's test) at the $P < 0.05$ level. [a] = significant difference between WISTAR and SHR, [b] = significant difference between WKY and SHR, [c] = significant difference between WKY and WISTAR.

Figure. 3-19.

Histogram of mean (± S.E.M.) percentage reduction in mean arterial pressure in > 20 week old male SH (n=10) and WKY (n=7) rats. Significant differences between SHR and WKY are indicated ($P < 0.05$).

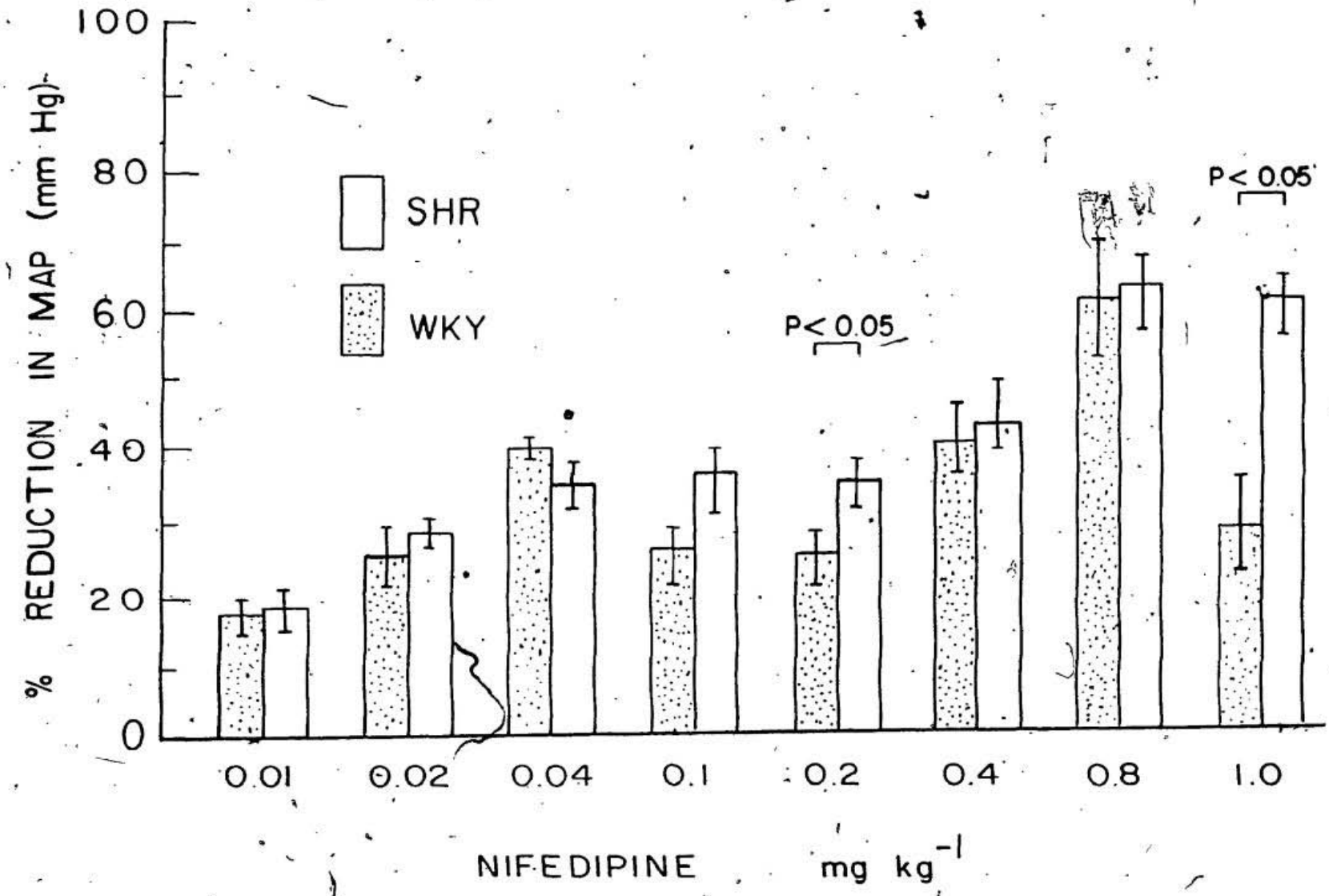

100
80
60
40
20
0
% REDUCTION IN MAP (mm Hg)
SHR
WKY
P< 0.05
P< 0.05
0.01
0.02
0.04
0.1
0.2
0.4
0.8
1.0
NIFEDIPINE mg kg⁻¹

Table 3-13: Mean percentage decrease in mean arterial pressure of > 20 week old male SH and WKY rats induced by nifedipine.

DOSE mg kg^{-1}	MAP DECREASE (%) SHR	WKY
0.01	18.4 ± 2.7	17.4 ± 2.7
0.02	28.1 ± 1.8	25.6 ± 3.3
0.04	35.3 ± 2.4	40.0 ± 1.8
0.10	36.1 ± 4.0	26.7 ± 3.3
0.20	35.0 ± 2.8	25.8 ± 3.1[a]
0.40	44.4 ± 4.5	41.3 ± 5.3
0.80	63.0 ± 5.0	61.6 ± 8.8
1.0	61.0 ± 4.2	30.0 ± 5.8[a]

The values are the means (± S.E.M.) of n=10 (SHR) and n=7 (WKY) rats. [a] = Significant difference (Unpaired Student's t test, $P < 0.05$) between WKY and SHR.

3.4. Radioligand Binding Studies.

3.4.1. Marker enzymes.

The activities of 5'ND and PDE_I were assessed in the post nuclear supernatant (PNS) and the microsomal fraction (MIC). The activity in the MIC fraction was divided by the activity in the PNS and a ratio obtained. This corresponds to the level of enrichment of plasma membrane. The ratios are shown in Table. 3-14. The level of enrichment was higher in WKY tail arteries than in SHR arteries, although no meaningful comparision can be made between the two strains as there were not sufficient observations for the SHR animals.

3.4.2. Radioligand binding studies.

Specific binding of ^{3}H-nitrendipine to the MIC fraction was saturable in presence of increasing concentrations of the ligand. Representative saturation curves and Scatchard plots are shown in Figure. 3-20 and 3-21 for SHR and WKY rats respectively. Scatchard analysis of the data revealed a straight line (Figure. 3-20 and 3-21 insert) with dissociation constant (K_d) values in the subnanomolar range (Table. 3-15). The B_{MAX} values were 194.0, 198.7, 224.0 and 477 fmoles mg^{-1} protein (Table. 3-15). In all assays, specific binding of labelled ligand was about 30% of the total binding.

Table 3-14: Level of enrichment of plasma indicated by levels of 5'ND and PDE_I in PNS and MIC fractions.

	RATIO OF MIC/PNS (O.D. min^{-1} mg^{-1} protein)	
	5'ND	PDE I
WKY (n=3)	9.48 ± 2.83	6.27 ± 1.02
SHR (n=1)	3.2	21.76

Table 3-15: K_d and B_{MAX} values derived from radioligand binding studies.

	B_{MAX} fmoles/mg protein	K_d (n*M*)	r -ve
SHR	198.7	0.187	0.97
WKY	224.0	0.361	0.97
WKY	477.0	0.26	0.95
WKY(denervated)	194.0	0.654	0.90

r = regression correlation.

(NB. No statistical test could be performed due to paucity of data.)

Figure. 3-20.

A representative example of the saturable binding of ^{3}H-nitrendipine binding to the membrane (MIC fraction) of tail artery from SHR animals. Each point is the mean of duplicates. Scatchard analysis of the binding data (inset) produced a straight line (r= -0.97). The dissociation constant (k_d) equalled 0.18n*M*. B_{MAX} (x axis intercept) was 198 fmoles mg^{-1} protein.

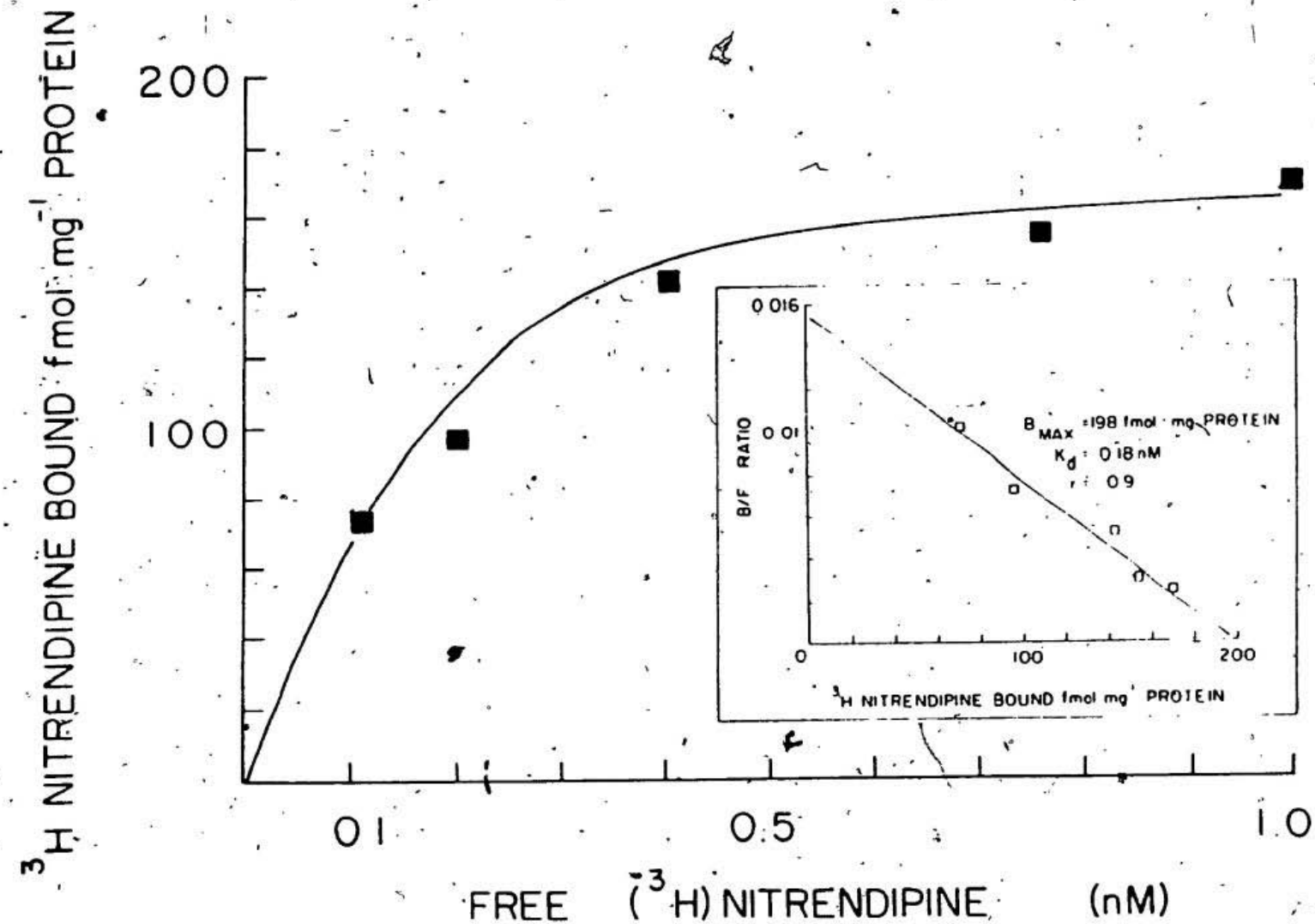
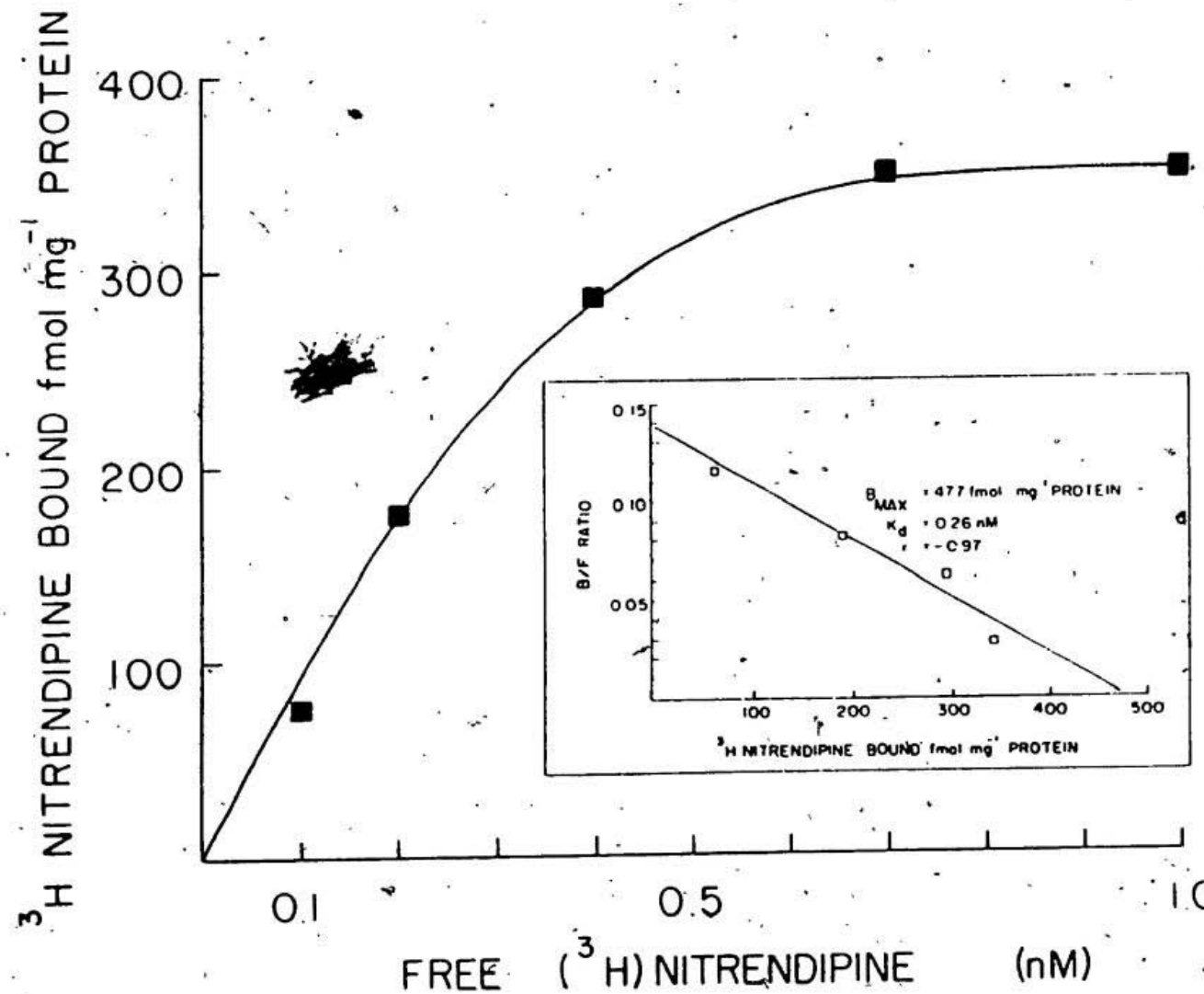

200
100
0.1
0.5
1.0
^{3}H NITRENDIPINE BOUND fmol mg^{-1} PROTEIN
FREE (^{3}H) NITRENDIPINE (nM)
0.016
0.01
B/F RATIO
B_{MAX} =198 fmol mg PROTEIN
K_d = 0.18 nM
r = 0.9
0
100
200
^{3}H NITRENDIPINE BOUND fmol mg^{-1} PROTEIN

Figure. 3-21.

A representative example of the saturable binding of ^{3}H-nitrendipine binding to the membrane (MIC fraction) of tail artery from WKY animals. Each point is the mean of duplicates. Scatchard analysis of the binding data (inset) produced a straight line (r= -0.95). The dissociation constant (k_d) equalled 0.26n*M*. B_{MAX} (x axis intercept) was 477 fmoles mg^{-1} protein.


^{3}H NITRENDIPINE BOUND fmol mg^{-1} PROTEIN
400
300
200
100
0.1
0.5
1.0
FREE (^{3}H) NITRENDIPINE (nM)
B/F RATIO
0.15
0.10
0.05
B_{MAX} = 477 fmol mg^{-1} PROTEIN
K_d = 0.26 nM
r = -0.97
100
200
300
400
500
^{3}H NITRENDIPINE BOUND fmol mg^{-1} PROTEIN

Chapter 4

DISCUSSION.

4.1. General Considerations.

4.1.1. Control and experimental animals.

In animal studies of essential hypertension, such as this thesis, where two or more strains of the same species are used, it is neccessary to define a reference point which would 'label' a strain 'hypertensive' or otherwise. In the clinical context, hypertension has been defined, by the World Health Organisation (WHO) as a blood pressure, that exceeds 160/95 mmHg, a 'normal' pressure being defined as 110/70 mmHg. In reality, it is to be expected that extensive variation in the blood pressures of animals will arise and the establishment of an acceptable pressure range thus helps to rid those animals which do not qualify as hypertensive. In experimental hypertension, animals with systolic blood pressures of at least 150 mmHg and over are considered to be, by most investigators to be hypertensive (Yamori, 1977). The development of hypertension in the SHR is thought to be a two stage process, where initially the high BP is a reflection of an increased cardiac output, but normal TPVR (5 -12 weeks), and subsequently an increase in TPVR but normal cardiac output. Thus hypertensive SHR can be considered so at the age of 12 weeks. The 'prehypertensive' stage has been defined

to encompass the ages of 6 to 12 weeks according to a number of reports, although pressures are significantly different at 5 - 6 weeks and even in 4 week old animals (Lais, Rios, Boutelle, DiBona & Brody, 1977). In the studies mentioned here, SHR and WKY rats were used mainly at the 10 - 12 week age group. In all the studies the systolic pressures of SHR exceeded 150 mmHg, indeed they were usually in the 170 mmHg range. These pressures were significantly different from those of WKY animals. Only in the 5 week age group of animals (*in vivo* study) were the systolic pressures below 150 mmHg. Nevertheless, the mean systolic pressures were significantly higher than those of WKY rats. These observations together with those of Lais *et al.*, (1977) indeed indicate that the 'prehypertensive' stage is at a lower age group than previously thought. It should be mentioned that in the in vivo study, the Wistar strain, which is the original parent strain for both SHR and WKY (Okamoto & Aoki, 1963), was used as an additional control in one of the studies. Whereas the mean systolic and diastolic pressures of SHR were significantly different from WKY and Wistar, there was no significant difference in mean systolic pressure between WKY and Wistar. In contrast to this, the mean diastolic pressure of Wistar rats was significantly higher than that of WKY rats (Table.3-10). The rats were all purchased from Charles River Inc., but at different times and in three different batches. Despite this, the systolic and diastolic pressures were found to be similar in all three batches.

The choice of the control animal, is important. The SHR and WKY, as mentioned above, were developed by in breeding (Okamoto & Aoki, 1963)

offsprings of Wistar rats which had a tendency to develop high pressure. In view of their common parental background (Wistar), the WKY can be considered to be 'abnormal', relative to the Wistar, as the SHR, the difference being in the blood pressures. McMurty, Wright & Wexler (1981) have argued that the use of WKY rats as the only control for SHR was not justified for various reasons (McMurty, Wright & Wexler, 1981). They report on the incompatibility of transplanted pituitary and adrenal glands between SHR and WKY rats but accepted by Sprague Dawley (SD) rats. Furthermore SD pups, but not WKY pups, nursed by SHR dams became hypertensive. In addition they base their contention on the dilution of strains as a result of the widespread disbursement of both SHR and WKY rats. I chose to include the Wistar strain in one of the *in vivo* studies (10 - 12 week age group) so that we could compare our data from the SHR to that of both the WKY and the Wistar.

4.1.2. Choice of vascular smooth muscle preparation.

Many early studies, directed towards the question of what role vascular contributions play in the development of hypertension, either involved experiments with perfused vascular beds or with large isolated vessels such as the thoracic aorta. The perfusion technique , which provides information regarding the combined effect of all vessels in a particular bed, does not, however, identify where within the bed specific changes in vascular smooth function are present. A number of studies with the rat have noted a significant fall in pressure in arteries with diameters larger than 100μm, indicating that a large proportion of TPVR is found proximal to the microcirculation (Fronek & Zweifach, 1975, Bohlen, Gore & Hutchins, 1977, Folkow, Hallback, Jones & Sutter, 1977). What then is the range of diameters one could use? In essence, all precapillary vessels (conduit, muscular & arterial) contribute, although to different degrees, to the total TPVR and therefore, by this definition, could be called resistance vessels. The term resistance vessel is thus used with respect to vessels which contribute significantly to TPVR. The range of diameters generally believed to constitute a resistance vessels is in the order of 100 - 500μm (Andersson, Hogestatt, Skarby & Uski, 1985), although recently, research concerned with isolated vessels, have utilised arteries having diameters in the range of 100 - 300μm (Mulvany, 1985). The tail artery has a diameter approximately of 500μm, or less, depending on the age of the rat and level within the tail, and thus should contribute to the TPVR in the rat.

4.2. In Vitro Study.

I have attempted to characterise calcium entry through receptor operated (ROC) and potential operated (POC) channels in blood vessels from both hypertensive and normotensive animals. In these studies I have used the agonist norepinephrine (NE) which induces contraction by mobilising Ca^{2+}, intracellularly, and by promoting Ca^{2+} influx through ROC and /or POC. KCl (K^+) on the other hand is known to depolarise VSM and induce Ca^{2+} entry through POC with resultant contraction. There is extensive pharmacologic evidence which is consistent with the notion that the 1,4 dihydropyridine class of compounds are potent and specific antagonists acting at a site or sites associated with Ca^{2+} channel function (Fleckenstein, 1977, Fleckenstein, 1983, Cauvin, Loutzenhiser & Van Breemen, 1983). Nifedipine has been shown to block Ca^{2+} entry through POC's in VSM (Godfraind, 1983, Triggle, 1984b).

Two protocols were used to assess the sensitivity of calcium channels to nifedipine. First I obtained responses to NE and K^+ after the tissues had been exposed to nifedipine for 30 minutes and related the tonic response to control tonic responses obtained in the absence of nifedipine. The tonic component is almost totally dependent on Ca^{2+}_{EXT} (Hurwitz & Suria, 1971, Steinsland, Furchgott & Kirpekar, 1973, Bevan, Garstka, Su & Su, 1973, Deth & van Breemen, 1974). Assessing the sensitivity of this component gives an indication of sensitivity of Ca^{2+} influx.

The second approach involved recording contractions in response to the cumulative addition of Ca^{2+} to tissues activated with NE or K^+, but maintained in a zero Ca^{2+} PSS. A third approach used by some investigators (Sutter, 1984), is to induce tone, and once stabilised, add the antagonist cumulatively. Of these three methods, the first two have been evaluated as being the most suitable for the investigation of the effects of CATS on blood vessels (Hof & Vuorela, 1983). In addition I used isotonic depolarising PSS (for K^+ activation) since tone induced by hypertonic depolarising buffers can have a component which is insensitive to the effects of CATS (Hof & Vuorela, 1983).

4.2.1. Post junctional sensitivity of tail artery to NE and K^+.

Tail artery ring preparations respond, in a dose-dependent manner, to NE and K^+. No significant differences in the sensitivity of tissues from both WKY and SHR to either NE or K^+ were found (Table. 3-3). The tissues from WKY tended to be slightly more sensitive (non significant) to NE in comparision to tissues from the SHR. I also compared the effect of cocaine on the NE dose- response curve since Laher & Triggle (1984a), using tail artery strip preparations from 14 week old SHR and WKY animals, had shown that $40\mu M$ cocaine caused a significantly greater leftward shift in the NE dose-response curve. The extent of shift was calculated as the ratio of NE ED_{50} (pre cocaine) to NE ED_{50} (post cocaine). They found ratios of about 4 for SHR vessels compared to 2 for WKY vessels. In this study the ratios were smaller for both SHR and WKY vessels. The ratio in SHR vessels (2.1 ± 0.32) was approximately half the value reported by Laher & Triggle

(1984a) whereas the values obtained for WKY vessels (1.83 ± 0.5) in the present study was similar to the earlier one. A major difference between the two studies relates to the source of the rats. Laher & Triggle (1984a) bred the animals used, at Memorial University, whereas in the present study all animals were obtained from commercial sources. After cocaine the vessels from WKY were still more sensitive than SHR vessels (Figure. 3-1). Mulvany, Nillson, Nyborg & Mikkelsen (1982) reported similar findings. In tail artery ring preparations from 14 week old SHR and WKY animals, Mulvany *et al.*, (1982), found a lower NE sensitivity in SHR vessels compared to WKY. Addition of 3 μM cocaine caused leftward shifts of equal magnitude in the NE dose-response curves of both SHR and WKY vessels. In contrast , Hermsmeyer (1976) and Webb, Vanhoutte & Bohr (1981), using helical strip preparations of rat tail arteries reported different results from mine. They both found the NE sensitivity of SHR strips, which had been chemically denervated by 6-OHDA, to be increased. Webb & Vanhoutte (1979) found that whilst NE sensitivity was not different in tail artery strips from SHR and WKY, the addition of cocaine caused a leftward shift of the NE dose-response curve only in tissues from SHR. The effect of cocaine is to inhibit the $Uptake_1$ mechanism (Iversen, 1962) which normally removes NE from the synaptic cleft. Comparing NE ED_{50} values in the absence and presence of cocaine, indirectly provides and index of the $Uptake_1$ mechanism. Many studies have indicated that the $Uptake_1$ mechanism functions at a more efficient rate in the hypertensive state. Why there are differences between results presented here and other studies cannot conclusively be explained, but differences in animals from different colonies,

technical differences, perhaps such as helical strips versus ring preparations may affect the results.

4.2.2. Nifedipine sensitivity.

In my studies, K^+ induced responses were more sensitive to nifedipine, than were NE induced responses. Whereas the difference in sensitivity to nifedipine was about one log unit between maximal NE (IC_{50} = 2.6 x $10^{-8}M$) and maximal K^+ (IC_{50} = 2.1 x $10^{-9}M$) induced responses in WKY animals, the difference was significantly larger in tissues from SHR animals (NE IC_{50} = 2.0 x $10^{-8}M$; K^+ IC_{50} = 3.9 x $10^{-10}M$). The higher sensitivity of K^+ induced responses is consistent with much of the literature published which indicate that in virtually all smooth muscle types, depolarization induced responses are more sensitive, than agonist induced responses, to CATS (Cauvin, Loutzenhiser & Van Breemen, 1983, Flaim, 1982).

These results are in contrast to receptor mediated contractions which exhibit varying sensitivities to CATS. This variation can exist both between blood vessels from the same animal or within a particular vascular bed (Cauvin, Loutzenhiser & Van Breemen, 1983, Flaim, 1982). The variation in sensitivity is thought to, indirectly, reflect the relative utilization of various Ca^{2+} mobilization routes, viz. ROC, POC and intracellular Ca^{2+} with different levels of activation of the receptor. Studies on rabbit aorta, superior mesenteric artery and mesenteric resistance vessels by Cauvin *et al.*, (1984) provide some evidence for one aspect of this concept. They found that the sensitivities of NE-induced contractions and $^{45}Ca^{2+}$ influx to diltiazem were inversely related to the ability of NE to liberate Ca^{2+}. In the rabbit aorta, at low concentrations of NE (1.0 x $10^{-8}M$, no Ca^{2+}_{INT}

release), the sensitivity to CATS was similar to that found in mesenteric resistance vessels. At higher concentrations of NE ($1.0 \times 10^{-5} M$, Ca^{2+}_{INT} release), the sensitivity to diltiazem was about four orders of magnitude lower than that observed in the mesenteric resistance vessels. In the latter vessels, $10\mu M$ NE induced Ca^{2+} influx was just as sensitive to diltiazem as 80 mM K^+ induced Ca^{2+} influx was. Sensitivity in the superior mesenteric artery, intermediate in terms of diameter of the vessel, was also intermediate between the other two vessels.

Thus one possible reason for the lower sensitivity of NE induced responses is due to intracellular release of Ca^{2+}. This internal mobilization is thought to be insensitive to antagonism by CATS (Saida & Van Breemen, 1983), although intracellular sites of action have been proposed (Church & Zsoter, 1980). In the Ca^{2+} sensitivity study (see below), stimulation of rat tail artery with NE, after removal of extracellular Ca^{2+}, resulted in a reduced phasic response and a severely reduced tonic component. Su, Swamy & Triggle (in press) in a study of tail artery strip preparations also found that after a 30 minute exposure to Ca^{2+} (no EGTA) PSS, NE at a concentration of $10\mu M$ induced a phasic response which on average was about 60% of the control, suggesting that Ca^{2+}_{INT} was being released. Cauvin *et al.*, (1984) have suggested that this intracellular Ca^{2+}, once released, may somehow decrease the sensitivity of the ROC to CATS. The present study does not allow us to verify this.

However studies in cardiac muscle cells and other types of cells (Miller, 1985)

provide some scope to explain a decrese in sensitivity. Bkaily & Sperelakis (1984) (Bkaily & Sperelakis, 1984) have provided data for cultured cardiac muscle cells which indicate that myocardial slow channels must be phosphorylated for the channel to be available for voltage activation. In neurones it has been shown that activation of protein kinase C (PrKC) augments (DeRiemer, Strong, Albert, Greengard & Kaczmarek, 1985) Ca^{2+} permeation through Ca^{2+} channels (i.e. POCs). Spedding (1986) has reported on the possibility of PrKC involvement in ROC mediated events. In addition to the facilitative functions, a role for negative feedback regulation of cell function has been ascribed to PrKC (Drummond & MacIntyre, 1985). In smooth muscle cells, phorbol esters, which activate PrKC, have been shown to promote α_1-adrenergic receptor uncoupling by phosphorylating the receptors (Leef-Lundberg, Cotechia, Caron & Lefkowitz, 1986). These observations provide sufficient basis to speculate upon the possibility that a protein kinase, probably calmodulin dependent, may in a similar manner 'act' at the ROC and affect its sensitivity to CATS at high doses of agonists.

The sensitivity of agonist-induced responses to CATS may also depend upon factors, other than coupling to post-receptor Ca^{2+} mobilization events, such as receptor subtype (α_1 or α_2) and the extent of receptor reserve. A possible role for postsynaptic α_2 adrenoceptors (see reviews by McGrath, 1982 & 1983) in addition to postsynaptic α_1 adrenoceptors contributing to vasoconstriction has been suggested based mainly on *in vivo* studies in the pithed rat (Timmermans & Van Zwieten, 1981). Definite evidence that postsynaptic α_2-adrenoceptors are involved

in VSM contraction have not been forthcoming clearly in studies with *in vitro* preparations from the arteriolar system. The characterization of the adrenoceptors mediating contraction in VSM is an area of intense investigation. The lack of availability of a highly selective full agonist at the α_2 receptor as well as the low specificity of the currently available α_2 antagonists compared to prazosin (α_1 antagonist) has contributed to a large number of inconclusive studies. Nevertheless α_2 adrenoceptors have been reported from *in vitro* preparations, canine venous smooth muscle (De Mey & Vanhoutte, 1981), feline cerebral vessels (Skarby, Andersson & Edvinsson, 1981) and rat tail arteries (Sprague-Dawley) (Medgett & Langer, 1984c, Weiss, Webb & Smith, 1983) and SHR (Medgett, Hicks & Langer; 1984a) rats. Furthermore it has been reported (Van Meel, Dejonge, Kalman, Wilfert, Timmermans & Van Zwieten, 1981, Van Zwieten, Van Meel & Timmermans , 1983), from *in vivo* studies that responses induced by α_2 adrenoceptor agonists are more sensitive to CATS than α_1-mediated responses. The sensitivity to CATS can therefore depend on where endogenously released NE acts (α_1 or α_2 adrenoceptors) and the relative dependencies of α_1 and α_2 adrenoceptor-mediated responses on Ca^{2+}_{EXT}.

The comparision of NE-induced responses in tail arteries, from SHR and WKY revealed no significant differences in sensitivity to nifedipine (Table. 3-4.). In addition, in so far as NE-induced responses are concerned, there were no differences in sensitivity to nifedipine between ED_{100} and ED_{50} levels of activation, in either of the strains. This suggests that (a) the ROC function is not

altered in the hypertensive state, and (b) the similarity of IC_{50} values for maximal and submaximal activation by NE, indicates that the role of Ca^{2+}_{EXT} is similar, irrespective of the strength of the stimulus. Paradoxically, however, there were differences in the magnitude of response to different doses of NE after Ca^{2+}_{EXT} removal (Ca^{2+} sensitivity study). It is interesting to note that Kannan & Seip (1986) found a differential sensitivity to nitrendipine in rat superior mesenteric artery when stimulated at ED_{25} and ED_{50} levels of NE. They speculated that the differential sensitivity was probably due to NE activating heterogenous receptors or that the Ca^{2+} channels associated with the NE response were heterogeneous. The lack of a differential sensitivity to nifedipine between the two doses of NE in my study could therefore be interpreted in terms of NE activating a less heterogeneous group of receptors or Ca^{2+} channels.

The sensitivity of K^+ induced responses to nifedipine, in VSM from SHR and WKY also do not show significant differences. When the IC_{50} values are compared between both strains at the ED_{100} level of activation, the difference in sensitivity is 3 fold ($IC_{50} = 3.9 \times 10^{-10} M$ and $1.3 \times 10^{-9} M$) for SHR and WKY respectively ($P > 0.05$). At the ED_{50} level of activation (30.6 ± 0.9 mM K^+) the sensitivity of WKY vessels does not change appreciably ($IC_{50} = 2.1 \times 10^{-9} M$), whereas in vessels from SHR there is a paradoxical decrease ($P > 0.05$) in sensitivity to nifedipine ($IC_{50} = 1.1 \times 10^{-9} M$). This suggests that POC function does not alter in the hypertensive state.

In a similar study, Su, Swamy & Triggle (in press), with tail artery strip preparations from 15-17 week old SHR and WKY animals found no significant difference in the sensitivities of K^+ , NE, phenylephrine (α_1 agonist) or BHT-920 (α_2 agonist) induced responses to the inhibitory actions of the calcium antagonist D600. They interpreted their results as suggesting a lack of difference in the 'calcium channel antagonist' pathway (ROC/POC) between SHR and WKY.

It is interesting to note that despite the differences in methodology and the calcium antagonist used by Su, Swamy & Triggle (in press) their results are similar to those presented in this thesis. In the first instance they used D600 (methoxyverapamil) a calcium antagonist, which is not as specific for VSM as is nifedipine. Secondly and perhaps more important is that in inducing responses by K^+ (80m*M*) they used hypertonic solutions. In my studies, where the ED_{100} dose was 60m*M* K^+, only isotonic buffers were used. The use of hypertonic depolarising buffers can compromise the sensitivity of responses to CATS as part of the response is insensitive to such drugs (Hof & Vuorela, 1983). In addition it has been shown that the use of hypertonic media results in the utilization of intracellular sources of Ca^{2+} (Andersson, Hellstrand, Johansson & Ringberg, 1972) which, thus, confounds the analysis of the results.

To date, the results of most studies relating to calcium movements in the hypertensive state have not conclusively demonstrated whether enhanced Ca^{2+} influx occurs through ROC or POC. Mochizuki, Yamamoto, Kondo, Aoki, Mizuno

& Hotta, (1979) reported that verapamil produced a greater reduction in 5-hydroxytryptamine (5-HT) or NE induced tension in aortae from SHR compared to WKY. In another study (Pedersen *et al.*, 1978), ring preparations of SHR thoracic aorta were reported to be more sensitive to nifedipine when stimulated both with NE (18μM) or K^+ (127mM). In the same study Ca^{2+} deprivation studies also showed that the NE and K^+ induced responses in SHR relaxed more rapidly and to a greater magnitude than those from WKY, indicating that the responses were more dependent on Ca^{2+}_{EXT} for the response. In contrast, Nghiem *et al.*, (1982) reported a decreased sensitivity of NE and K^+ induced responses, in SHR aorta, to D600, compared to WKY aortae. However, when Ca^{2+}_{EXT} was removed and the tissues exposed to D600, the NE response in SHR aorta was more sensitive than tissues from WKY. The degree of inhibition of K^+ induced responses under the same conditions, in the presence of D600 was similar for both SHR and WKY. Recently Kazda, Garthoff & Knorr (1985) assessed the sensitivity of α_1 (phenylephrine) and α_2 (BHT-920) induced responses to nisoldipine, an analog of nifedipine. They found that α_2 induced responses were more sensitive, than α_1 induced responses, in aorta from stroke prone spontaneously hypertensive rats (SHRSP), suggesting a higher availability of Ca^{2+}, mediated through a Ca^{2+} entry pathway associated with α_2 receptors. They did not, however, assess sensitivity to K^+ induced responses. It has yet to be shown as to what extent α_2 induced responses in the aorta depend on Ca^{2+}_{EXT}. As mentioned before, α_1 and α_2 adrenoceptors have been reported to be present, in rat tail arteries, with a predominance of the former. Furthermore it has been proposed that α_2

adrenoceptor, but not α_1 adrenoceptor mediated responses are dependent more on Ca^{2+}_{EXT} (Medgett & Rajanayangam, 1984b). Such α_2 adrenoceptors have been reported to mediate the vasoconstrictor response to NE (endogenous and exogenous) to a greater extent in SHR tail arteries (Medgett *et al.*, 1984a). Recently Hicks, Tierny & Langer (1985) found that α_2 adrenoceptor mediated responses in SHR tail arteries were more sensitive to diltiazem than were α_1 adrenoceptor mediated responses in WKY tail arteries. Collectively these studies suggest a greater Ca^{2+} influx through ROC in the hypertensive state. It should be emphasized that the above described results are from perfused segments of rat tail arteries. Similar results have not been demonstrated when helical strip preparations of rat tail arteries have been studied (Su *et al.*, in press).

A possible reason for the differences in results could relate to the role of the endothelium as a modulator of contractile agonist effects. In the rat aorta responses to the α_2 agonist clonidine are much lower than to NE (Egleme, Godfraind & Miller, 1984). Removal of the endothelium enhanced the response to both NE and clonidine, such that the response to the latter became almost equal to that produced by NE. Similar responses in the rat aorta have also been reported with oxymetazoline and UK 14-304 (Godfraind, Egleme & Osache, 1985). Indeed, in the perfused rat tail artery, Matsuda, Kuon, Holtz & Busse (1985) have shown that responses induced by luminal application of α_2 agonists were augmented after endothelial removal. In contrast, α_1 agonist-induced responses were not affected by endothelial removal.

Table 4-1: Summary of vascular smooth muscle sensitivity to calcium antagonists.

Tissue	Agonist	Antagonist	Finding	Ref
Aortic Strips	NE	D600	ND between SHR & WKY (normal calcium levels)	(1)
			↑ sensitivity in SHR (low calcium levels)	(1)
Aortic Strips	NE	D600 Felodipine	ND between SHR & WKY	(2)
Aorta	NE	Nifedipine	↑ sensitivity in SHR	(3)
Aortic Strips	NE	D600	ND between SHR & WKY	(4)
			↑ sensitivity in SHR (at low D600 concn.)	(4)
Aortic Strips	KCl	D600 Felodipine	ND between SHR & WKY	(2)
Aorta	KCl	Nifedipine	↑ sensitivity in SHR	(3)
Aortic Strips	KCl	D600	↓ sensitivity in SHR	(5)
			ND between SHR & WKY (no external calcium)	(5)
Aortic Strips	KCl	Verapamil	↑ sensitivity in SHR (at low verapamil concn.)	(6)
			↓ sensitivity in SHR (at high verapamil concn.)	(6)

continued..

Table. 4-1 continued..

Tissue	Agonist	Antagonist	Finding	Ref
Aortic Strips	Clonidine	D600	ND between SHR & WKY	(4)
			↓ sensitivity in SHR (at high D600 concn.)	(4)
Aortic strips	PGE_2	Verapamil	↓ sensitivity in SHR	(6)
	Methoxamine	Verapamil	↑ sensitivity in SHR	(6)
Carotid art Strips	NE	D600	↓ sensitivity in SHR	(5)
			↑ sensitivity in SHR (no external calcium	
Iliac art Strips	NE	D600	ND between SHR & WKY	(5)
			↑ sensitivity in SHR (no external calcium)	
Carotid art Strips	KCl	D600	↓ sensitivity in SHR	(5)
			↑ sensitivity in SHR (no external calcium)	
Iliac art Strips	KCl	D600	ND between SHR & WKY	(5)
			↑ sensitivity in SHR (no external calcium)	

continued..

Table. 4-1 continued..

Tissue	Agonist	Antagonist	Finding	Ref
Tail art. Strips	NE Phenylephrine B-HT 920	D600	ND between SHR & WKY	(8)
Tail art. Ring	NE	Nifedipine	ND between SHR & WKY	(9)
Tail art. strip	KCl	D600	ND between SHR & WKY	(8)
Tail art. Ring	KCl	Nifedipine	↓ sensitivity in SHR (at ED_{50} levels of KCl)	(9)
Portal V. Strip	NE	D600	↓ sensitivity in SHR	(1)
Portal V. Strip	NE	Nifedipine Nitrendipine Nisoldipine	ND between SHR & WKY	(7)
Portal V. Strip	KCl	Felodipine	ND between SHR & WKY	(2)
Portal V. Strip	KCl	Nifedipine Nisoldipine Nitrendipine	ND between SHR & WKY	(7)

ND= No difference in sensitivity.

(1)= Pang & Sutter (1981), (2)= Sutter (1985), (3)Pedersen *et al.*, (1980)
(4)= Nghiem *et al.*, (1980), (5) Nghiem *et al.*, (1982b), (6) Levy (1975),
(7)=Harris *et al.*, (1983), (8)=Su *et al.*, (in press), (9)= This study.

The use of the portal vein, in preference to conduit vessels such as the aorta, as a model of a resistance vessel, because of Ca^{2+}_{EXT} dependency and spontaneous discharges, has been advocated by Sutter *et al.*, (1977). Harris, Swamy & Triggle (1983) and Pang & Sutter (1981) both assessed the sensitivity of NE and K^+ induced responses to CATS and found no differences in Ca^{2+} translocation or permeability sites. These and other studies are summarised in Table. 4-1.

The methods used for assessing sensitivity reflect the CATS sensitive influx route, however, utilization of Ca^{2+}_{INT} may confound the interpretation of these results. In the second part of this study the latter factor was controlled. Thus the utilization of Ca^{2+}_{INT} was minimised by Ca^{2+}_{INT} depletion in a Ca^{2+} free media followed by activation in a zero Ca^{2+} buffer. Extracellular Ca^{2+} levels were then increased and responses measured. Thus Ca^{2+} entry into the cell was assessed. The results from these studies are discussed below.

4.3. Calcium Sensitivity Study.

In an attempt to explain an enhanced NE sensitivity in 6 week old SHR, Mulvany & Nyborg (1980) measured the Ca^{2+} sensitivity of mesenteric resistance vessels in the presence of NE and K^+. When the vessels were fully activated with 10μM NE or 125mM K^+, and after Ca^{2+}_{INT} had been depleted, the SHR vessels had a higher Ca^{2+} sensitivity. This increase in sensitivity was only observed when the vessels were stimulated with NE and not with K^+. At submaximal doses of NE and K^+ the Ca^{2+} sensitivities were generally lower but the tissues from SHR were still more sensitive than comparable tissues form the WKY. They reasoned that either the 'NE channels' (ROC) were more permeable or there were more of them. I have in this study demonstrated, under similar conditions, that there is indeed an enhanced Ca^{2+} sensitivity in tail arteries from SHR but not in WKY, when stimulated with NE. Responses to exogenous Ca^{2+} are shown in Figures. 3-6 and 3-7 in presence of NE and K^+ respectively. Ca^{2+} responses in the presence of submaximal doses of K^+ and NE reached maximum at Ca^{2+} concentrations of 5mM whereas under maximal stimulation the 100% response was measured at 10mM Ca^{2+}. The pD_2 values calculated from results obtained in the presence of submaximal doses of NE and K^+ were all significantly higher (greater Ca^{2+} sensitivity) than corresponding values obtained when maximal doses of NE and K^+ were used (Table. 3-6). Only in WKY vessels stimulated with NE was there no significant difference. This in contrast to the study in rat mesenteric resistance vessels where the Ca^{2+} sensitivities are lower at submaximal doses of activation with either NE (1μM) or K^+ (50mM) (Mulvany & Nyborg, 1980).

A significant increase in Ca^{2+} sensitivity in SHR (pD_2 = 3.45 ± 0.04 SHR; 3.26 ± 0.06 WKY) is only evident at the ED_{50} (0.70 μM) level of stimulation with NE and not at ED_{100} (10μM) levels. Mulvany, Nyborg & Mikkelsen, (1982) also found no significant difference in Ca^{2+} sensitivity in rat tail artery from SHR when stimulated at a ED_{100} (10μM) dose of NE. Such a high concentration of NE presumably results in the optimal activation and opening of NE operated channels, but whether this is of physiological significance is debatable. The use of supramaximal doses of NE merits further mention. In the rat tail artery, whose resting E_m is about -54mV (Hermsmeyer, 1976, Hermsmeyer, Trapani & Abel, 1981), contractions induced by KCl will not occur unless the membrane potential is reduced to at least -49mV (Cheung, 1984). In my study the threshold for KCl induced responses was at 15mM (Figure. 3-3), which shifts the E_m to about -45mV (Hermsmeyer, 1976).About 90% of the contraction occurs when the E_m is changed to between -25mV to -20mV (Hermsmeyer *et al.*, 1981). This shift in E_m can be achieved by increasing the K^+ concentration to about 60mM (Hermsmeyer, 1976, Hermsmeyer *et al.*, 1981). Presumably most of the POC are open during this change in E_m from the threshold E_m. Therefore in order to activate POC's, using depolarising buffers, one does not need to use supramaximal levels (eg. 127mM Pedersen *et al.*, 1981) of K^+. Such high concentrations of K^+ will probably totally eliminate membrane potential and there could be changes in Ca^{2+}_{INT} and phosphorylated protein pools thought to control contraction (Conti, Adelstein, 1980).

Based on the results obtained from this study it can be concluded that the effects of submaximal doses of agonists should also be studied since such conditions most likely reflect physiological conditions and any differences observed can thus be related to the disease process.

Thus whilst my data are in contrast to those of Mulvany *et al.*, (1982) in rat tail artery, they are consistent with previous studies where enhanced Ca^{2+} sensitivities were found in mesenteric resistance vessels (Mulvany & Nyborg, 1980) and femoral resistance vessels (Mulvany *et al.*, 1982). The studies described were conducted on animals which were 12 weeks old and as such the increased Ca^{2+} sensitivity could either be a primary defect, or secondary and thus a consequence of the increase in pressure. In addition, neurogenic influence may also affect the sensitivity. Mulvany, Korsgaard & Nilsson (1981) have, however reported increased Ca^{2+} sensitivities in SHR pretreated with 6-OHDA from birth until the third week. Chemical denervation reduced pressure in both SHR and WKY, but did not affect the difference in Ca^{2+} sensitivity present in adult untreated rats. Thus, despite a reduction in pressure and removal of possible neurogenic influences, the Ca^{2+} sensitivity differences persisted. Furthermore, prevention of hypertension, in the same model, by hydralazine and felodipine treatment did not affect the changes in heart/body weights, media/lumen ratios, and the increased Ca^{2+} sensitivites (Mulavny, Mikkelesen, Pedersen, Nyborg & Jepersen, 1983). In an another study (Mulvany & Korsgaard, 1983), the relation between the three cardiovascular parameters mentioned above and blood pressure in SHR/WKY F_2-hybrids were

assessed. Of the three only Ca^{2+} sensitivity of resistance vessels showed a significant correlation with systolic blood pressure. These results, together with earlier reports of increased Ca^{2+} sensitivity at the 4 week age group, suggest that increased blood pressure could be due to increased Ca^{2+} influx and that this influx is mediated through NE regulated channels (ROCs). A word of caution is in order at this stage. The finding of increased Ca^{2+} sensitivity does not imply that altered channel function is necessarily the cause of increased blood pressure, since a net increase in Ca^{2+}_{INT} can result from other changes such as changes in Ca^{2+}_{INT} metabolism (see section 1.4).

In studies with portal vein from SHR and WKY, Harris, Swamy & Triggle (1983) found a higher sensitivity to Ca^{2+} in presence of K^+ in the hypertensive state. With NE no such differences were apparent (Harris, Swamy, Triggle & Walters, 1980). Lipe & Moulds (1985) found no significant differences between digital arteries, from hypertensive and normotensive patients, in Ca^{2+} pD_2 values determined in the presence of K^+ or NE in a Ca^{2+} free medium. Nor were there significant differences in the ability of verapamil to reduce maximal responses induced by NE. Lipe & Moulds (1985) interpreted their results in terms of there being no abnormality of the mechanisms regulating Ca^{2+} ion entry and release in VSM, at least in human digital arteries.

In view of the reported Ca^{2+} sensitivity changes, I further extended my study to assess the effect of nifedipine on Ca^{2+} entry through ROC and POC, into the cell.

Ca^{2+} sensitivity was thus assessed after exposure of the vessels to varying concentrations of nifedipine. Nifedipine (0.05 and 1.0n*M*) affected the NE activated Ca^{2+} response curve equally in both SHR (Figure. 3-12.) and WKY (Figure. 3-13.) tail arteries. This is clearly shown if one compares the change in Ca^{2+} pD_2 from control valus to that in presence of nifedipine (Table. 3-7). The reduction in Ca^{2+} sensitivity is non-significant ($P > 0.05$). When the nifedipine concentration was increased to 100n*M* a significant rightward shift was observed for both SHR ($P < 0.01$) and WKY ($P < 0.05$) Ca^{2+} dose-response curves. Responses to Ca^{2+} in the presence of NE were more sensitive to nifedipine in WKY vessels than in SHR, suggesting a possible alteration in ROC. The Ca^{2+} response curve (WKY), in presence of 1.0n*M* nifedipine, had a greater maximal response than the combined control response curve. No explanation can be offered for this anomaly, particularly since control responses had been reproducible.

A rightward shift in Ca^{2+} dose-response curves is observed in K^+ activated vessels from both SHR (Figure. 3-14.) and WKY (Figure. 3-15.) at the two lower doses of nifedipine (0.05 and 1.0n*M*). The resultant reduction in Ca^{2+} pD_2 (ie. reduced sensitivity) is greater ($P < 0.05$) in tissues from SHR vessels (Table. 3-8) exposed to 0.05n*M* nifedipine. At higher concentrations the shift in Ca^{2+} pD_2 is significant in both SHR ($P < 0.05$) and WKY ($P < 0.05$) vessels. Also apparent in this study is the selectivity of nifedipine for Ca^{2+} responses induced by depolarisation over that induced by NE. This is clearly illustrated by the almost complete inhibition of the Ca^{2+} responses in presence of 100n*M* nifedipine when

vessels were activated by K^+. At this concentration of nifedipine, the Ca^{2+} responses in NE activated vessels were about 30% (WKY) and 60% (SHR) of the control response. The results collectively suggest a lower sensitivity of the ROC and a higher sensitivity of POC in the SHR, to nifedipine.

A lower sensitivity to nifedipine has been reported by Kannan, Seip & Crankshaw (1986) in the aorta of SHR. They reported that Ca^{2+} induced responses in K^+ activated SHR aorta were more resistant to nifedipine than were WKY aorta as indicated by greater rightward shifts in the Ca^{2+} dose-response curves, in the latter, with increasing nifedipine concentrations. No differences between SHR and WKY tissues with regards to Ca^{2+} responses were observed, under similar conditions, in the superior mesenteric artery. They also reported that aortic responses to K^+ were more dependent on Ca^{2+}_{EXT} than responses to K^+ in the superior mesenteric artery. It was argued by Kannan *et al.*, (1986) that the decrease in sensitivity to nifedipine could be due to either alterations in the activation process for POCs or that the extent of depolarisation in SHR aorta was less than that in WKY. Alternatively, although not suggested by Kannan *et al.*, (1986), SHR aorta may be more dependent on internal Ca^{2+} mobilization in response to K^+ stimulation and that internal release is enhanced in vascular tissue from SHR. Whether depolarisation is different in the aorta is not known, but at least in the rabbit aorta, NE induced responses are not 'electro-mechanically coupled' (Cauvin *et al.*, 1985) (ie. response is more or less due to operation of ROC and Ca^{2+}_{INT}). Aortic tissue from the SHR, compared to WKY has also been

reported to be less sensitive to verapamil in the concentration range of 10^{-8} to $10^{-6}M$ (Levy, 1975).

The lower sensitivity of ROC to nifedipine and the increase in sensitivity to Ca^{2+} in the presence of NE (previous study) requires comment. Why should there be an apparent decrease in sensitivity? In the first place the increase in Ca^{2+} sensitivity is only observed at ED_{50} levels of NE, there being no difference at the ED_{100} level. The nifedipine study investigated Ca^{2+} responses under maximal levels of activation with NE. At such levels of activation, a higher proportion of Ca^{2+} channels are presumably open through which Ca^{2+} enters. The amount of Ca^{2+} entering would therefore be expected to be higher than at submaximal levels of the agonist. McGrath (1985) has reported that in the isolated rat anococcygeus smooth muscle preparation, responses to submaximal levels of agonist are nifedipine sensitive whereas maximal responses were nifedipine insensitive. It is therefore possible that the higher amount of Ca^{2+} in the cell, in some manner, affects the sensitivity of ROCs to nifedipine and that this decreased sensitivity is more significant in tissues from the SHR. Indeed inactivation of POCs by Ca^{2+}_{EXT} has been described by Hurwitz, McGuffe, Smith & Little (1982) in the guinea pig ileal longitudinal muscle.

In any case, such changes in sensitivity, in the presence of CATS are not new. Kawaguchi, Aoki, Yamamoto & Hotta (1982) reported a decreased sensitivity to Ca^{2+} in the SHR mesenteric artery when activated with either NE or K^+. The

sensitivity of the NE activated Ca^{2+} responses, to diltiazem, was increased in SHR arteries compared to responses in WKY, suggesting changes in ROCs and POCs.

Further studies with more potent 1,4,dihydropyridines on Ca^{2+} responses have been reported by Nyborg, Byg-Hansen & Mulavny (1985). Using felodipine, which is more potent (1000 fold) than either nifedipine, or D600, as an antagonist of either K^+ or NE responses (Nyborg & Mulvany, 1984), it was reported that mesenteric resistance arteries from SHR were more sensitive to the antagonist than WKY arteries. Unfortunately, only the sensitivity of NE activated arteries, and not K^+ activated arteries was assessed.

The studies mentioned here have provided evidence, that in hypertensives there may be a geater role for calcium channels in mediating Ca^{2+} influx into VSM cell, which ultimately leads to increased tone and hence increased TPVR. Most investigators interpret increased sensitivity to CATS in terms of there being an abnormality of the 'system' on which such compounds act. Since CATS preferentially act at POCs it would seem logical to extrapolate such findings and postulate that the role of POCs is altered in the hypertensive state. It has been argued that this extrapolation may not necessarily be true (Nyborg *et al*, 1985), since NE activation can be membrane potential sensitive ie., NE activation of vessels causes membrane depolarization, such that the response to NE is probably in part due to Ca^{2+} influx through POCs. They further suggest that the differences lie in the ROCs, if activation of these leads to greater depolarisation in

SHR vessels ie., more ROC activation leading to greater depolarisation. Hermsmeyer *et al.*, (1981) have postulated that the membrane potential of VSM is the major factor controlling VSM tone. In SHR tail arteries, Hermsmeyer has reported that the extent of depolarisation upon increasing doses of exogenous NE is greater in SHR than in WKY (Hermsmeyer, 1976). The basis for this increase in depolarisation is an apparent decrease in intracellular K^+ in SHR vessels resulting in a decreased K^+ equilibrium potential (E_K). A reduced E_K in arterial muscle would increase the sensitivity to vasoactive agents, such as NE, which mediate their action through membrane depolarisation. Agonist stimulation which depolarises E_m, leads to increases in ionic conductances, including Ca^{2+}, through voltage sensitive channels (Haeusler, 1983, Harder & Sperelakis, 1979). Therefore it would not be inconsistent to suggest that as a result of greater depolarisation, more POCs are activated in SHR tail arteries. For a given concentration of NE more Ca^{2+} could enter the cell through these POCs. This increase in utilization of POCs could occur with or without changes in ROC function.

Some evidence for this comes from studies on arteries which have intrinsic (myogenic) tone. As mentioned in the Introduction, this tone is the most important determinant of vascular resistance. It is present even in the absence of external stimuli and may have an autoregulatory function (Bevan, 1985). Myogenic tone is dependent on Ca^{2+}_{EXT} (Hwa & Bevan, 1986). In cat and rat cerebral arteries, elevation of transmural pressure results in cell depolarisation and action potential generation (Harder, 1984, Halpern, Mongeon & Root, 1984) which

appear to be due to pressure mediated increases in Ca^{2+} permeabilities. In middle cerebral artery from SHR and WKY rats, the effect of increasing transmural pressure is a corresponding depolarisation. However this depolarisation is greater in middle cerebral arteries from SHR animals than WKY animals (Harder, Smeda & Lombard, 1985). Whether this enhanced depolarisation is due to alterations in fluxes of Ca^{2+} or some other ionic mechanisms is not known, but in SHR arterial muscle enhanced Ca^{2+} and other divalent cation permeabilities have been demonstrated (Shibata, Kurahuchi & Kuchii, 1973, Jones, 1974, Bohr, 1974, Noon, Rice & Baldessarini, 1978, Goldberg & Triggle, 1977). For a recent review see Jones (1982). Irrespective of the underlying cause of enhanced depolarisation, the end result is the same: enhanced Ca^{2+} entry (through POCs) into the VSM cell (Johansson & Somlyo, 1980) and therefore tone. An indication of this is provided by the work of Harder *et al.*, (1985). In addition to measuring E_m changes with increasing pressures they also assessed the effect of verapamil on tone in response to pressure changes. They found that at transmural pressures of 100mmHg and above, active tone was greater in SHR cerebral arteries than in WKY arteries. In WKY vessels the sensitivity to verapamil decreased with increasing pressure.

Therefore even in the absence of NE stimulation, enhanced Ca^{2+} entry can take place through POCs. However, whether this phenomenon is of physiological significance does not need to be determined, although it would seem logical to assume that pressure related changes in tone should contribute to TPVR. An increase in myogenic activity with enhanced contraction in response to stretch, the

Bayliss response, might be expected to elevate peripheral resistance, under conditions of increased cardiac output. In the SHR, the developmental phase of hypertension is characterised by an increase in cardiac output, but normal TPVR (Albretcht, 1974, Preutitt & Dowell, 1978).

Whether an enhanced Ca^{2+} sensitivity, as found in the tail artery in the studies described in this thesis or in other tissues (Mulvany & Nyborg, 1980) is related to the rise in BP can be questioned. It is possible that there is no such relationship and that the observed differences are due to the unique genetic makeup of the strains. That enhanced Ca^{2+} sensitivity may not be related to high BP is supported by studies on SHR, WKY and Wistar rats (Mulvany & Nyborg, 1983). They reported enhanced NE activated Ca^{2+} sensitivity in both SHR (MAP= 138 mmHg, Ca^{2+} pD_2=4.09) and normotensive Wistar (MAP= 110 mmHg, Ca^{2+} pD_2= 4.03) mesenteric resistance vessels. The sensitivity (Ca^{2+} pD_2= 3.8) of WKY (MAP=111 mmHg) vessels was, however, lower.

Nevertheless, on the basis of the Ca^{2+} sensitivity study indicating differential effects of CATS in hypertensive than in normotensive vessels, greater effects on peripheral resistance should be found under *in vivo* conditions in hypertensive animals and humans. Studies related to this hypothesis are discussed in the next section.

4.4. In Vivo Study.

In the present study, acute intravenous (i.v.) infusion of nifedipine caused dose dependent reductions of mean arterial pressure (MAP) in anesthetised SHR, WKY and Wistar rat. This dose dependent decrease in MAP was observed in all the three age groups studied. In the 5 week age group of animals, which is considered by some to be 'prehypertensive' in SHR (see Lais, Boutele, DiBona & Brody, 1977. for references) , the MAP of SHR was significantly higher ($P < 0.05$) than that of WKY rats. Lais *et al.*, (1977) also reported significantly higher pressures in 4 week old SHR than in age matched WKY rats and thus suggested that only those SHR of 3 weeks of age or younger should be classed prehypertensive.

In the 5 week old age group the effect of nifedipine was not significantly different between SHR and WKY rats. In contrast to this, nifedipine lowered MAP to a significantly greater ($P < 0.05$) extent in SHR compared with WKY or Wistar rats in the 10-12 week age group. With respect to WKY rats the fall in pressures were not significantly different from SHR at the low doses of nifedipine (0.01, 0.02 & 0.04 mg kg^{-1}), only at 0.1, 0.2 and 0.4 mg kg^{-1} nifedipine were there significant differences. When one compares the sensitivity of WKY and Wistar rats to SHR rats, the Wistar is more resistant to the hypotensive effect of nifedipine than the WKY strain. Within the 20 week age group, there is a reduction in sensitivity to nifedipine .i.e. the differential sensitivity to nifedipine observed in the 10-12 week age group is reduced.

Nifedipine is known to reduce/inhibit excitation contraction coupling in VSM by inhibiting Ca^{2+} entry through POCs (Godfraind, 1983, Triggle, 1984b). The antihypertensive effect of CATS has been suggested to result from vasodilation of peripheral vessels (Vater, Kroneberg, Hoffmeister, Kaller, Merg, Oberdof, Puls, Schlossman & Stoepel, 1972, Ogura & Hashimoto, 1974). With this knowledge, and together with other *in vivo* data, it has been postulated that there is an abnormally high Ca^{2+} influx in the hypertensive state (Kwan, 1985b) coupled with a reduced membrane Ca^{2+}ATPase. Furthermore, and in view of the relative selectivity of CATS such as nifedipine, nitrendipine and nicardipine for POCs, and the differential sensitivity in hypertensives, it has been argued that this postulated higher Ca^{2+} influx is mediated through POCs (Ishii *et al.*, 1980, Robinson, 1985).

Similar results to those presented here have been reported by other researchers in different animal models of hypertension. In SHR, nifedipine administered i.p., at a dose which produced a marked fall in pressure had no effect in normotensive controls (Iriuchijima, 1980). In DOCA-NaCl hypertensive rats the hypotensive effect of nifedipine (p.o), was greater than that induced by hydralazine. Hydralazine itself was more potent in normotensive control animals. A selective hypotensive effect following acute administration has also been shown in the Dahl-Salt-Sensitive (DSS) rat model of hypertension (Sharma *et al.*, 1984). These and other studies are summarised in Table. 4-2.

Such animal studies are complemented by studies on human essential

Table 4-2: Antihypertensive effects of calcium antagonists.

Drug	Dose mg/kg (Route)	Animal	Finding	Ref
Verapamil	0.5 (i.v.)	SD/Wistar	↓ MAP 49%	(1)
Nifedipine	0.5 - 5.0 (i.v.)	DSS(LS)	↑ sensitivity compared to DSR(LS)	(2)
		DSS(HS)	↑ sensitivity compared to DSR(HS)	
	5.0 (p.o.)	SHR	↓ SAP 49%	(3)
		WKY	↓ SAP 22%	
	0.3 (i.p.)	SHR	↓ SAP 16%	(4)
		DOCA-NaCl	↓ SAP 37%	
		WKY	NS in SAP	
	3.0 (p.o.)	SHR	↓ MAP 30%	(5)
		WKY	↓ MAP 18%	
Nitrendipine	3.1 (p.o.)	SHR	↓ SAP 30%	(6)
		DOCA-NaCl	↓ SAP 31%	
		WISTAR	↓ MAP 19%	
Nisoldipine	1.0 - 3.1 (p.o.)	SHR	↓ SAP 12 - 18%	(8)
		Rat Renal(1K.1C)	↓ SAP 8 - 39%	

continued

Table. 4-2. continued...

Drug	Dose mg/kg (Route)	Animal	Finding	Ref
Nifedipine	0.01 - 1.0 (i.v.)	SHR*#	↓ MAP (5 week animals)	(7)
		WKY*	↓ MAP. NS with SHR (5 week animals)	
	0.01 - 1.0 (i.v.)	SHR*	↓ MAP (10 - 12 week animals	(7)
		WKY*#	↓ MAP[a] (10 - 12 week animals)	
		WISTAR*	↓ MAP[b] (10 - 12 week animals)	
	0.01 - 1.0 (i.v.)	SHR*	↓ MAP (> 20 week animals)	(7)
		WKY*	↓ MAP[c] (> 20 week animals)	

MAP, mean arterial pressure; SAP, systolic arterial pressure; HR, heart rate; *, anesthetised; DSS, Dahl Salt Sensitive; DSR, Dahl Salt Resistant; HS, High Salt Diet; LS, Low Salt Diet; 1K.1C, 1 Kidney 1Clip; NS, no significant difference;

a, significant differences only at higher doses. *b*; more insensitive to nifedipine than WKY rats; *c*, loss in differential sensitivity in SHR compared to 10 - 12 week age group;

(1)=Oates, 1979 ; (2)=Sharma *et al.*, 1984; (3)=Ishii*et al.*, 1980; (4)=Iriuchijima, 1980; (5)=Kubo *et al.*, 1981; (6)=Stoepel *et al.*, 1981; (7)=This study; (8)=Kazda *et al.*, 1980.

hypertension. These again show a differential sensitivity to CATS (Aoki, Mochizuki, Yoshida, Kab, Kato & Takikawa, 1978, Pedersen, Christensen & Ramsch, 1980, Aoki, Kawaguchi, Sato, Kondo & Yamamoto, 1982). Enhanced vasodilation in hypertensives during Ca^{2+} channel blockade with verapamil has also been reported by Hulthen *et al.*, (1982). This effect is apparently related to the level of BP prior to CATS administration (Buhler *et al.*, 1982, McGregor *et al.*, 1983, Yoshimira *et al.*, 1983). BP is directly related to vacular resistance and the latter increases with increasing severity of hypertension (Lund-Johansen, 1977). A significant relationship between the nifedipine induced decreases in BP and systemic vascular resistance has been shown (Olivari, Bartorelli, Polese, Fiorentini, Moruzzi & Guazzi, 1979). In contrast to the findings of Olivari *et al.*, (1979) the report by Sharma *et al.*, (1984) indicates that DSS rats prior to the induction of hypertension with an elevated NaCl diet, are also more sensitive to the anti-hypertensive action of nifedipine.

Since vascular resistance is a function of peripheral tone, and in view of the dependence of tone on Ca^{2+}_{EXT} and the effectiveness of CATS in hypertension, it is not unreasonable to expect changes in Ca^{2+} channel function.

Most of the studies reporting the selective effect of CATS in hypertension have either been chronic or the drug was administered p.o. It is probable that what one is observing is not the the effect of Ca^{2+} influx blockade, but a composite effect masked by autoregulatory adaptations. Very few acute studies have been done

where the drug is administered i.v. The method used here is acute, and the effect seen would be expected to be a more faithful reflection of Ca^{2+} influx blockade at its maximum.

Based on the results obtained it appears that vasoconstriction at the 'prehypertensive' stage in hypertension in SHR is not a result of enhanced Ca^{2+} influx through POCs, and therefore, is unlikely as a primary pathological factor of disturbance in this model. This would appear to be consistent with the etiology of hypertension in SHR animals i.e., normal peripheral vascular tone but raised cardiac output. In the human case of hypertension somewhat similar results have been reported by Hulthen, Bolli & Buhler (1985). They reported that nicardipine and verapamil produced a significantly greater reduction in forearm vascular resistance (FVR) in moderate hypertensive patients (diastolic pressure $>$ 85 mmHg) than in normotensive or mild hypertensives (diastolic pressure $<$ 85 mmHg). Furthermore, sodium nitroprusside, a 'non-specific' general vasodilator had no such effect. They interpreted their results in terms of enhanced Ca^{2+} influx mediated vasoconstriction in moderate, but not mild hypertensives.

The results in the present studies with the 10-12 week age group show significant differences in the antihypertensive effect of nifedipine. The enhanced response to nifedipine can be interpreted in either of two ways. In the first instance it might reflect a functional change in VSM. Alternatively, it might be due to a non-specific enhancement, consequent to structural modifications of blood vessels as

suggested by Folkow (1978). As a consequence of structural changes, the effects of vasodilator and vasoconstrictor agents on vascular resistance may be enhanced in the hypertensive state. That the enhanced vasodilator response to CATS is not a consequence of structural changes has been shown by Robinson, Dobbs & Bayley (1982). They assessed the vasodilator response to verapamil and sodium nitroprusside in hypertensive and normotensive patients. In the former the increase in forearm blood flow was greater after verapamil infusion. The increase in flow was, however, less after sodium nitroprusside infusion in hypertensive patients compared to normotensives. Clearly then, if structural changes were responsible for an enhanced response, the increase in blood flow ought to have been the same for both types of vasodilators. The fact that the vasodilation is not the same points to some other factor other than structural changes being the cause of a differential response.

It could be argued that the greater reduction in MAP of SHR, could be due to an abnormal baroreceptor function in this strain. If baroreflex function was optimal in both groups, blood pressure should be maintained at pre-nifedipine levels in both hypertensives and normotensives. Conceivably the differential sensitivity between DSS (Sharma *et al.*, 1984) and Dahl Salt-Resistant rats (DSR), which is greater than that between SHR and WKY (this study), may reflect the defective baroreceptor reflex which has been described in the DSS rat by Gordon, Matsuguchi & Mark (1981). Indeed Gordon *et al.*, (1981) note a hyperresponsiveness to phenylephrine in DSS rats on a 0.4% salt diet indicating

that the baroreceptor reflex defect is a primary change and thus not secondary to the elevated BP. A baroreceptor defect does not appear to be the major cause of the hyperactive sympathetic nervous system and the resultant hypertension in young SHR (Judy & Farrell, 1979). The greater sensitivity, in acute studies, of DSS rats compared to the SHR to the hypotensive action of nifedipine may thus relate to such alterations in baroreceptor activity between the two strains of hypertensive rat. Whilst such alterations may explain differences in sensitivity between the two strains, baroreflex differences between the SHR and WKY strains could also explain the results observed.

Struyker-Boudier, Evenall, Smits & van Essen (1982) studied baroreflex sensitivity (BRS) in SHR and WKY rats from a young (4 week) age and during the development of hypertension till the animals were 20 weeks old. They reported that in 4 week old SHR and WKY the mean BRS was similar. However as the animals grew older, the BRS increased only in WKY rats, such that at the 20 week period, BRS was significantly higher ($P < 0.05$) than that observed in age matched SHR rats. The BRS in SHR rats did not change appreciably over the age range studied. Therefore for a given dose of nifedipine, the ability of SHR animals to effectively counteract the drop in pressure would be less than that of WKY animals, who, apparently, have a 'normal' BRS. Due to this reduced BRS, the drop in pressure would be expected to be greater in SHR than in an animal with a normal (or relatively more effecient) BRS (WKY).

Pedersen, Christensen & Ramsch (1980) found a significant correlation between the decrease in vascular resistance of the forearm and the increase in heart rate in normotensives; but no such correlation was found in the hypertensive subjects suggesting an inefficient compensatory mechanism in the hypertensive in response to nifedipine. Thus whilst an impaired BRS may contribute to an increased reduction in pressure, with hypertensive drugs, the extent of this correlation is probably not sufficient to account for all differences. Furthermore a defective baroreceptor system cannot readily account for the differential sensitivity of hypertensives to nifedipine compared to, for instance, sodium nitroprusside.

Since the currently described studies were carried out in rats anesthetised with pentobarbital and since anesthetics exert cardiovascular and baroreceptor depressant actions (Altura, 1980), it is possible that the effect of nifedipine may be influenced. However, similar findings which have been reported from studies in conscious animals (Ishii *et al.*, 1980, Iriuchijima, 1980) and humans, argue against a confounding influence of the anesthetic on the interpretation of the data.

The least sensitive of the two control strains was the Wistar strain (Table. 3-12 and Figure. 3-18). This is not surprising if one considers the origins of the SHR and WKY strains. Both the latter were derived from Wistar parent strains and in reality the WKY may also be considered, in comparision to other rat strains, as a genetic anomaly. At two of the doses of nifedipine there were siginificant differences in the hypotensive effect compared to WKY (Table. 3-12 and Figure.

3-18). Some *in vitro* data also suggest differences between WKY and Wistar strains in handling of Ca^{2+}. It has been reported that tissues from hypertensive animals respond to non-physiological cations such as La^{3+} (Shibata *et al.*, 1973, Bohr, 1974, Goldberg & Triggle, 1977). The La^{3+} response is thought to reflect alterations in Ca^{2+} binding and permeability properties of VSM membrane. This La^{3+} response is absent in ordinary Wistar rats (Goldberg & Triggle, 1977) but is prominent in rats derived from the Wistar Kyoto rat (Triggle & Laher, 1985). Triggle & Laher (1985) concluded, however, that although the La^{3+} response was a genetically determined phenomenon, present in SHR and absent in Wistar or Sprague Dawley rats, the magnitude of the La^{3+} response did not correlate to the degree of hypertension in SHR and genetically related offspring and thus was not related to hypertension. In the present study it appears that nifedipine sensitivity of WKY and Wistar rats parallels the sensitivity of the tissues from these animals to La^{3+}.

If indeed there is an enhanced hypotensive effect of nifedipine, in hypertension, then enhanced Ca^{2+} influx must be demonstrated in the hypertensive state. This has been difficult to show directly in view of the complexity of intracellular Ca^{2+} stores in VSM and the ubiquitous nature of the ion. Only indirect extrapolations have been possible such as those reported by Buhler *et al.*, (1985). They quantified α_1 and α_2 adrenoceptor mediated vasoconstrictor tone in normo- and hypertensive patients by measuring the vasodilation in the forearm, following intra-arterial infusions of prazosin (α_1 antagonist) and yohimbine (α_2 antagonist). They found

that, in hypertensives, the increase in forearm flow (indicating vasodilation) was greater with prazosin and yohimbine. They interpreted the results in terms of enhanced Ca^{2+} influx mediated via α_1 and α_2 adrenoceptors. They did not, however, address the possibility of Ca^{2+} release from intracellular stores and thus their conclusion must be viewed with caution. In the same study they reported a greater dilator effect in hypertensives with nitrendipine, suggesting enhanced Ca^{2+} influx through POCs. An enhanced role for α_2 adrenoceptors in hypertension, based on studies performed on perfused tail artery segments from the SHR compared to the control WKY, has been suggested by Medgett *et al.*, (1984a). Furthermore, responses mediated by an α_2 agonist, TL 99, in the SHR were more sensitive to the inhibitory effects of diltiazem (Hicks *et al.*, 1985) whereas diltiazem inhibited to an equal degree α_1 mediated responses in tissues from SHR and WKY. Hicks *et al.*, (1985) also demonstrated that the effects of electrical field stimulation of the perfused tail artery were antagonised by diltiazem in the SHR but not in WKY. Implicit in these findings is the suggestion of increased α_2 adrenoceptor mediated Ca^{2+} influx through ROCs and/or α_2 adrenoceptors linked to POCs. This enhanced Ca^{2+} influx could occur when there is an alteration in the properties of α-adrenoceptors (both α_1 and α_2).

The alteration(s) may manifest as differences in receptor number (B_{MAX}) and/or affinity. A direct way to assess such changes, if they exist, is to conduct radioligand binding studies.

Unfortunately binding studies in animal models of hypertension do not reveal a clear cut trend in changes of α adrenoceptors and the results are often confusing and contradictory. The topic of adrenergic receptors in hypertension has been recently been reviewed (Rosendorff, Susanni, Hurwitz & Ross, 1985). In any case altered numbers or affinities of α adrenoceptors may not be relevant to possible functional implication. One may still have the same affinity of number of binding sites on VSM, but an amplification mechanism (second messengers) that is more effecient (enhanced receptor effector coupling) such that occupation of a receptor (or receptor site) could produce a larger repsonse than it normally does would probably suffice.

So far the action of nifedipine has been discussed in terms of Ca^{2+} influx blockade. Studies with DSS and DOCA-NaCl hypertensive rats indicate that the sensitivity to nifedipine (in vivo) is apparently greater in these models of hypertension than in SHR (Ishii *et al.*, 1980, Iriuchijima, 1980, Kazda, Garthoff & Thomas, 1983,). A differential sensitivity to nifedipine which was reported in human hypertension (MacGregor, Markandu, Rotellar, Smith & Sagnella, 1983) was enhanced further if the patients were sodium loaded (MacGregor, Markandu, Smith & Sagnella, 1985). These studies suggest that either VSM tone in salt induced forms of hypertension is more dependent on Ca^{2+} or that Ca^{2+} antagonists might have an additional effect besides peripheral vasodilation in these forms of hypertension. Indeed it has now been shown that CATS, in contrast to minoxidil and sodium nitroprusside, actually decrease Na^{+} and water retention,

leading to an overall volume load reduction (Garthoff, Kazda, Knorr, Thomas, 1983). Similar increases in Na^+ excretion in acutely saline loaded rats has also been reported in SHR rats (Garthoff, Kazda, Knorr & Thomas, 1982). Thus the antihypertensive effect of CATS may not only be due to peripheral vasodilation.

The results obtained at the 20 week or greater group are surprising in that the difference in sensitivity to nifedipine is somewhat reduced. Only at two doses were there significant decreases. At this age, the BP is higher than that in the younger age groups (Tables. 3-9 and 3-10). This stage is considered as the 'established hypertension' stage. If one compares the percentage reduction in MAP in the SHR at the 10-12 week age group and the 20 week age group, for each dose of nifedipine there is a slight loss in sensitivity i.e., the percentage reduction in MAP in the 20 week age group is, if anything, slightly less than that of the 10-12 week age group. One possible reason for this could be that in this age group the BP is now more dependent upon structural changes of the blood vessel than Ca^{2+} influx into the VSM cells. As previously reviewed, however, Buhler *et al.*, (1982) and MacGregor *et al.*, (1983) have reported a significant correlation between effectiveness of CATS and the pretreatment levels of BP. The study reported by Buhler *et al.*, (1982) was, however, a chronic study whereas the current study reports the comparative results following acute administration. The possibility of differences between batches must also be considered since the rats were bought at different times for each of the age groups.

Notwithstanding the results for this age group, the other results suggest that there may an altered Ca^{2+} influx through POC. The results from the *in vivo* and *in vitro* data offer indirect evidence for altered role of POC function.

4.5. Binding Studies.

The altered sensitivity to nifedipine of K^+ induced responses(*in vitro* and *in vivo*) and increased Ca^{2+} sensitivity of NE induced responses are indicative of alterations in Ca^{2+} handling by VSM form the SHR. Such differences may reflect differences in the affinity of nifedipine for binding sites on POCs or that the number of binding sites is increased and coupling to the receptor thus enhanced, in hypertensive conditions. With this in view, preliminary studies were conducted on binding assays of tail arteries form SHR and WKY rats.

The binding results cannot be quantitatively compared owing to paucity of the data. Nevertheless what data there is, indicates that there is relatively high affinity binding to plasma membrane fractions from tail arteries. The k_d values, which are in the sub nanomolar range (Table. 3-15) are consistent with other studies which report similar affinities. These include bovine aorta[k_d = 0.10n*M*] (Sarmiento, Janis, Jenkins, Katz & Triggle, 1984), canine aorta[k_d=0.31n*M*], canine mesenteric artery[k_d=0.25n*M*] and rat mesenteric artery[k_d=0.1n*M*] (Triggle, Agrawal, Bolger, Daniel, Kwan, Luchowski & Triggle, 1983), guinea pig ileum[k_d=0.16n*M*] (Bolger, Gengo, Klockowski, Siegel, Janis, Triggle & Triggle, 1983), rat myometrium[k_d=0.18n*M*], rat stomach[k_d=0.15n*M*] and guinea pig bladder[k_d=0.15n*M*] (Triggle, 1984b). These data were obtained at 25°C and low protein concentration, conditions under which the present data were determined (25°C and 15-35μg protein). At higher temperatures (37°C) and high protein concentrations, lower binding affinities have been reported (Triggle & Janis, 1984).

The B_{MAX} values obtained in this study suggest a high density binding in the tail artery. This would be in contrast to the low density binding found in other smooth muscle mentioned above. In general, for blood vessels, the range of B_{MAX} is about 80 -125 fmol/mg protein, whereas that for other smooth muscles the binding levels are somewhat higher. One possible reason for this could be the isolation procedure itself. The plasma fraction (sarcolemma enriched fraction) I obtained was by differential centrifugation, which is the method used in isolating similar fractions in bovine aorta (Sarmiento *et al*, 1984), canine aorta, canine and rat mesenteric artery (Triggle *et al.*, 1982), although these authors extended their purification by sucrose density centrifugation. A potential source of the difference in apparent B_{MAX} could therefore be related to the degree of purification inherent in the method used. An indication of the enrichment is given by the enhancement of membrane marker enzymes in the microsomal fractions. In this study, the level of enrichment of 5'ND, which is a putative marker for plasma membranes is consistent with the level of enrichment of 5'ND activity reported by Triggle *et al.*, (1982) in canine aorta, canine and rat mesenteric arteries.

Triggle & Janis (1984), have argued that the observed high B_{MAX} of nitrendipine in guinea pig ileal smooth muscle (1100 fmol/mg protein) is not due to differences in enrichment. A second possible reason for the high B_{MAX} observed in this study is the binding of nitrendipine to the nerve endings. Nitrendipine is known to bind with high affinity to brain tissue (Bellemann, 1982, Gould, Murphy & Snyder, 1982, Belleman, Schade & Towart, 1980). The rat tail artery is a richly innervated

blood vessel, more so than the mesentric artery or the aorta. Thus it is possible that included in the B_{MAX} values is binding to neuronal tissue in the blood vessel. In contrast to the low density of binding sites in blood vessels, areas rich in synapses of the brain exhibit a higher density of binding (Murphy, 1982).

The data presented here do not permit any conclusions concerning possible changes in POC affinity or number. The k_d values obtained are close to the IC_{50} values obtained in the *in vitro* study. This is consistent with the good 1 : 1 linear correlation shown for a series of K^+ induced mechanical responses in guinea pig ileal longitudinal smooth muscle and inhibition of specific ^{3}H-nitrendipine binding in the same tissue (Bolger, Gengo, Klockowski, Siegel, Janis, Triggle & Triggle, 1983). To date very few studies have been done on ^{3}H-nitrendipine binding in SHR and WKY. Ishii, Kano, Kurobe & Ando (1983) report a significant increase in B_{MAX} of ^{3}H-nitrendipine in SHR brain tissues whereas k_d values did not change. In the same study they found no significant differences in either B_{MAX} or k_d values for cardiac tissue from 9 wk old SHR and WKY. Chatelain, Demol & Roba (1984) also found no significant differences in either the k_d or B_{MAX} for ^{3}H-nitrendipine binding to cardiac tissue from 9 week old SHR and WKY. In contrast the same group reported significant differences in k_d and increases in B_{MAX} from cardiac tissue from 24 wk old SHR compared to WKY. The relevance of these findings is not yet clear.

4.6. Concluding remarks.

In view of the critical dependence of VSM tone on the level of intracellular Ca^{2+}, it is not suprising that altered Ca^{2+} handling has been suggested as one of the causes of increased tone in VSM from hypertensives (Kwan, 1985). This alteration can manifest itself as changes in intracellular Ca^{2+} regulatory mechanisms or increased Ca^{2+} influx. This thesis was an attempt to characterise two pathways through which Ca^{2+} influx occurs, namely ROC and POC (see section. 1.3.1.1), in tail arteries. Each pathway was selectively activated by stimulating the artery with either NE (Ca^{2+} entry through ROC) or K^+ (Ca^{2+} entry through POC). The sensitivity of NE and K^+ responses to nifedipine gives a measure of ROC and POC sensitivity to nifedipine.

NE responses, from both SHR and WKY, were equally sensitive to nifedipine. K^+ responses were significantly more sensitive to nifedipine than NE responses. However, there was no difference in sensitivity of K^+ responses to nifedipine between SHR and WKY tail arteries. These results suggest that neither POC nor ROC function is altered in hypertension.

The Ca^{2+} sensitivity of SHR tail artery preparations was found to be elevated when vessels were activated by ED_{50}, but not ED_{100}, levels of NE. This suggests alterations in ROC function. What is clear from this study is that in addition to ED_{100} levels of agonists, ED_{50} doses should also be used in assessing sensitivity. Nifedipine also significantly reduced the Ca^{2+} sensitivity of K^+ activated vessels

(SHR) at a concentration which had an insignificant effect on Ca^{2+} sensitivity in WKY vessels. This again points to possible alterations in POC function. A similar protocol with NE activation revealed that the Ca^{2+} responses in vessels from SHR were more resistant to nifedipine than WKY vessels, suggesting a downregulation of ROC.

In order to correlate the studies mentioned above, further studies were done to assess the effect of i.v. nifedipine in SHR and WKY animals. These results suggest that in young animals (prehypertensive phase of hypertension) altered Ca^{2+} influx may not be significant. At the 10-12 week age group, a differential sensitivity to nifedipine is observed in SHR animals. In addition Wistar animals were more insensitive to nifedipine than WKY. This observation, together with the report (Laher & Triggle, 1984b) that blood vessels from WKY animals also (in addition to SHR) responded to La^{3+}, indicates that caution should be exercised in interpreting data from SHR and control. The decrease in differential sensitivity observed in the 20 week old animals suggests that at this age the high pressure is maintained by some other mechanism than increased Ca^{2+} influx.

Although the results mentioned above provide further indirect evidence for changes in channel function, direct evidence is lacking. No doubt, use of techniques such as radioligand binding studies, which would allow direct measurement of the channel properties, would be helpful. Another approach would be to determine what influence do intracellular messengers have on the channel functional properties and whether this is altered in hypertension.

References

Abboud, F.M. The sympathetic nervous system in hypertension. *Clin. Exp. Hyp.*, 1984, *6*, 43-60.

Albretcht, I. The hemodynamics of early stages of spotaneous hypertension in rats. Part 1. Male study. *Japan. Circ. J.*, 1974, *38*, 985-990.

Altura, B.M. Cardiovascular actions of general anesthetics. *Fed. Proc.*, 1980, *39*, 1574-1599.

Andersson, C., P. Hellstrand., B. Johansson & A. Ringberg. Conraction of vascular smooth muscle induced by hyperosmolality. *Acta. Physiol. Scand.*, 1972, *84*, 48A-49A.

Andersson, K.E., E.D. Hogestatt., T. Skarby & T.K. Uski. Some aspects of the pharmacology of resistance vessels. *Prog. appl. Microcirc.*, 1985, *8*, 19-31.

Aoki, K., Y. Kawaguchi., K. Sato., S. Kondo & M. Yamamoto. Clinical and pharmacological properties of calcium antagonists in essential hypertension and spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, 1982, *4*, S298-S302.

Aoki, K., A. Mochizuki., T. Yoshida., S. Kab., K. Kato & K. Takikawa. Antihypertensive effect of cardiovascular Ca^{2+} antagonist in hypertensive patients in the absence and presence of beta adrenergic blockade. *Am. Heart. J.*, 1978, *96*, 218-226.

Aoki, K., N. Yamamitsha, K. Tazumi and K. Hotta. ATPase activity and Ca^{2+} binding activity of subcellular membrane of arterial smooth muscle in spontaneously hypertensive rat. *Japan. Heart. J.*, 1974, *15*, 180-181.

Aprigliano, O. and K. Hermsmeyer. In vitro denervation of the portal vein and caudal artery in the rat. *J. Pharmacol. Exp. Ther.*, 1976a, *198*, 568 - 577.

Arkesteijn, C.J.M. A kinetic method for serum 5'-nucleotidase using stabilised glutamate dehydrogenase. *J. Chem. Clin. Biochem.*, 1976, *14*, 155-.

Bell, D.R., R.C. Webb and D.F. Bohr. Functional basis for individualities among vascular smooth muscles. *J. Cardiovasc. Pharmacol.*, 1984, *7(Suppl. 3.)*, S1-S11.

Belleman, P., A. Schade & R. Towart. Dihydropyridine receptor in rat brain labelled with [^{3}H]nimodipine. *2356-2360*, 1980, *80*, 1983.

Bellemann, P., D. Ferry., F. Lubbecke and H. Glossmann. [^{3}H]-Nimodipine and

[^{3}H]-nitrendipine as tools to directly identify the sites of action of 1,4-dihydropyridine calcium antagonists in guinea-pig tissues. *Arzneim.-Forsch. Drug. Res.*, 1982, *32(I)*, 361 - 363.

Bergland, G., O. Andersson and L. Wilhelmsen. Prevalence of primary and scondary hypertension. Studies in a random population sample. *Br. Med. J.*, 1976, *2*, 554-556.

Bevan, J. Vascular, myogenic or stretch dependent tone. *J. Cardiovasc. Pharmacol.*, 1985, *7(Suppl. 3.)*, S1299-S136.

Bevan, J.A., W. Garstka., C. Su & M.O. Su. The bimodal basis of the contrcatile response of the rabbit ear artery to norepinephrine and other agonists. *Eur. J. Pharmacol.*, 1973, *22*, 47-53.

Bianchi, G. U. Fox & E. Imbasciati. The development of a new strain of spontaneously hypertensive rats. *Life Science.*, 1973, *14*, 339-347.

Bkaily, G & N. Sperelakis. Injection of protein kinase inhibitor into cultured heart cells blocks calcium slow channels. *Am. J. Physiol.*, 1984, *246*, H630-H634.

Blaustein, M.P. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. *Am J. Physiology.*, 1977, *232(3)*, C165-173.

Blaustein, M.P. Sodium transport and hypertension. Where are we going? *Hypertension.*, 1984, *6*, 445-453.

Bohlen, H.G., R.W. Gore and P.M. Hutchins. Comparision of microvascular pressures in spontaneously hypertensive and normotensive rats. *Microvasc. Res.*, 1977, *13*, 125-130.

Bohr, D.F. Vascular smooth muscle: dual effect of calcium. *Science*, 1963, *139*, 597-599.

Bohr, D.F. Vascular smooth muscle updated. *Circ. Res.*, 1973, *32*, 665-672.

Bohr, D.F. Reactivity of vascular smooth muscle from normal and hypertensive rats: effects of several cations. *Fed. Proc.*, 1974, *33*, 127 - 132.

Bolger, G.T., P. Gengo., R. Klockowski., H. Siegel., R.A. Janis., A.M. Triggle & D.J. Triggle. Characterisation of binding of Ca^{2+}- channel antagonist, [^{3}]-nitrendipine, to guinea pig ileal smooth muscle. *J. Pharmacol. Exp Ther.*, 1983, *225*, 291-309.

Bolton, T.B. Mechanisms of action of transmitters and other substances on smooth muscle. *Physiological Reviews.*, 1979, *59*, 606 - 718.

Bou, J., J. Llenas and R. Massingham. Calcium entry blocking drugs, "calcium antagonists" and vascular smooth muscle function. *J. Auton. Pharmacol.*, 1984, *3*, 219 - 232.

Bradford, M.M. A rapid and sensitive method for quantitation of microgram quantities of protein binding utilising the principle of protein-dye binding. *Anal. Biochem.*, 1976, *72*, 248 - 253.

Brann, L.R., D.J. Root and W. Halpern. Intrinsic tone and contractile responsiveness of small cerebral artery in SHR and WKY rats. *Fed. Proc.*, 1980, *39*, 1191.

Brody, M.J & B.G. Zimmerman. Peripheral circulation in arterial hypertension. *Prog. Cardiovasc. Pharmacol.*, 1976, *18*, 323-340.

Brody, M.J., J.E. Faber., M.M. Mangiape & J.P.Porter. Central, neural and humoral regulation of arterial pressure in hypertension. In H.H. Ong & J.C. Lewis (Eds.), *Hypertension. Physiological basis and treatment*, Orlando, Florida.: Academic Press, 1984.

Brody, M.J., J.R. Haywood and K.B. Torvin. Neural mechanims in hypertension. *Ann. Rev. Physiol*, 1980, *42*, 441 - 453.

Buhler, F.R., U.L. Hulthen., F.B. Muller., W. Kiowski & P. Bolli. The place of calcium antagonist verapamil in antihypertensive therapy. *J. Cardiovasc. Pharmacol.*, 1982, *4(Suppl.3.)*, 350-357.

Casteels, R. Electro and pharmacomechanical coupling in vascular smooth muscle. *Chest*, 1980, *78*, 150-156.

Casteels, R and C. van Breemen. Active and passive calcium fluxes across cell membranes of the guinea pig taenia coli. *Pflugers. Arch.*, 1975, *359*, 197-207.

Cauvin, C., S. Lukeman., J. Cameron., O. Hwang and C. van Breeman. Differences in norepinephrine activation and diltiazem inhibition of calcium channels in isolated rabbit and mesenteric resistance vessels. *Circ. Res.*, 1985, *56*, 822-828.

Cauvin, C and C. van Breemen. Different Ca^{2+}channels along the arterial tree. *J. Cardiovasc. Pharm.*, 1985, *7(Suppl. 4)*, S4 - S10.

Cauvin, C., R.L. Loutzenhiser & C. van Breemen. Mechanism of calcium antagonist induced vasodilation. *Ann. Rev. Pharmacol. Toxicol.*, 1983, *23*, 373-396.

Cauvin, C., S. Lukeman., J. Cameron., K. Meisheri., H. Yamamoto and C. van

Breemen. Theoretical basis for vascular selectivity of Ca^{2+} antagonists. *J. Cardiovasc. Pharm.*, 1984b, *6(Suppl. 4)*, S630 - S638.

Cauvin, C., K. Saida and C. van Breemen. Extracellular Ca^{2+} dependence and diltiazem inhibition of contraction in rabbit conduit arteries and mesenteric resistance vessels. *Blood Vessels.*, 1984a, *21*, 23 - 31.

Cavero, I & M. Spedding. "Calcium Antagonists": A class of drugs with a bright future. Part I. Cellular calcium homeostatis and calcium as a coupling messenger. *Life Science*, 1983, *33*, 2571-2581.

Chatelain, P., D. Demol and J. Roba. Comparision of [^{3}H]-nitrendipine binding to heart membranes of normotensive and spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, 1984, *6*, 222 - 223.

Cheng, J.B and S. Shibata. Pressor response to 5-Hydroxytryptamine and KCl in the perfused hindquarter preparation from spontaneously hypertensive rat. *J. Pharmacol. Exp. Ther.*, 1980, *214*, 488-495.

Cheung, D.W. Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol.*, 1982, *328*, 461 - 468.

Cheung, D.W. Neural regulation of electrical and mechanical activities in the rat tail artery. *Pflugers Arch.*, 1984, *400*, 335 - 337.

Church, J & T.T. Zsoter. Calcium antagonistic drugs. Mechanism of action. *Can. J. Physiol. Pharmacol.*, 1980, *58*, 254-264.

Clark. D.W.J., D.R. Jones., E.L. Phelan and C.E. Devine. Blood pressure and vascular resistance in genetically hypertensive rats treated at birth with 6-hydroxydopamine. *Circ. Res.*, 1978, *43*, 293 -300.

Cohen, M.L and B.A. Berkowitz. Decreased vascular relaxation in hypertension. *J. Pharmacol. Exp. Therap.*, 1976, *196*, 396-.

Collis, M.G., C. De Mey and P.M. Vanhoutte. Renal vascular recativity in young SHR. *Hypertension.*, 1980, *2*, 45-52.

Conti, M.A & R.S. Adelstein. Phosphorylation by cyclic adenosine 3'5'-monophosphate dependent protein kinase regulates myosin light chain kinase. *Fed. Proc.*, 1980, *39(5)*, 1569-1573.

Conway, J. A vascular abnormality in hypertension - A study of blood flow in the forearm. *Circulation.*, 1963, *27*, 520-529.

Corbett, D.A., M.T. Goldberg., V.C. Swamy., C.R. Triggle and D.J. Triggle.

Reactivity of vas deferentia from spontaneously hypertensive and normotensive Wistar rats. *Can. J. Physiol. Pharmacol.*, 1980, *58*, 656-665.

Dahl, L.K., M. Heine and L. Tassinari. Effects of chronic excess salt ingestion. *J. Exp. Med.*, 1962, *115*, 1173 - 1190.

Daniel, E.E. The use of subcellular membrane fractions in analysis of control of smooth muscle function. *Experienta.*, 1985, *41*, 905-913.

Daniel, E.E., A.K. Grover and C.Y. Kwan. Isolation and properties of plasma membrane from smooth muscle. *Fed. Proc.*, 1982, *41*, 2898-2904.

Daniel, E.E., C.Y. Kwan., R.M.K.W. Lee and J. Smeda. Early structural changes in precapillary vessels in hypertension and their relationship to functional changes. *J. Cardiovasc. Pharmacol.*, 1984, *6(Suppl.4)*, S671 - S682.

DeMey, J & P.M. Vanhoutte. Uneven distribution of postjunctional alpha$_1$- and alpha$_2$-like adrenoceptors in canine arterial and venous smooth muscle. *Circ. Res.*, 1981, *48*, 875-884.

DeFeo, T.T & K.E. Morgan. Calcium force relationship as detected with aequorin in two different vascular smooth muscles of the ferret. *J. Physiol.*, 1985, *369*, 269-282.

DeRiemer, S.A., J.A. Strong., K.A. Albert., P. Greengard & L.K. Kaczmarek. Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C. *Nature.*, 1985, *313*, 316-316.

Deth, R & C. van Breemen. Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during induced activation of the rabbit aorta. *Pflugers. Arch.*, 1974, *348*, 13-22.

Droogmans, G., L. Raeymakers and R. Casteels. Electro and pharmacomechanical coupling in smooth muscle cells of rabbit ear artery. *J. Gen. Physiol.*, 1977, *70*, 129-148.

Egleme, C., T. Godfraind & R.C. Miller. Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. *Br. J. Pharmacol.*, 1984, *81*, 16-18.

Field, F.P., R.A. Janis & D.J. Triggle. Aortic reactivity of rats with genetic and experimental renal hypertension. *Can. J. Physiol. Pharmacol.*, 1972, *50*, 1072-1079.

Filo, R.B., D.F. Bohr and J.C. Ruegg. Glycerinated skeletal and smooth muscle: calcium and magnesium dependance. *Science*, 1965, *147*, 1581-1583.

Finch, L and G. Haeusler. Vascular resistance and reactivity in hypertensive rats. *Blood Vessels.*, 1974, *11*, 145-158.

Finch, L., G. Haeusler & H. Thoren. Failure to induce experimental hypertension in rats after intraventricular injection of 6-hydroxydopamine. *Br. J. Pharmacol.*, 1972, *44*, 356-357.

Fitzpatrick, D.F. and A. Szentivanyi. The relationship between increased myogenic tone and hyporesponsiveness in vascular smooth muscle of spontaneously hypertensive rats. *Clin. Exp. Hypertens.*, 1980, *2(6)*, 1023-1037.

Flaim, S.F. Comparitive pharmacology of calcium blockers based on studies of vascular smooth muscle. In S.F. Flaim and R. Zelis (Ed.), *Calcium Blockers*, Baltimore, MD: Urban and Schwarzenberg, 1982.

Fleckenstein, A. Die bedentung der energiereichen phosphate. fur kontraktiltaet und tonus der myocards. *Verh. Dtsch. Ges. Inn. Med.*, 1964, *70*, 81-99.

Fleckenstein, A.L. Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. *Ann. Rev. Pharmacol. Toxicol.*, 1977, *17*, 149-166.

Fleckenstein, A. *Calcium antagonism in heart and smooth muscle. Experimental facts and therapeutic prospects.* New York, N.Y:Wiley Interscience., 1983.

Fleming, W.W., D.P. Westfall., I.S. De La Lande and L.B. Jellet. Log-normal distribution of equieffective doses of norepinephrine and acetylcholine in several tissues. *J. Pharmacol. Exp. Ther.*, 1972, *181*, 339 - 345.

Folkow, B. Structural, myogenic, humoral and nervous factors controlling peripheral resistance. In M. Harrington (Eds.), *Hypotensive Drugs*, London: Pergammon Press, 1956.

Folkow, B. Cardiovascular structural adaptation: its role in the initiation and maintenance of primary hypertension. *Clin. Sci. Mol. Med.*, 1978, *55*, 347-504.

Folkow, B. Physiological aspects of primary hypertension. *Physio. Rev.*, 1982, *62*, 347-504.

Folkow, B., M. Gurevich., M. Hallback., Y. Lundgren and L. Weiss. The hemodynamic consequence of regional hypotension in spontaneously hypertensive and normotensive rats. *Acta. Physiol. Scand.*, 1971, *83*, 532-541.

Folkow, B., M. Hallback, J.V. Jones & M.S. Sutter. Dependence on external calcium for the noradrenaline contractility of the resistance vessels in spontaneously hypertensive and renal hypertensive rats, as compared with normotensive controls. *Acta Physiol. Scand.*, 1977, *101*, 84 - 97.

Folkow, B., M. Hallback., Y. Lundgren., R. Sivertsson and L. Weiss. Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and spontaneously hypertensive rats. *Circ. Res.*, 1973, *32/33(Suppl. 1.)*, I2-I16.

Friedman, S.M. Sodium ions and regulation of vascular tone.—In B.M. Altura (Eds.), *Advances in microcirculation. Ionic regulation of microcirculation*, Basel: Karger, 1982.

Frohlich, E.D., R.C. Tarazi. and H.P. Dunstan . Rexamination of the hemodynamics of hypertension. *Am. J. Med. Sci.*, 1969, *257*, 9-23.

Fronek, K & B.W. Zweifach. Microvascular pressure distribution in skeletal muscle and the effect of vasodilation. *Am. J. Physiol.*, 1975, *228*, 791-796.

Fuchs, F. Striated muscle. *Ann. Rev. Physio.*, 1974, *36*, 461-502.

Garthoff,B., S. Kazda., A. Knorr & G. Thomas. Nitrendipine increases sodium excretion in acutely saline loaded rats. *Biochem. Pharmacol.*, 1982, *31*, 3015-.

Garthoff, B., S. Kazda., A. Knorr & G. Thomas. Factors involved in the antihyperyensive action of calcium antagonists. *Hypertension.*, 1983, *5 (Suppl.II.)*, II34-II38.

Godfraind, T. Actions of nifedipine on calcium fluxes and contraction in isolated rat arteries. *J. Pharmacol. Exp. Ther.*, 1983, *224*, 443-450.

Godfraind, T., C. Egleme & I.A. Osache. Role of endothelium in the contractile response of rat aorta to α-adrenoceptor agonists. *Clinical Science.*, 1985, *68 (Suppl. 10)*, 65s-71s.

Goldberg, M.T. and C.R. Triggle. An analysis of the action of lanthanum on aortic tissue from normotensive and spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.*, 1977, *55*, 1084 - 1090.

Goldberg, M.T. and C.R. Triggle. Elevated vascular reactivity in the timolol treated spontaneously hypertensive rat. *Can. J. Physiol. Pharmacol.*, 1978, *56*, 1072 - 1075.

Gould, R.J., K.M.M. Murphy & S.H. Snyder. [^{3}H]Nitrendipine labelled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc. Natl. Acad. Sci.*, 1982, *79*, 3656-3660.

Greenberg, S and D.F. Bohr. Venous smooth muscle in hypertension. Enhanced contractility of portal vein in SHR. *Circ. Res.*, 1975, *36/37(Suppl. 1.)*, I208-I215.

Grover, A.K. Calcium handling studies using isolated smooth muscle membranes. In A.K. Grover & E.E. Daniel (Eds.), *Calcium and contractility*, Clifton, N.J.: Humana Press, 1985.

Grover, A.K., C.Y. Kwan and E.E. Daniel. Na - Ca exchange in rat myometrium membrane vesicles highly enriched in plasma membrane. *Am. J. Physiol.*, 1981, *240*, C175-182.

Grover, A.K., C.Y. Kwan., J. Crankshaw., D.J. Crankshaw., R.E. Garfield and E.E. Daniel. Characterisation of calcium transport and binding by rat myometrium plasma membrane. *Am. J. Physiol.*, 1980, *239*, C66-C74.

Haack, D.W., J.J. Schaffer and J.G. Simpson. Comparision of cutaneous microvessels from spontaneously hypertensive, normotensive wistar-kyoto and normotensive wistar rats. *Proc. Soc. Exp. Biol. Med.*, 1980, *164*, 453-458.

Haeusler, G. Relationship between noradrenaline induced depolarisation and contraction in vascular smooth muscle. *Blood Vessels.*, 1983, *15*, 46-54.

Haeusler, G and L. Finch. Vascular reactivity and serotonin and hypertension in the rat. *Naunyn-Schmiedeberg's. Arch. Pharmacol.*, 1972, *272*, 101-116.

Hallback, M. Consequence of social isolation on blood pressure, cardiovascular reactivity and design in spontaneously hypertensive rats. *Acta. Physiol. Scand.*, 1975, *93*, 455-465.

Halpern, W., S.A. Mongeon & D.T.Root. Sress, tension and myogenic aspects of small isolated extraparenchymal rat arteries. In W.L. Stephens (Eds.), *Smooth muscle contraction.*, New York: Dekker., 1984.

Hansen, T.R and D.F. Bohr. Hypertension, transmural pressure and vasculsr smooth muscle response in rats. *Circ. Res.*, 1975, *36*, 590-598.

Harder, D.R. Pressure dependent membrane depolarisation in cat middle cerebral artery. *Circ. Res.*, 1984, *55*, 197-202.

Harder, D.R and K. Hermsmeyer. Membrane mechanisms in arterial hypertension. *Hypertension.*, 1983, *5*, 404-408.

Harder, D.R & H. Sperelakis. Action potentials induced in Guinea Pig arterial smooth muscle by tetraethylammonium. *Am. J. Physiol.*, 1979, *237*, C75-C80.

Harder, D.R., S.J. Contney., W.J. Williams and W.J. Stekiel. Norepinephrine effect on in situ venous membrane potential in spontaneously hypertensive rats. *Am J. Physiol.*, 1981, *24*, H837-H842.

Harder, D.R., J. Smeda and J. Lombard. Enhanced myogenic depolarisation in hypertensive cerebral arterial muscle. *Circ. Res.*, 1985, *57*, 319-322.

Harris, A.L., V.C. Swamy and D.J. Triggle. Calcium reactivity and antagonism in portal veins from spontaneously hypertensive and normotensive rats. *Can. J. Physiol. Pharmacol.*, 1984, *62*, 146 - 150.

Harris, A.L., V.C. Swamy., D.J. Triggle & D.H. Walters. Responses to norepinephrine of portal vein strips from normotensive and spontaneously hypertensive rats. *J. Auton. Pharmacol.*, 1980, *1*, 61-66.

Henry, P.D. Comparitive pharmacology of calcium antagonists: Nifedipine, verapamil and diltizem. *Am. J. Cardiol.*, 1980, *46*, 1047-1058.

Hermsmeyer, K. Electrogenesis of increased norepinephrine sensitivity of arterial vascular smooth muscle in hypertension. *Circ. Res.*, 1976, *38*, 362 - 367.

Hermsmeyer, K. Membrane electrical contribution to increased arterial reactivity in hypertension. In J.A. Bevan, T. Godfraind, R.A. Maxwell & P.M. Vanhoutte (Eds.), *Vascular neuroeffector mechanisms.*, New York: Raven Press, 1980.

Hermsmeyer, K., A. Trapani and P.W. Abel. Membrane potential-dependent tension in vascular muscle. In P.M. Vanhoutte and I. Leusen (Ed.), *Vasodilation,* New York: Raven Press., 1981.

Hess, P. B. Lansman & R.W. Tsien. Different modes of Ca^{2+} channel gating behaviour favored by dihydrpyridine Ca^{2+} agonists and antagonists. *Nature*, 1984, *311*, 538-544.

Hicks, P.E., C. Tierny and S.Z. Langer. Preferential antagonism by diltiazem of α_2-adrenoceptor mediated vasoconstrictor responses in perfused tail arteries of spontaneous hypertensive rats. *Naunyn-Schmiedeberg's Arch Pharmacol.*, 1985, *328*, 388 - 395.

Hof, R.P. and J. Vuorela. Assessing calcium antagonism on vascular smooth muscle: a comparision of three methods. *Journal of Pharmacological Methods*, 1983, *9*, 41 - 52.

Holloway E.T and D.F. Bohr. Reactivity of smooth muscle in hypertensive rats. *Circ. Res.*, 1973, *33*, 678-685.

Hudgins, P and G.B. Weiss. Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmacol. Exp. Ther.*, 1968, *159*, 91-97.

Hulthen, U.L., P. Bolli & F.R. Buhler. Vasodilatory effect of nicardipine and verapamil in the forearm of hypertensive as compared with normotensive man. *Br. J. Clin. Pharmacol.*, 1985, *20*, 62S-66S.

Hulthen, U.L., P. Bolli., F.W. Amann., W. Kiowski and F.R. Buhler. Enhanced vasodilation in essential hypertension by calcium channel blockade with verapamil. *Hypertension.*, 1982, *4(2)*, 26-31.

Hurwitz, L & A. Suria. The link between agonist action and response in smooth muscle. *Ann. Rev. Pharmacol.*, 1971, *11*, 303-326.

Hurwitz, L., L.J. McGuffee., S.A. Little & H. Blumberg. Evidence for two distinct types of potassium activated calcium channels in an intestinal smooth muscle. *J. Pharmacol. Exp. Ther.*, 1980, *214*, 574-580.

Hutchins, P.M. and A.E. Darnell. Observation of a decreased number of small arterioles in spontaneously hypertensive rats. *Circ. Res.*, 1979, *34/35(Suppl. 1.)*, I161-I165.

Hwa, J.J & [illegible] A. Bevan. Stretch dependent (myogenic) tone in rabbit ear resistance arteries. *Am. J. Physiol.*, 1986, *250*, H87-H95.

Iriuchijima, J. Sympathetic discharge rate in spontaneously hypertensive rat. *Japan. Heart. J.*, 1973, *14*, 350-356.

Iriuchijima, J. Effect of calcium antagonist, nifedipine, on blood pressure of various hypertensive rats. *Hirosh. J. Med.*, 1980, *29*, 15-19.

Ishii, K., T. Kano., Y. Kurobe., J. Ando. Binding of ^{3}H-nitrendipine to heart and brain membranes from normotensive and spontaneously hypertensive rats. *Eur. J. Pharmacol.*, 1983, *88*, 277-278.

Ishii, H., K. Itoh and T. Nose. Different antihypertensive effects of nifedipine in conscious experimental hypertensive and normotensive rats. *Eur. J. Pharmacol.*, 1980, *64*, 21 - 29.

Iversen, L.L. The inhibition of noradrenaline uptake by drugs. In N.J. Harper & A.B. Simmonds (Eds.), *Advances in drug research.*, London, UK.: Academic Press, 1965.

Janis, R.A & A. Scriabine. Sites of action of Ca^{2+} channel inhibitors. *Biochem. Pharmacol.*, 1983, *32*, 3499-3507.

Johansson, B & A.P. Somlyo. Electrophysiology and excitation-contraction coupling. In D.F. Bohr., A.P. Somlyo & H.V. Sparks (Eds.), *Handbook of Physiology. The Cardiovascular System.*, Washington: American Physiological Society., 1980.

Johnsson, E.M & R.A. Macia. Unique resistance to guanethedine induced chemical sympathectomy of spontaneously hypertensive rats. *Circ. Res.*, 1979, *45*, 243-249.

Jones, A.W. Altered ion transport in large and small arteries from spontaneously hypertensive rats. *Circ. Res.*, 1974, *34(Suppl. I)*, I117-I122.

Jones, A.W. Content and fluxes of electrolytes. In D.F. Bohr, A.P. Somlyo & H.V. Sparks (Eds.), *Handbook of physiology. Vacular smooth muscle*, Baltimore: William & Wilkins, 1980.

Jones, A.W. Ionic dysfunction and hypertension. In B.M. Altura (Eds.), *Advances in microcirculation. Ionic regulation of the microcirculation*, Basel: Karger, 1982.

Jones, A.W and R.C. Hart. Altered ion transport in aortic smooth muscle during DOCA hypertension in the rat. *Circ. Res.*, 1975, *37*, 333-341.

Judy, W.V & S.K Farrell. Arterial baroreceptor reflex control of sympathetic nerve activity in the spontaneously hypertensive rat. *Hypertension.*, 1979, *1*, 605-614.

Judy, W.V., A.M. Watanabe., D.P. Henry., H.R. Besch., W.R. Murphy and G.M. Hockel. Sympathetic nerve activity. Role in regulation of blood pressure in SHR. *Circ. Res.*, 1976, *38 (Suppl. II)*, II21-II29.

Judy, W.V., A.M. Watanabe, W.R. Murphy, B.S. Aprison & P.L. Yu. Sympathetic nerve activity and blood pressure in normotensive back cross rats genetically related to the spontaneously hypertensive rat. *Hypertension*, 1979, *1*, 598-604.

Kannan, M.S., A.E. Seip & D.J. Crankshaw. Effect of nifedipine on calcium-induced contractions to potassium in the aorta and mesenteric arteries of spontaneously hypertensive and normotensive rats. *Can. J. Physiol. Pharmacol.*, 1986, *64*, 310-314.

Katz, A.M. Basic cellular mechanisms of action of calcium channel blockers. *Am. J. Cardiol.*, 1985, *55*, 2B-9B.

Katz, A.M., W.D. Hager., F.C. Messino & A.J. Puppano. Cellular actions and pharmacology of the calcium channel blocking drugs. *Am. J. Medicine*, 1984, *77(2B)*, 2-10.

Kazda, S., B. Garthoff and A. Knorr. Interference of the calcium antagonist nisoldipine with the abnormal response of vessels from hypertensive rats to alpha-adrenergic stimulation. *J. Cardiovasc. Pharmacol.*, 1985, *7(Suppl. 6)*, S61-S65.

Keatinge, W.R. Ca concentration and flux in Ca-deprived aretries. *J. Physiol.*, 1972, *224*, 35-59.

Kubo, P.I. Increased pressor responses to pressor agents in SHR. *Can. J. Physiol. Pharmacol.*, 1979, *57*, 59-64.

Kubo, P.I. Cardiovascular reactivity in renal and spontaneously hypertensive rats. *Arch. Int. Pharmacodyn. Ther.*, 1980, *234*, 49-57.

Kubo, T & M. Hashimoto. Effects of intra-ventricular and spinal 6-hydroxydopamine on blood pressure of spontaneously hypertensive rats. *Arch. Int. Pharmacodyn.*, 1978, *213*, 166-176.

Kuriyama, H., I. Yushi., H. Suzuki., K. Kitamura and I. Itoh. Factors modifying contraction relaxation cycle in vascular smooth muscle cells. *Am. J. Physiol.*, 1982, *243*, H641-H662.

Kwan, C.Y. Dysfunction of calcium handling by smooth muscle in hypertension. *Can. J. Physiol. Pharmacol.*, 1985a, *6*, 366 - 374.

Kwan, C.Y. Calcium handling defects and smooth muscle pathophysiology. In A.K. Grover and E.E. Daniel (Ed.), *Calcium and Contractility*, Clifton, N.J.: Humana Press., 1985b.

Kwan, C.Y., L. Belbeck and E.E. Daniel. Abnormal biochemistry of vascular smooth muscle plasma membrane as an important factor in the initiation and maintenance of hypertension. *Blood Vessels.*, 1979, *16*, 259-268.

Kwan, C.Y., L. Belbeck and E.E. Daniel. Characteristics of arterial plasma membrane in renovascular hypertension in rats. *Blood. Vessels.*, 1980, *17*, 131-140.

Kwetnacky, R., R. McCarthy., N.B. Thoa., R.C. Lake & I.J. Kopin. Sympathoadrenal responses of spontaneously hypertensive rats to immobilzation. *Am. J. Physiol.*, 1979, *236*, H457-H462.

Laher, I. and C.R. Triggle. Pharmacological studies of smooth muscle from Dahl salt-sensitive and salt-resistant rats. *Can. J. Physiol. Pharmacol.*, 1984a, *62*, 101 - 104.

Lais, L.T. and M.J. Brody. Vasoconstrictor hyper-responsiveness: an early pathogenic mechanism in the spontaneously hypertensive rat. *Eur. J. Pharmacol.*, 1978, *47*, 177 - 189.

Lais, L.T., R.K. Bhatnagar & M.J. Brody. Inhibition by dark adaptation of the progress of hypertension in the spontaneously hypertensive rat (SHR). *Circ. Res.*, 1974, *34/35 (Suppl.I)*, I155-I160.

Lais, L.T., L.L. Rios., S. Boutelle., G.F. DiBona & M.J. Brody. Arterial pressure development in neonatal and young spontaneously hypertensive rats. *Blood Vessels.*, 1977, *14*, 277-284.

Lais, L.T., R.A. Schaffer and M.J. Brody. Neurogenic and humoral factors controlling vascular resistance in the spontaneously hypertensive rat. *Circ. Res.*, 1974, *35*, 764 - 774.

Lau, K and B. Eby. The role of calcium in genetic hypertension. *Hypertension*, 1985, , 657-667.

Leef-Lundberg, L.M.F., S. Cotechia., M.C. Caron & R.J. Lefkowitz. Agonist promoted desensitization and phosphorylation of α_1-adrenergic receptors coupled to stimulation of phospatidylinositol mrtabolism. *Fed. Proc.*, 1986, *45*, 928.

Levy, J.V. Verapamil and diazoxide antagonism of agonist induced contractions of aortic strips from normal and spontaneously hypertensive rats. *Res. Commun. Chem. Pathol. Pharmacol.*, 1975, *11*, 387-404.

Lipe, S & R.F.W. Moulds. Calcium, drug action and hypertension. *Pharmacology & Drugs*, 1983, *22*, 299-330.

Lipe, S & R.F.W Moulds. In vitro calcium dependence of arterial smooth muscle in human hypertension. *Clin. Exp. Pharmacol. Physiol.*, 1985, *12*, 319-323.

Loutzenhiser, R., P. Leyten., K. Saida and C. van Breemen. Calcium compartments and mobilization during contraction of smooth muscle. In A.K. Grover and E.E. Daniel (Ed.), *Calcium and Contractility*, Clifton, N.J.: Humana Press., 1985.

Loutzenhiser, R and C. Van Breemen. The influence of receptor occupation on calcium influx mediated vascular smooth muscle contraction. *Circ. Res.*, 1983, *52(Suppl.1.)*, I97-I103.

Lund-Johansen, P. Hemodynamic alterations in hypertension - spontaneous changes and effects of drug therapy. A review. *Acta. Med. Scand.*, 1977, *609(Suppl)*, 1-14.

Lundin, S.H & P. Thoren. Renal function and sympathetic activity during mental stress in normotensive and spontaneously hypertensive rats. *Acta. Physiol. Scand.*, 1982, *115*, 115-124.

MacGregor, G.A. Sodium is more important than calcium in essential hypertension. *Hypertension*, 1985a, 7, 623-637.

MacGregor, G.A., N.D. Markandu., C. Rotellar., S.J. Smith and G.A. Sagnella. The acute effect of nifedipine is related to pre-treatment blood pressure. *Postgrad. Med. J.*, 1983, *59(Suppl.2)*, 91-94.

MacGregor, G.A., N.D. Markandu. S.J. Smith and G.A. Sagnella. Does nifedipine reveal a functional abnormality of arteriolar smooth muscle cell in essential hypertension. The effect of altering sodium balance. *J. Cardiovasc. Pharmacol.*, 1985, *7(Suppl. 6)*, S178-S181.

Matsuda, H., E. Kuon., J. Holtz & R. Busse. Endothelium mediated dilations contribute to the polarity of the arterial wall in vasomotion induced by α_2-adrenergic agonists. *J. Cardiovasc. Pharmacol.*, 1985, 7, 680-688.

McCarron, D.A. Is calcium more important than sodium in the pathogenesis of essential hypertension? *Hypertension*, 1985, 7, 607-627.

McGrath, J.C. Evidence for more than one type of postjunctional α-adrenoceptor. *Biochem. Pharmacol.*, 1982, *31*, 467 - 482.

McGrath, J.C. The variety of vascular α-adrenoceptors. *Trends Pharmacol. Sci.*, 1983, *4*, 14 - 18.

McGrath, J.C. α-Adrenoceptor and the Ca^{2+} dependence of smooth muscle contraction: evidence for subtypes of receptors or for agonist-dependent differences in the agonist-receptor interaction? *Clinical Science.*, 1985, *68 (Suppl. 10)*, 55s-63s.

McGregor, D.D and F.H. Smirk. Vascular responses to to 5-Hydroxytryptamine in genetic and renal hypertensive rats. *Am. J. Physiol.*, 1970, *219*, 687-690.

McMurty, J.P., G.L. Wright & B.C. Wexler. Spontaneous hypertension in cross-suckled rats. *Science*, 1981, *211*, 1173-1175.

Medgett, I.C. and S.Z. Langer. Heterogeneity of smooth muscle α-adrenoceptors in rat tail artery *in vitro*. *J. Pharmacol. Exp. Ther.*, 1984c, *229*, 823 - 229.

Medgett, I.C. and S. Rajanayangam. Effects of reduced calcium concentration and of diltiazem on vasoconstrictor responses to noradrenaline and sympathetic nerve stimulation in rat isolated tail artery. *Br. J. Pharmac.*, 1984b, *83*, 889 - 898.

Medgett, I.C., P.E. Hicks and S.Z. Langer. Smooth muscle α_2-adrenoceptors mediate vasoconstrictor responses to exogenous norepinephrine and to

sympathetic stimulation to a greater extent in spontaneously hypertensive than in wistar kyoto rat tail arteries. *J. Pharmacol. Exp. Ther.*, 1984a, *231*, 159 - 165.

Meisheri, K.D., O. Hwang and C. van Breemen. Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. *J. Membrane Biol.*, 1980, *59*, 19 - 25.

Mellander, S & B. Johansson. Control of resistance, exchange, and capacitance functions in the peripheral circulation. *Pharmacological Reviews.*, 1968, *20*, 117-196.

Meuller, E and C. Van Breemen. Role in intracellular calcium sequestration in beta adrenergic relaxation of smooth muscle. *Nature*, 1979, *281*, 582-583.

Miller, R.C. Second messengers, phosphorylation and neurotransmitter release. *Trends in Neurosciences.*, 1985, *8*, 436-465.

Mochizuki, A., Y. Yamamoto., S. Kondo., K. Aoki., T. Mizuko and K. Hotta. Tension development and Ca^{2+} uptake of isolated aorta from spontaneously hypertensive rats. *Japan. Circ. J.*, 1979, *43*, 853-854.

Morel, N., M. Wibo and T. Godfraind. A calmodulin stimulated Ca^{2+} pump in rat aorta plasma membranes. *Biochim. Biophys. Acta.*, 1981, *644*, 82-88.

Mulvany, M.J., E. Mikkelesen., O.L. Pedersen., N. Nyborg and L.T. Jepersen. Cardiovascular abnormalities in spontaneously hypertensive rats. Causes or consequences of increased pressure? In R. Jacob., R.W. Gulch & G. Kissling (Ed.), *Cardiac adaptation to hemodynamic overload, training and stress.*, Darmstadt: Dr. D. Steinkopff Verlag, 1983.

Mulvany, M.J. Investigations of resistance vessels. *Prog.appl. Microcirc.*, 1985, *8*, 1-6.

Mulvany, M.J & N. Korsgaard. Correlations and otherwise between blood pressure, cardiac mass and resistance vessels characteristics in hypertensive, normotensive and hypertensive/normotensice hybrid rats. *J. Hypertension.*, 1983, *1*, 235-244.

Mulvany, M.J. and N. Nyborg. An increased calcium sensitivity of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. *Br. J. Pharmac.*, 1980, *71*, 585 - 596.

Mulvany, M.J & N. Nyborg. Correlations and otherwise between blood pressure, cardiac mass and resistance vessel characteristics in hypertensive, normotensive and hypertensive/normotensive hybrid rats. *J. Hypertension.*, 1983, *1*, 235-244.

Mulvany, M.J., C. Aalkajaer and J. Christensen. Changes in noradrenaline sensitivity and morphology of arterial resistance vessels during development of high blood pressure in spontaneously hypertensive rats. *Hypertension.*, 1980, *2*, 664-671.

Mulvany, M.J., P.K. Hansen and C. Aalkajaer. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. *Circ. Res.*, 1978, *43*, 854-864.

Mulvany, M.J., N. Korsgaard, N. Nyborg and H. Nilsson. Chemical denervation does not affect medial hypertrophy and increased calcium sensitivity of mesenteric resistance vessels from spontaneously hypertensive rats. *Clinical Science.*, 1981a, *61*, 61s - 63s.

Mulvany, M.J., H. Nilsson,, N. Nyborg and E. Mikkelsen. Are isolated femoral resistance vessels or tail arteries good models for the hindquarter vasculature of spontaneously hypertensive rats. *Acta Physiol Scand.*, 1982, *116*, 275 - 283.

Murphy, K.M., R.J. Gould and S.H. Snyder. Autoradiographic visualization of [^{3}H]-nitrendipine binding sites in rat brain: Localization to synaptic zones. *Eur. J. Pharmacol.*, 1982, *81*, 517 - 519.

Nagaoka, A & W. Lovenberg. Regional changes in the activities of aminergic biosynthetic enzymes in the brains of hypertensive rats. *Eur. J. Pharmacol.*, 1977, *43*, 297-306.

Nakamura, K & K. Nakamura. Activation of central noradrenergic and adrenergic neurons in young and adult spontaneously hypertensive rats. *Japan Heart. J.*, 1978, *19*, 635-636.

Nghiem, C., V.C. Swamy and D.J. Triggle. Inhibition by D 600 of norepinephrine and clonidine induced responses of the aortae from normotensive (WKY) and spontaneously hypertensive rats (SHR). *Life Sci.*, 1982b, *30*, 45 -49.

Noon, J.P., P.J. Rice and R.J. Baldessarini. Calcium leakage as a cause of the high resting tension in vascular smooth muscle from the spontaneously hypertensive rat. *Proc. Natl. Acad. Sci.*, 1978, *75*, 1605 - 1607.

Nyborg, N.C.B and M.J. Mulvany. Effect of felodipine, a new dihydropyridine vasodilator, on contractile responses to potassium, noradrenaline, and calcium in mesenteric resistance vessels of the rat. *J. Cardiovasc. Pharmacol.*, 1984, *6*, 499-505.

Nyborg, N.C.B., J. Byg-Hansen and M.J. Mulvany. Effect of felodipine on

resistance vessels from spontaneously hypertensive and normotensive rats. *J. Cardiovasc. Pharmacol.*, 1985, *7(Suppl.6)*, S43-S46.

Ogura, K & K. Hashimoto. Qualitative and comparitive studies of pharmacological features in the coronary femoral and renal circulations with different coronary vasodilators. *Jpn. J. Pharmacology.*, 1974, *24*, 227-233.

Okamoto, K and K. Aoki. Development of a strain of spontaneously hypertensive rats. *Japan. Circ. J.*, 1963, *27*, 282 - 293.

Okamoto, K., S. Nosaka., Y. Yamori & Y. Matsumoto. Participation of neural factors in the pathogenesis of hypertension in the spontaneously hypertensive rat. *Japan. Heart. J.*, 1967, *8*, 168-180.

Olivari, M.Y., C. Bartorelli., A. Polese., C. Fiorentini., P. Moruzzi & M.D. Guazzi. Treatment of hypertension with nifedipine, a calcium, antagonist agent. *Circulation.*, 1979, *59*, 1056-1062.

Page, I.H and J.W. McCubin. The physiology of arterial hypertension. In W.F. Hamilton & P. Dow Waverly (Eds.), *Handbook of Physiology. Circulation*, Washington: American Physiological Society, 1965.

Pang, C.C.Y & M.C. Sutter. Differential effects of D600 on contractile response of aorta and portal vein from spontaneously hypertensive rats. *Blood Vessels.*, 1981, *18*, 120-127.

Paul, O. Epidemiology of hypertension. In J. Genest, E. Koiw & O. Kachel (Ed.), *Hypertension.*, New York: McGraw Hill, 1977.

Pedersen, O.P., N.J. Christensen & K.D. Ramsch. Comparision of acute effects of nifedipine in arterial hypertension. *J. Cardiovasc. Pharmacol.*, 1980. *2*, 357-366.

Pedersen, O.L., E. Mikkelsen and K.-E. Anderson. Effects of extracellular calcium on potassium and noradrenaline induced contractions in the aorta of spontaneously hypertensive rats - Increased sensitivity to nifedipine. *Acta. Pharmacol. et Toxicol.*, 1978, *43*, 137 - 144.

Pickering, G.W. *The nature of essential hypertension.* London:Churchill, 1961.

Postnov, Y.V and S.N. Orlow. Cell membrane alteration as a source of primary hypertension. *J. Hypertension*, 1984, *2*, 1-6.

Preutitt, R.L & R.F. Dowell. Structural vascular adaptations during the developmental stages of hypertension in the spontaneously hypertensive rat. In D.H. Lewis (Eds.), *Current advances in basic and clinical microcirculatory research.*, Basel: Karger, 1978.

Prosser, C.L. Smooth muscle. *Ann. Rev. Physiol.*, 1974, *36*, 503-535.

Provoost, A.P & W. De Jong. Differential development of of renal, DOCA-salt, and spontaneous hypertension in the rat after neonatal sympathectomy. *Clin. Exp. Hypertens.*, 1978, *1*, 177-189.

Raeymaekers, L and R. Casteels. Measurement of calcium uptake in the endoplasmic reticulum of the smooth mucsle cells of rabbit ear artery. *Arch. Int. Physiol. Biochim.*, 1981, *89*, 33-34.

Reuter, H. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature*, 1983, *301*, 569-574.

Reuter, H., H. Porzia., S. Kokubun and B. Prod'hum. 1,4, dihydropyridines as tools in the study of Ca^{2+} channels. *Trends in Neuroscience*, 1985, , 396-400.

Rickstein, S.E & P. Thoren. Characteristics of the sympathetic nervous activity in awake normotensive and hypertensive rats. *Acta. Physiol. Scand.*, 1979, *105*, 31A-32A.

Robinson, B.F. Altered calcium handling as a cause of primary hypertension. *Journal of Hypertension.*, 1984, *2*, 453 - 460.

Robinson, B.F., R.J. Dobbs & S. Bayley. Response of the forearm resistance vessels to verapamil and sodium nitroprusside in normotensive and hypertensive men: evidence for a functional abnormality of vascular smooth muscle in primary hypertension. *Clin. Sci.*, 1982, *63*, 33-42.

Rosendorff, C., E. Susanni., M.L. Hurwitz & F.P. Ross. Adrenergic receptors in hypertension. Radioligand binding studies. *J. Hypertension.*, 1985, *3*, 57F-581.

Saavedra, J.M., H. Grobecker & J. Axelrod. Changes in central catecholaminergic neurons in spontaneously (genetic) hypertensive rat. *Circ. Res.*, 1978, *42*, 529-534.

Saida, K & C. Van Breemen. Mechanism of Ca^{2+} antagonist induced vasodilation: intracellular actions. *Circ. Res.*, 1983, *53*, 137-142.

Sarmiento, J.G., R.A. Janis., D. Jenkins., A.M. Katz & D.J.Triggle. Characteristics of [^{3}H]nitrendipine binding to vascular smooth muscle. In A. Scriabine, S. Vanov & K. Deck (Eds.), *Nitrendipine*, Baltimore, MD.: Urban & Schwarzenberg, 1984.

Scatchard, G. Attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, 1949, *51*, 660 - 672.

Schomig, A., R. Dietz., W. Pascher. J.B. Luth., J. Mann., M. Schmidt & J. Weber. Sympathetic vascular tone in spontaneous hypertension of rats. *Klin. Wschr.*, 1978, *56(Suppl. I.)*, 131-138.

Schramm, M., G. Thomas, R. Towart and G. Franckowiak. Activation of calcium channels by novel 1,4-dihydropyridines: a new mechanism for positive inotropics or smooth muscle stimulants. *Arzneim-Forsch. Drug Res.*, 1983a, *33*, 1268 - 1272.

Schramm, M., G. Thomas, R. Towart and G, Franckowiak. Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. *Nature.*, 1983b, *303*, 535 - 537.

Sharma, J.N., P.G. Fernandez, I. Laher and C.R. Triggle. Differential sensitivity of Dahl salt-sensitive and Dahl salt- resistant rats to the hypotensive action of acute nifedipine administration. *Can. J. Physiol. Pharmacol.*, 1984, *62*, 241 - 243.

Shibata, S., M. Kuchii & T. Taniguchi. Calcium influx and binding in the aortic smooth muscle from spontaneously hypertensive rat. *Blood Vessels.*, 1975, *12*, 279-289.

Shibata, S., K. Kurahuchi. and M. Kuchii. A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, 1973, *185*, 406-417.

Sivertsson, R. Hemodynamic importance of structural cardiovascular factors in primary hypertension. *Acta. Physiol. Scand.*, 1970, *79(Suppl. 343.)*, 1-56.

Skarby, T., K.E. Andersson & L. Edvinsson. Characterisation of the postsynaptic α adrenoceptor in isolated feline cerebral arteries. *Acta. Physiol. Scand.*, 1981, *112*, 105-107.

Smirk, F.H. Pathogenesis of essential hypertension. *Br. Med. J.*, 1949, *1*, 791-797.

Sokal, R.R and F.J. Rohlf. *Biometry. The principles and practice of statistics in biological research.* San Francisco, California.:W.H. Freeman & Company., 1969.

Somlyo, A.V and A.P. Somlyo. Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmacol. Exp. Therap.*, 1968, *159*, 129-145.

Somlyo, A.P and A.V. Somlyo. Vascular smooth muscle I. Normal structure, pathology, biochemistry and biophysics. *Pharmacol. Rev.*, 1968, *20*, 197-272.

Somlyo, A.P and A.V. Somlyo. Calcium, magnesium and vascular smooth muscle function. In J. Genest, O. Kuchel, P Hamet & M. Cantin (Eds.), *Hypertension. Physiopathology and treatment. 2nd Edn.*, New York: McGraw Hill Book Co, 1983.

Spedding, M. The mechanism of the contractile effects of phorbol esters in smooth muscle is not primarily via Ca^{2+} channel activation. *Br. J. Pharmacol.*, 1986, *87*, 62P.

Steinsland, O.S., R.F. Furchgott & S.M. Kirpekar. Biphasic vasoconstriction of the rabbit ear artery. *Circ. Res.*, 1973, *32*, 49-58.

Stone, P.H., E.M. Antman., J.E. Miller and E. Braunwald. Calcium channel blocking agents in the treatment of cardiovascular disorders. Part II. Hemodynamic effects and clinical applications. *Ann. Intern. Med.*, 1980, *93*, 886 - 904.

Struyker - Boudier, H.A.J., R.T. Evenwel., J.F.M. Smits and H. Van Essen. Baroreflex sensitivity during the development of spontaneous hypertension. *Clin. Sci.*, 1982, *62*, 589-594.

Su, C., J.A. Bevan and B.C. Ursillo. Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. *Circ. Res.*, 1964, *15*, 20-27.

Sutter, M.C. Ionic permeability and blood pressure. *Can. J. Physiol. Pharmacol.*, 1985, *63*, 375 - 379.

Sutter, M.C., M. Hallback., V. Jones and B. Folkow. Contractile responses to noradrenaline. Varying dependence on external calcium of consecutive vascular segments of perfused rat hindquarter. *Acta. Physiol. Scand.*, 1977, *99*, 166-172.

Swamy, V.C and D.J. Triggle. The reactivity of illiac vascular strips from spontaneously hypertensive and normotensive rats. *Blood Vessels.*, 1980a, *17*, 246-256.

Swamy, V.C and D.J. Triggle. The responses of carotid vascular strips from spontaneously hypertensive and normotensive rats. *Can. J. Physiol. Pharmacol.*, 1980b, *58*, 53-59.

Tallarida, R.J & R.B. Murray. *Book manual of Pharmacological Calculations.* New York.:Springer Verlag, 1981.

Tanase, H., Y. Suzuki., A. Ooshima., Y. Yamori & K. Okamoto. Genetic analysis of blood pressure in spontaneously hypertensive rats. *Japan. Circ. J.*, 1970, *34*, 1197-1212.

Timmermans, P.B.M.W.M & P.A. van Zwieten. Minireview: the postsynaptic α_2-adrenoceptor. *J. Auton. Pharmacol.*, 1981, *1*, 171-183.

Touster, O., N.N. Aronson., J.T. Dulaney and P. Hendrickson. Isolation of rat liver plasma membranes. Use of nucleotide pyrophosphatase and phosphodiesterase 1 as marker enzymes. *J. Cell. Biol.*, 1970, *47*, 604-618.

Touw, K.B., J.R. Haywood., R.A. Schaffer & M.J. Brody. Contribution of the sympathetic nervous system to vascular resistance in young and old spontaneously hypertensive rats. *Hypertension.*, 1980, *2*, 408-418.

Triggle, D.J. Some aspects of the chemical pharmacology of calcium channel antagonists. In H.H. Ong & J.C. Lewis (Eds.), *Hypertension. Physiological basis and treatment.*, Orlando, FL: Acad. Press., 1984.

Triggle, D and R.A. Janis. Nitrendipine: Binding sites and mechanism of action. In A. Scriabine., S. Vanov and K. Deck (Ed.), *Nitrendipine*, Baltimore, MD.: Urban & Schwarzenberg., 1984.

Triggle, D.J & R.A. Janis. The 1,4-Dihydrpyridine receptor: A regulatory component of the Ca^{2+} channel. *J. Cardiovasc. Pharmacol.*, 1984, *6*, S949-S955.

Triggle, C.R. and I. Laher: A review of changes in vascular smooth muscle functions in hypertension: isolated tissue versus in vivo studies. *Can. J. Physiol. Pharmacol.*, 1985, *63*, 355 - 365.

Triggle, D.J. & V.C. Swamy. Pharmacology of agents that affect calcium. *Chest*, 1980, *78(Suppl)*, 174-180.

Triggle, C.R., D.K. Agrawal., G.T. Bolger., E.E. Daniel., C.Y. Kwan., E.M. Luchowski and D.J. Triggle. Calcium channel antagonist binding to isolated vascular smooth muscle membranes. *Can J. Physiol. Pharmacol.*, 1983, *60*, 1738 - 1741.

Van Breemen, C. Calcium requirements for activation of intact smooth muscle. *J. Physiol.*, 1977, *272*, 317-329.

Van Breemen, C and R. Loutzenhiser. Calcium movements in smooth muscle. *Chest*, 1980, *78(Suppl)*, 157-165.

Van Breemen, C., P. Aaronson and R. Loutzenhiser. Na-Ca intercations in mammalian smooth muscle. *Rev. Pharmacol.*, 1979, *30*, 167-208.

Van Breemen, C., B.R Farinas., P. Gerba and E.D. McNaughton. Excitation contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. *Circ. Res.*, 1972, *30*, 44-54.

Van Meel, J.C.A., A. Dejonge., H.O. Kalman., B. Wilfert., P.B.M.W.M. Timmermans & P.A. van Zwieten. Organic and inorganic calcium antagonists reduce vasoconstriction *in vivo* mediated by postsynaptic α_2 adrenoceptors. *Naunyn - Schmiedeberg's Arch. Pharmacol.*, 1981, *316*, 288-293.

Van Zwieten, P.A., J.C.A. van'Meel & P.B.M.W.M. Timmermans. Functional interaction between calcium antagonists and vasoconstriction induced by the stimulation of postsynaptic α_2 adrenoceptors. *Circ. Res.*, 1983, *52(Suppl.1)*, 77-80.

Vater, V.W., G. Kroneberg., F. Hoffmeister., H. Kaller., K. Merg., A. Oberdof., W. Puls., K. Schlossman & K. Stoepel. On the pharmacology of 4-(2'-nitrophenyl)-2, 6-dimethyl-3, 5 dicarbomethoxy-1,4-dihydropyridine.(Nifedipine, Bay a 1040). *Arzneim. Forsch.*, 1972, *22*, 1-14.

Webb, R.C and D.F. Bohr. Recent advances in the pathogenesis of hypertension: Consideration of sructural, functional, and metabolic vascular abnormalities resulting in elevated arterial resistance. *Am. H. Journal.*, 1981, *102*, 251 - 264.

Webb, R.C and R.C. Bhalla. Altered calcium sequestration by subcellular fractions of vascular smooth muscle from spontaneously hypertensive rat. *J. Moll. Cell. Cardiol.*, 1976, *8*, 651-660.

Webb, R.C and D.F.Bohr. Potassium relaxation of vascular smooth muscle cell from spontaneous hypertensive rats. *Blood Vessels.*, 1979, *16*, 71-79.

Webb, R.C. and P.M. Vanhoutte. Sensitivity to noradrenaline in isolated tail arteries from spontaneously hypertensive rats. *Clinical Science.*, 1979, *57*, 31s - 33s.

Webb, R.C., P. Vanhoutte & D.F. Bohr. Adrenergic neurotransmission in vascular smooth muscle from spontaneously hypertensive rats. *Hypertension*, 1981, *3*, 193-203.

Wei, R., R.A. Janis and E.E. Daniel. Calcium accumulation and enzymatic activities of subcellular fractions from aortae and ventricles of genetically hypertensive rats. *Circ. Res.*, 1976, *39*, 133-140.

Weiss, R.J., R.C. Webb & C.B. Smith. Alpha$_2$-adrenoceptors on arterial smooth muscle selective labelling by [^{3}H]clonidine. *J. Pharmacol. Exp. Ther.*, 1983, *225*, 599-605.

Yamori, Y. Pathogenesis of spontaneous hypertension as a model for essential hypertension. *Japan. Circ. J.*, 1977, *41*, 259-266.

Yamori, Y. Physiopathology of various strains of spontaneously hypertensive rats. In J. Genest, O. Kuchel, P. Hamet & M. Cantin (Eds.), *Hypertension. Physiopathology and treatment*, New York: McGraw Hill Book Co., 1983.

Yamori, Y., M. Matsumoto., H. Yanabe & K. Okamoto. Augmentation of spontaneous hypertension by chronic stress in rats. *Japan Circ. J.*, 1969, *33*, 399-409.

Yamori, Y., A. Ooshima & K. Okamoto. Genetic factors involved in spontaneous hypertension in rats. An analysis of F_2 segregrate generation. *Japan Circ. J.*, 1970, *36*, 561-568.

Yamori, Y., H. Yamabe., W. De Jong., W. Lovenberg & A. Sjoerdsma. Effect of tissue norepinephrine depletion by 6-hydroxydopamine on blood pressure in spontaneously hypertensive rats. *Eur. J. Pharmacol.*, 1972, *17*, 135-140.

Zsoter, T.T., C. Wolchinsky., N.F. Henein and L.C. Ho. Calcium kinetics in the aorta of the spontaneously hypertensive rats. *Cardiovasc. Res.*, 1977, *11*, 353-357.