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A STUDY OF THE PHARMACOLOGICAL REACTIVITY OF AORTAE FROM SPONTANEOUSLY HYPERTENSIVE RATS

by

© Mark T. Goldberg, B.Sc.

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

Increased vascular reactivity to pressor agents has often been observed in hypertensive states. A widely accepted hypothesis for this phenomenon attributes the reactivity changes to structural changes, such as vascular smooth muscle hypertrophy, that are thought to take place subsequent to the onset of hypertension. The work presented here studied vascular reactivity in an animal model of essential hypertension and offers an alternative explanation for this phenomenon.

Spontaneously hypertensive rats (SHR) treated from conception with timolol, a β-adrenergic blocker, did not develop high blood pressure. Thoracic aorta from these normotensive SHR exhibited increased reactivity to raised extracellular K^+, when compared with tissues from timolol-treated Kyoto Wistar control rats. The normotensive SHR aorta also showed significant responsiveness to La^{3+} and to high extracellular Ca^{2+} concentrations without previously depolarizing the tissue with high K^+. The response to La^{3+} was shown to be primarily mediated through a precipitation of the buffer in normal Krebs solution, resulting in a decrease in the pH of the bathing media. A large contractile response was recorded when these conditions were mimicked by the addition of HCl to the Krebs solution bathing the SHR aorta.

These observations indicate that altered reactivity in SHR aorta is not a consequence of hypertension. Reactivity changes were attributed to a deficient control of Ca^{2+} homeostasis in

this tissue, which may prove to be a causative factor in the etiology of hypertension in the SHR.

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LIST OF ABBREVIATIONS

Adren.	adrenaline
ATP	adenosine triphosphate
c-AMP	adenosine-3',5'-cyclic monophosphate
c-GMP	guanosine-3',5'-cyclic monophosphate
DOCA	desoxycorticosterone acetate
5-HT	5-hydroxytryptamine (serotonin)
GHR	New Zealand strain of genetically hypertensive rat
Max.	maximum
NA	noradrenaline
SHR	Japanese strain of spontaneously hypertensive rat
VSM	vascular smooth muscle
WKY	Kyoto Wistar rat

I INTRODUCTION

Hypertension, defined as a systolic blood pressure over 160 mm Hg or a diastolic pressure over 95 mm Hg afflicts over 18% of adults in the United States, making it the most common disease in that country (Health - United States, 1976-77). Hypertension, if left untreated, can lead to acute health problems such as heart disease (the leading cause of death in Canada and the U.S.A.), stroke, or kidney failure. It has been estimated that up to 90% of people with high blood pressure have what is known as "essential" hypertension; that is high blood pressure of unknown etiology.

To investigate possible causes of essential hypertension several animal models of the disease have been developed. The most widely studied of these models is the Spontaneously Hypertensive Rat (SHR), developed by Okamoto and Aoki (1963) by systematic inbreeding. Inheritance of hypertension in the SHR is polygenic (Hansen, 1972; Louis et al., 1969a) and determined by a few genes which act additively (Tanase et al., 1972). The pathogenesis of hypertension in this animal has been studied extensively and is reviewed below.

A. Etiology of Hypertension in the Spontaneously Hypertensive Rat

It has become evident that the pathogenesis of hypertension in the SHR is multifactorial, having both genetic and environmental components. The genetic factors are the predominant determinants of elevated blood pressure; their degree of influence having been calculated at over 80% (Tanase et al., 1970), while environmental factors tend to have an additive or diminutive effect on the rate of development and extent of

blood pressure elevation. At least 4 different genetically determined causes of high blood pressure have been studied and results are summarized here. The studies reported often seem to offer conflicting evidence. However, choice of controls and methodology vary and, for this reason, studies are often difficult to compare directly.

1. Environmental Influences

Selye (1974) has described stress as a phenomenum characterized by feelings of helplessness or depression. Its pathophysiological effects are probably mediated through circulating levels of adrenal corticotrophic hormone (ACTH). Henry and Cassel (1969) (see: Henry and Stephens, 1977) reviewed the effects of Selyean stress on incidence of hypertension in man and found a strong correlation in many cultures.

Although it is difficult to extrapolate stress measurements to animal studies, the rate of development of high blood pressure in the SHR does appear to be influenced by various types of environmental alterations. The thermal stress of cooling to 32°C increased the blood pressure of SHR, but not of Kyoto Wistar (WKY) control (Yen et al., 1978). Low temperature (16°C) also has more pronounced effects on lowering membrane potential of vascular smooth muscle (VSM) from SHR than from WKY (Hermsmeyer, 1976). Thermal resistance, as determined by survival time at high ambient temperatures, was lower in the SHR than in the Sprague-Dawley rat (Wright et al., 1977).

Immobilization, as well as combined visual, auditory and electrical stimuli increased the degree of hypertension in the SHR (Yamori et al., 1969). Even young SHR showed hyperreactive cardiovascular responses to brief periodic altering stimuli such as light flashes, noise and

vibration (Hallback and Folkow, 1974).

Ambient light levels can effect blood pressure in the SHR as well: Litters of SHR raised in darkness had significantly lower blood pressures than SHR raised in 12 hours of light per day (Lais et al., 1974a).

Dietary salt intake has been implicated as an environmental influence on development of human hypertension (Dahl, 1972). Interestingly, salt restriction does not appear to effect development of high blood pressure in the SHR (Lais et al., 1974; Louis et al., 1969b), although SHR show a preference for NaCl solution over tap water when given access to both (Catalanotto et al., 1972; Fregly, 1975; McConnell and Henkin, 1973).

2. Genetic Factors

a. Hormonal Mechanisms

Levels of circulating hormones play a direct role in the control of blood pressure. Aldosterone promotes retention of Na^+ and consequently retention of water. Vasopressin also promotes water retention and has a direct stimulatory effect on vascular smooth muscle. Adrenaline, noradrenaline, and angiotensin II all have direct pressor effects on vascular smooth muscle tone. Thus, an abnormality in circulating levels of any of these agents or factors which alter their release could provide a pathogenic mechanism for hypertension.

Fischer-Ferraro and co-workers (1971) found evidence of an intrinsic brain renin-angiotensin system in the rat. Subsequent investigations (Genten et al., 1975) revealed that the SHR has elevated cerebrospinal fluid levels of angiotensin II as compared to levels in Sprague Dawley controls.

Dietz and co-workers (1978) found decreased aldosterone secretion and increased secretion of corticosterone in the SHR, while a study by Yamori et al., (1973a) found no abnormality in corticosterone levels in the SHR. The renin-angiotensin system in the SHR was found to be normal (Dietz et al., 1978), except in adult, but not young, stroke-prone SHR (Matsunaga et al., 1975), a substrain which usually succumbs to stroke.

Parabiosis with a normotensive rat produced no increase in blood pressure in the normotensive animal, suggesting circulating factors play minimal role in blood pressure elevation in the SHR (Yamori, 1971).

In summary, the evidence implicating altered hormonal levels in the pathogenesis of hypertension in the SHR is scanty.

b. Renal Mechanisms

Guyton's theory of hypertension (Coleman et al., 1975; Guyton et al., 1974) suggests that the major flaw in genetic hypertension is the kidney's inability to excrete Na^+ at rates that will maintain normal extracellular fluid volume. He proposed that as Na^+ accumulates, the extracellular fluid volume expands, leading to VSM and cardiac muscle hypertrophy, as well as resetting of the baroreceptors. The resulting elevation in blood pressure is sufficient to force blood through the glomeruli at a high enough rate to gradually return extracellular fluid volume to normal levels, but leaving blood pressure high. The physiological findings in hypertensive patients correspond well with this theory; cardiac output is initially high, then once hypertension is established, cardiac output is low, but total peripheral resistance is elevated.

Sakai et al. (1978) reported significantly elevated cardiac output in young SHR compared to old SHR, and significantly higher total peripheral

resistance in old vs. young SHR.

Dietz and co-workers (1978), in further support of Guyton's theory, found young SHR retain more Na^+ than age-matched WKY. Fractional excretion of Na^+ in the SHR increased with age, as does blood pressure. However, this group also found extracellular fluid volume was significantly lower in the SHR, in opposition to the theory, while other investigators (Trippodo et al., 1978) found no difference in plasma volume among SHR, WKY, and Wistar rats.

The most convincing evidence for a renal mechanism of pathogenesis comes from kidney transplantation studies: When kidneys of SHR are transplanted to F_1 hydrids of SHR and Wistar rats, they too became hypertensive, while F_1 hybrids receiving kidneys from Wistar rats did not (Kawabe et al., 1978).

c. Neurogenic Factors

The sympathetic nervous system is intimately involved in the minute to minute control of blood pressure, heart rate, vasomotor tone, circulating adrenaline levels and to some extent, renin levels. A genetic abnormality associated with sympathetic output could conceivably initiate hypertension.

Immunosympathectomy (Folkow, et al., 1972a) prevented, while treatment with 6-hydroxydopamine (a compound that destroys catecholaminergic nerve terminals) delayed (Yamori et al., 1972) or prevented (Linch et al., 1972) high blood pressure development in the SHR. Onset of hypertension in the SHR can also be delayed by α- or β-adrenergic blockers (Folkow et al., 1972b, Numao and Irichijima, 1974).

Involvement of the sympathetic nervous system in the pathogenesis of high blood pressure in the SHR has also been assessed by studies of enzyme activity in the CNS, which are often conflicting.

Nakamura and Nakamara (1978) found dopamine β-hydroxylase activity was elevated in the locus coeruleus, area A2 of the medulla and in spinal intermediolateral cells of young but not old SHR, suggesting increased adrenergic activity may be present in these areas in the initial stages of hypertension. In contrast, Nagatsu et al., (1976) found dopamine β-hydroxylase activity in the locus coeruleus significantly lower in the SHR than in the WKY, although dopamine β-hydroxylase activity was elevated in the periphery in this study.

Another enzyme associated with adrenergic and noradrenergic neurons in the central nervous system, aromatic L-amino acid decarboxylase, had lower activity in SHR than in Wistar rat brains (Yamori et al., 1970); however activity of this enzyme was equally low in both SHR and WKY brains (Yamori et al., 1973b). This enzyme is also necessary in the production of serotonin, another neurotransmitter that is involved in the central control of blood pressure (bulbospinal neurons with cell bodies in the vasomotor areas of the brainstem are a mixture of catecholaminergic and serotonergic nerves - Chalmers, 1975). Buckingham et al., (1976) found that 5,6-dihydroxytryptamine, a compound that destroys serotonergic nerve terminals, when injected intracerebroventricularly in young SHR delayed the onset of hypertension. These authors suggested central serotonergic neurons play a role in the etiology of hypertension in the SHR.

Tyrosine hydroxylase, the rate-limiting enzyme in the production of adrenaline and noradrenaline, was not different in areas A1, A2, or the locus coeruleus in SHR, WKY or Sprague-Dawley rats (Renaud et al.,

1979). Nor was there a difference in MAO activity in central blood vessels obtained from 15 week old SHR and WKY (Lai and Spector, 1978).

Studies of the peripheral sympathetic nervous system are as inconclusive as those of central sympathetic activity: Splanchnic and renal nerves (Irichijima, 1973; Judy et al., 1976; Okamoto et al., 1967) had a higher discharge rate in the SHR than controls. Denervation greatly reduced the initially high blood pressure in the SHR isolated hindlimb preparation (Nosaka et al., 1972). However, Lais and co-workers (1974b) reported that vascular resistance in SHR remained significantly higher than in Wistar rats after bilateral lumbar sympathectomy. Bohlen and Lobach (1978) found the cremaster muscle arterioles in young WKY dilated significantly more after denervation than comparable vessels in the SHR. These two studies imply vascular resistance and arteriole diameter is determined more by the structural or myogenic properties than by nervous input.

The sympathetic nervous system may also contribute to a renal pathogenic mechanism, since renal denervation in young SHR delayed onset of elevated blood pressure (Dietz et al., 1978; Liard, 1977).

In summary, while an abnormality in the function of central and peripheral aminergic neurons is suggested by these studies, the involvement of these neurons in the etiology of hypertension in the SHR has not been firmly established.

d. Vascular Factors

There is evidence that both structural and reactivity changes occur in the vasculature of the SHR that may be related to the development

and maintanence of hypertension. Folkow and co-workers (1970, 1973) have postulated that increased reactivity reported in hypertension is primarily a result of VSM hypertrophy secondary to development of hypertension. They suggest that enlargement of the media of resistance vessels occurs at the expense of lumen diameter. As the VSM hypertrophies, the arteriolar lumen gets narrower and presents more resistance to blood flow. Others (Doyle and Fraser, 1961; Mendlowitz and Naftchi, 1958) suggested the idea that reactivity changes are a cause, not a consequence of elevated pressure.

Folkow's hypothesis is supported by the work of Mulvany and co-workers (Mulvany and Halpern, 1977; Mulvany et al., 1978; Warshaw et al., 1979). They report that mesenteric arteries from SHR showed medial hypertrophy as well as smaller lumen diameter and thicker media than comparable WKY arteries. Yamori and Sasagawa (1975) also reported medial hypertrophy or hyperplasia in SHR using histometrical methods. In contrast to these reports, Hutchins and Darnell (1974) found the small resistance vessels of the cremaster muscle in the SHR had a larger diameter than normotensive controls, but found there were 50% fewer arterioles in the SHR. These findings were confirmed by Heinrick et al. (1978) in the mesenteric bed in SHR. Bohlen (1979) studied the cremaster muscle in SHR and WKY and found fewer open arterioles in the SHR but no significant difference in arteriole diameter, wall thickness or cross-sectional area in the two animals. Thus, histometric studies of VSM considered alone yield conflicting results.

Hypertrophy of VSM is usually associated with increased collagen synthesis, since collagen provides a structural framework to which the smooth muscle cells attach. There have been reports of increased

rates of incorporation of proline, a precursor of collagen, in aorta and mesenteric arteries of older SHR (Yamori, 1976). Prolyl hydroxylase activity, a measure of collagen synthesis, was elevated in SHR vessels (Ooshima et al., 1974). Incorporation of labelled lysine into non-collagenous protein was found to be elevated in young SHR (Yamabe and Lovenberg, 1974).

There is evidence that vascular hypertrophy does not occur early in the development of hypertension in the SHR. Newman and Langner (1978) reported significantly high rates of labelled proline incorporation occur only after 23 weeks of age, when hypertension was well established.

In summary, structural changes that could result in an increased resistance to flow probably occur in response to elevated pressure. But the question of increased reactivity as a causative factor in spontaneous hypertension remains unsettled. A review of this problem follows.

B. The Pharmacological Relationship Between Vascular Reactivity and Hypertension

1. Theoretical Considerations

a. Determining Vascular Reactivity

"Vascular reactivity" is a term that loosely corresponds to the responsiveness of the vasculature to a given stimulus. The stimulus can be a pharmacological one or it can be a change in the perfusion pressure or flow rate through a vessel or vascular bed. Vascular reactivity has been measured with the use of various preparations. The advantages and disadvantages of these different preparations are dealt with elsewhere, but it should be stressed that different aspects of reactivity are measured, depending on the preparation and on the method of data presentation.

When in vivo determinations are made, the hindlimb, hindquarter or whole animal is usually perfused with recirculated blood or a physiological saline through a large artery at a constant rate of flow or at a constant pressure. The relationship between flow (F), changes in perfusion pressure (ΔP) and vascular resistance (R) is an analogue to Ohm's law which expresses the relationship between current, voltage differential and electrical resistance, and can be approximated by the equation:

$$F = \frac{\Delta P}{R}$$ (Burton, 1972; Folkow and Neil, 1971)

Thus, in these constant flow perfusion studies, changes in perfusion pressure are measured and reflect changes in vascular resistance. Responses to controlled increases in flow or to pharmacological agents added to the perfusion fluid which effect vascular smooth muscle contractility are recorded as changes in perfusion pressure. Shortening

of the VSM cell results in a reduction in lumen diameter: A small decrease in lumen diameter results in a large increase in measured resistance since from Poiseuille's Law, resistance (R) to flow through a rigid tube (which approximates flow through a blood vessel) is a function of the inverse of the 4th power of the tube's radius (r):

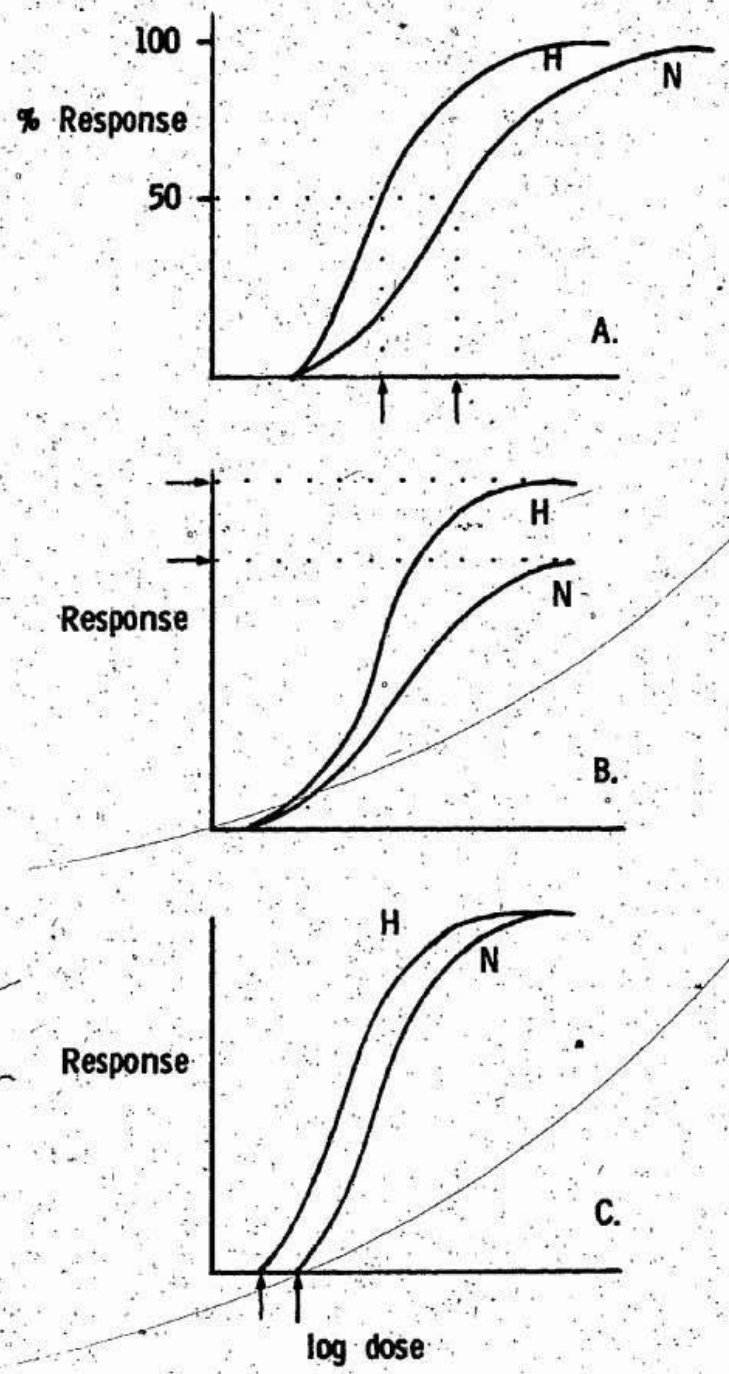
$$R = \frac{8\eta}{\pi r^4}$$ (η in this equation represents viscosity) (Burton, 1972)

In vitro studies of vascular reactivity usually use isolated strips or rings of vascular tissue, although isolated perfused arteries are also used, in which case the above considerations are applicable. In the isolated strip or ring preparations, tension development recorded isometricly is usually reported. Responses to pharmacological agents added to the bathing solution are then recorded as tension development and can be subjected to analysis for vascular reactivity.

There are several parameters to consider when analysing vascular reactivity to pharmacological agents from dose-response data. These are illustrated in Figure 1. Kalsner (1974) demonstrated that when considering supersensitive phenomena, if just dose-ratio is considered (Figure 1A) then hyperresponsiveness due to alterations in the response of the effector tissue itself rather than response of its drug receptors may be missed. He suggests alterations in the threshold (Figure 1C), maximum response (Figure 1B) and the slope of the dose-response curve (Figure 1A) should also be considered.

Johansson (1974) pointed out that there are several different cellular factors that determine vascular reactivity: (1) drug-receptor interactions, (2) availability of Ca^{2+} and/or ATP to the contractile proteins, (3) the state of the contractile proteins themselves, (4) the

Figure 1: Shows hypothetical examples of different parameters of increased vascular reactivity seen in dose-response curves; (A) shows steeper slope and lower ED_{50} in the hypertensive, (B) shows greater maximum response in hypertensive and (C) shows lower threshold in hypertensive. H - hypertensive, N - normotensive.


100
% Response
50
H
N
A.
H
N
Response
B.
H
N
Response
C.
log dose

contribution of passive elastic elements and (5) mechanical factors related to the structure of intact blood vessels. But of these factors, control of Ca^{2+} homeostasis appears to be the intimate link between many types of stimuli and the responsiveness of VSM (see: Fleish, 1974; Weiss, 1977). The control of free intracellular Ca^{2+} levels is briefly reviewed below.

b. Control of Intracellular Ca^{2+} in Smooth Muscle

It is known from studies of glycerinated (Filo et al., 1965) and chemically skinned (Endo et al., 1977) smooth muscle, that to be in a relaxed state, the concentration of free (unbound) intracellular Ca^{2+} must be less than 10^{-7} M and that maximal contractile activity occurs at concentrations of 10^{-5} to 10^{-4} M. The extracellular fluid concentration is in the millimolar range, so an inward Ca^{2+} gradient is maintained even in a maximally contracted state. Yet extracellular Ca^{2+} is necessary for contractile activity; in Ca^{2+}-free solution, VSM loses responsiveness (see: Brading, 1979; Weiss, 1977).

There appear to be several routes via which Ca^{2+} may enter the cell. Passive diffusion down its concentration gradient is an obvious one. There probably exist voltage-dependant Ca^{2+} channels which open when the cell membrane depolarizes and allows Ca^{2+} to enter down its concentration gradient, carrying an inward current (Tomita, 1975). Apparently, other channels exist which admit Ca^{2+} in response to receptor stimulation with little or no membrane depolarization; so called pharmacomechanical-coupling (Casteels et al., 1977; Somlyo and Somlyo, 1968). Intracellular stores of Ca^{2+} may also be released by receptor-mediated events, as in the case of the initial (phasic) response to noradrenaline in rabbit aorta which does not require extracellular Ca^{2+} (van Breemen et al., 1973).

Intracellular storage sites for Ca^{2+} include mitochondria, sarcoplasmic reticulum, nucleus and plasma membrane (Somlyo and Somlyo, 1975). However, the storage sites are saturable and clearly the cell must extrude Ca^{2+} to maintain an inward gradient. It has been suggested that 2 mechanisms exist to extrude Ca^{2+}; an ATP-dependant pump (Janis et al., 1977) and a Na^{+}-Ca^{2+} exchange system (Ma and Bose, 1977). The latter mechanism has been demonstrated in a number of nerve and muscle preparations (see:Blaustein, 1974), but has not been unequivocally demonstrated in smooth muscle (see:van Breemen et al., 1979). The subject of Na^{+}-Ca^{2+} exchange is considered in detail in the Discussion chapter.

If the intracellular free Ca^{2+} concentration is by some means increased in a given vascular tissue, that tissue will show an increased reactivity. There are several possible mechanisms which singly or in combination could account for such a change:

1. An increased extracellular Ca^{2+} concentration would increase the inward Ca^{2+} gradient and the rate of passive diffusion.
2. An increased membrane permeability to Ca^{2+} due to
   a) depolarization and opening of voltage-dependant channels
   b) increased pharmacomechanical coupling
   c) increased passive leakage
3. Intracellular sources of Ca^{2+} more labile due to
   a) reduced storage capacity
   b) increased regenerative release
   c) increased release in response to receptor stimulation
4. Ineffective removal of Ca^{2+} from the VSM cell.

c. Synthesis of a Theoretical Approach to Vascular Reactivity in Hypertension

One can predict the consequences of alterations of different determinants of vascular reactivity in terms of changes they might produce in dose-response curves, as outlined in Figure 1:

If, as Folkow suggests (Folkow et al., 1973) in hypertensive states the VSM hypertrophies and encroaches on vessel lumen, then the lumen will become narrower. If reactivity is then studiedwith perfusion techniques in a constant flow experiment, the narrower lumen will result in a higher baseline pressure than in a comparable vessel or vascular bed from a normotensive animal. If a dose-response curve is then constructed to a pressor agent, the hypertensive tissue should show a greater maximum response (Figure 1B) and a steeper slope (Figure 1A) due to the exponential relationship between lumen radius and resistance, but the threshold should remain the same, since the sensitivity of receptors and the mechanisms controlling the availability of Ca^{2+} should not be altered by hypertrophy.

In isolated ring or strip studies of reactivity, hypertrophy might be expected to produce a <u>decrease</u> in maximum response, since the VSM cells are larger, their ability to shorten is physically impeded by neighbouring hypertrophied cells. The threshold should remain unaltered as in perfused preparations.

If the increased reactivity described is not totally attributable to VSM hypertrophy, but also involves a hypersensitive tissue response quite different effects on dose-response relationships can be anticipated: Both <u>in vivo</u> and <u>in vitro</u> preparations should show a lowered threshold (Figure 1C) since the tissue is either more sensitive to excitation at the receptor level, or perhaps, since more Ca^{2+} is made available to the

contractile proteins through one or more of the mechanisms outlined earlier.

The literature on vascular reactivity in hypertensive states will now be reviewed in the context of these theoretical considerations.

2. Empirical Considerations

Early investigators of hypertension found evidence of increased vascular reactivity to pressor agents in hypertensive patients (Doyle and Black, 1955; Lee and Holze, 1951) and in various experimental animals (Brown and Malgraith, 1941; McQueen, 1956; Smirk, 1949). To reiterate, there are two hypotheses to account for these observations: One hypothesis suggests the increased reactivity reflects a supersensitivity of the vascular smooth muscle in hypertensives. Furthermore, this supersensitivity is probably transmitted genetically and may be a causative factor in this disease. The opposing hypothesis was expounded by Folkow and co-workers (1973) and proposes that the increase in reactivity occurs secondary to structural changes in the resistance vessels; that these changes are the result of vascular smooth muscle (VSM) hypertrophy in response to high blood pressure. In other words, this second hypothesis suggests that the increase in reactivity is not a causative factor, but rather a direct result of hypertension. Folkow suggests hypertrophy of the VSM subsequent to the development of hypertension leads to a narrowing of the vessel lumen. Hypertrophy of the medial smooth muscle of the renal and superior mesenteric arteries has been demonstrated in human hypertension (Barrett, 1963; Furuyama, 1962) but is not apparent in smaller vessels (Short, 1966) from hypertensive humans. A small narrowing of the arteriolar radius will be reflected as a large change in resistance since in accordance with Pouseille's relationship, resistance in a vessel is inversely proportional to the 4th power of the radius. Thus, any encroachment of the hypertrophied muscle into the lumen will appear to increase the resistance in a study that measures

resistance to flow. Folkow suggests this explains the apparent reactivity increase reported in hypertension.

A review of the literature follows, comparing the supporting evidence for each of these hypotheses in both human and experimental hypertension. Care has been taken to reevaluate the data presented to assess the different parameters of reactivity and to offer interpretation in light of them which may, in some cases differ from the authors' original conclusions.

a. Human Vascular Reactivity Studies

Since it is difficult to obtain living arterial smooth muscle from human subjects, studies reported are usually in vivo experiments using intact perfused vascular beds. Most investigators have used the forearm or digital vascular beds since the arterial blood supply is accessable (for the infusion of vasoactive agents) and since changes in blood flow can be readily estimated by plethysmography. Often in these experiments, the contralateral hand or forearm serves as a control. It is assumed that concentrations of infused vasoactive substances are so small that they do not get into systemic circulation in significant concentrations, do not effect heart rate or have reflex effects on blood pressure.

Using this technique increased reactivity in hypertensives was seen to infused 5-methyl-isourea and angiotensin (Doyle and Black, 1955), noradrenaline (Greisman, 1952; Mendlowitz and Naftchi, 1958), 5-hydroxytryptamine (Doyle, Fraser and Marshall, 1959) and adrenaline (Duff, 1956). It is difficult to verify claims of lowered threshold in these studies, as corrections for higher baseline resistance to flow in patients with established hypertension were rarely made. One study

avoided this problem by using a visual measurement of the state of contraction of VSM. Lee and Holze (1951) found the precapillary sphincter in the bulbar conjunctiva of hypertensives were more sensitive to topically applied adrenaline than vessels in the normotensive control group.

These studies appear to support the increased sensitivity hypothesis. However, Folkow and co-workers have published data that supports the increased resistance theory: Sivertsson and Olander (1968) found that resistance to blood flow to the hand in hypertensives was raised even at maximal vasodilation, but found no difference in the threshold sensitivity to noradrenaline when compared to matched normotensive controls.

Conway (1963) found that the increase in flow following reactive hyperemia was similar in hypertensive and normotensive patients. Although this study looked at the effect of vasodilation by metabolites rather than constriction by vasoactive agents, it does lend support to Folkow's hypothesis, and confirmed an earlier study by Folkow et al., (1958).

The studies outlined above have a common flaw: They are studies on patients with established essential hypertension. Since hypertrophy is the natural consequence of prolonged elevation of pressure, it is difficult to separate secondary structural changes due to hypertrophy or hyperplasia from those due to innate myogenic mechanisms. One study of human essential hypertension by Doyle and Fraser (1961) attempted to transcend this difficulty by studying the offspring of hypertensives: Noradrenaline was infused into the forearm in hypertensives and their sons, who were as a group normotensive. The resistance to flow developed was measured by plethysmography. Both the hypertensives and their normotensive sons were found to have increased reactivity. This study suggests that the

increased reactivity to vasoconstrictors seen in hypertension may be an intrinsic property of the VSM and not a secondary effect due to ensuing hypertrophy.

In summary, although most clinical studies of vascular reactivity in hypertension found increased reactivity to vasoactive agents, they are inconclusive with respect to the mechanism. This is so because in vivo reactivity can only be estimated by changes in resistance to a constant flow. Most studies found resistance to be elevated in hypertension, probably as a consequence of hypertrophy of VSM. Once hypertrophy has taken place it is difficult to discriminate between apparent reactivity changes, induced by a narrower lumen, and real ones caused by an increase in VSM sensitivity to vasoconstrictors.

b. Vascular Reactivity in Experimental Hypertension

There are several methods used to produce hypertension in experimental animals; the hypertension usually produced is caused by increased blood volume, renin secretion, mineral corticoid levels, Na^+ retention and/or by genetic predisposition.

A renal model was described in 1934 by Goldblatt and co-workers in which one renal artery was partially constricted with a clamp and the contralateral kidney was removed. This so-called "one-kidney Goldblatt" model resulted in permanent elevation of blood pressure to pathological levels. Hypertension also results when just one renal artery is clamped. The former procedure produces a volume-expansion induced hypertension, while in the latter model, the renin/angiotensin system is thought to be responsible for the increase in blood pressure resulting (Tobian, 1974).

A second model of experimentally produced hypertension is the adrenal corticosterone-induced model. The hypertension is produced by the administration of desoxycorticosterone-acetate (DOCA), usually given in combination with a high salt diet (Selye et al., 1943). In this model, salt is retained rather than excreted by the kidneys. Water retained with the salt leads to a volume-expansion type of high blood pressure.

A third model is genetic hypertension, and is the one which most closely approximates human essential hypertension (i.e., hypertension of unknown etiology). Several strains of hypertensive rats have been described, but the two most widely used are the New Zealand genetically hypertensive rat (GHR) described by Smirk and Hall (1958) and the Japanese spontaneously hypertensive rat (SHR) of Okamoto and Aoki (1963).

Tissues from these models have been used in a number of preparations each with its own advantages and disadvantages for studying vascular smooth muscle reactivity. Perfused whole animals or perfused hindquarters are useful for measuring total peripheral resistance in constant flow experiments. Isolated, perfused vessels or vascular beds provide a means of measuring resistance in a given vessel or vasculature, usually in the absence of autonomic nervous influence. Artificial perfusate can also be used to eliminate the influence of circulating hormones and metabolites in the blood. Isolated vascular beds (eg., the mesenteric bed) are perhaps better preparations than isolated vessels for reactivity studies, because they contain the small muscular arterioles that are the primary determinants of vascular resistance (Folkow and Neil, 1971).

Another preparation of vascular smooth muscle is the isolated strip or ring bathed in physiological solution in a tissue bath. This preparation greatly reduces structural effects and is relatively free of hormonal and

nervous influences, providing a direct measure of contractile tension developed to known concentrations of pressor agents added.

With these preparations and models of hypertension, several investigators have studied reactivity of vascular smooth muscle from hypertensive animals. These studies are tabulated in Tables I, II and III with respect to altered parameters of vascular reactivity. Table I demonstrates that it is difficult to assess reactivity changes on the basis of ED_{50} slope of the dose-response curve alone, as many authors have in the past. In Table II the majority of studies show increased maximum response to pressor agents in perfused preparations and decreased maximum response in isolated rings or strips of aorta, as predicted by the theoretical consequences of hypertrophy. One noteworthy exception is a study by Greenberg and Bohr (1975) which showed an increased maximum response to several pressor stimuli in an isolated perfused portal vein preparation from the SHR. This study is exceptional because the venous side of the circulation is not subjected to high pressure and would not be expected to hypertrophy or show increased reactivity if Folkow's hypothesis were correct.

Table III shows a lower threshold was reported to many stimuli in several different preparations and models of hypertension, supporting the hypothesis postulating increased VSM sensitivity in hypertension.

The discrepancies in results from the same preparation may reflect differences in controls and methodology:

Clineschmidt et al. (1970) has shown significant differences in maximum tension development between 2 normotensive strains, the Carworth Farms Wistar and the NIH-bred Wistar rat. The tabulated studies constitute a wide variety of normotensive controls. There is no consistancy in the

Table I: Vascular Reactivity to Pressor Agents in Hypertensive Rats*

Pressor Agent	Isolated Rings or Strips (aorta unless otherwise specified)			Perfused Vessels (mesenteric artery unless otherwise specified)			Perfused Hindquarters or Hindlimbs			Perfused Whole Animals		
	SHR	DOCA	Renal	SHR	DOCA	Renal	SHR	DOCA	Renal	SHR	DOCA	Renal
NA	≈40, ≈107 ↑62 ↑214 ↑213 ↑226	≈168 ≈200	↑97 ↑100 ≈168 ≈200	↑164, ↑14 ≈98	↑236sm ↑237sc ↑67 ↑65 ↑2361m	↑65 ↑164 ↑236sm ↑237sc ↑2361m	↑68 ↑76	↑68	↑68 ↑166 186	↑59	↑65	↑65
Adren.		≈168 ≈110	≈168	≈98				≈34			↑34	
K^+	↑62 ↑226 ↑62 ↑213		↑100	≈98								
5-HT			↑100	↑103 ↑165	↑103 ↑103r ↑237sc ↑67	↑98 ↑165 ↑237sc						
Ca^{2+} Depol.		↑122		≈68	≈68	≈68						
ATP					↑67							
La^{3+}	√213 ↑ 23c	↑23c										
Mn^{2+}	√213 ↑ 23c	↑23c										
Sr^{2+}	√213 ≈98pv ↑23c	↑23c										

Table I Continued

Table I (Continued)

Pressor Agent	Isolated Rings or Strips (aorta unless otherwise specified)		
	SHR	DOCA	Renal
Ba^{2+}	↓23c +213 +214 ≈98pv	↓23c	

Legend: ↑ steeper slope or lower ED_{50} in hypertensive rat compared to normotensive control

↓ hypertensive rat dose response curve shows less steep slope or higher ED_{50} compared to normotensive control

≈ similar responses reported

✓ response only seen in hypertensive

a aorta

m mesenteric arteries

r renal artery

sc sub-cutaneous arterioles

sm small mesenteric arteries

lc large mesenteric arteries

c carotid artery

pv portal vein

* Numbers refer to articles in the List of References

Table II: Maximum Response to Pressor Agents in VSM from Hypertensive Rats*

Pressor Agent	Isolated Aortic Rings or Strips			Isolated Perfused Vessels			Isolated Perfused Hindquarters
	SHR	DOCA	RENAL	SHR	DOCA	RENAL	SHR
NA	↑62, ↑213 ↓170(GHR)		↑62	↑98(pv) ↓104(m) ↓133(m)	↑67(m) ↓104(m) ↓25(f)	↓10(f)	↑77 ↓150
K^+	↑62 ↓170(GHR)	↓110		↑98(pv)	↓25(f)		
Adren.				↑98(pv)	↓122(f)		
5-HT			↑41	↓103(m)	↑ 67(m) ↓103(m)		
Depol. Ca^{2+}					↓25(f)		
Sr^{2+}				↑98(pv)			
Ba^{2+}				↑98(pv)			↓150
ATP					↑67(m)		

Legend:
- ↑ higher maximum response in hypertensive rat
- ↓ lower maximum response in hypertensive rat
- pv portal vein
- m mesenteric artery
- f femoral artery
- GHR New Zealand genetically hypertensive rat

* Numbers refer to articles in the List of References

Table III: Threshold Response to Pressor Agents in VSM from Hypertensive Rats

Pressor Agents	Isolated Aortic Strips or Ring			Isolated Perfused Vessels or Hindquarters		
	SHR	DOCA	RENAL	SHR	DOCA	RENAL
Noradrenaline	↓62 ≈213		↓62	≈68(m) ≈76(hq) ↓150(hq)	↓25(f) ≈67(m) ≈67(m)	↓10(f) ↓68(m)
K^+	↓62	↓110	↓62		↓25(f)	
5-HT	↓41			↓ 8(f) ↓103(f)	↓8(f) ↓103(f) ≈67(m)	↓8(f) ↓103(f)
Adrenaline					↓122(f)	
Ca^{2+} after depolarization					— ↓25(f)	
ATP					≈67(m)	

Legend: ↓ Lower threshold in hypertensive rat
≈ Similar threshold in hypertensive and normotensive rat
m Mesenteric artery
f Femoral artery
hq Isolated perfused hindquarters

* Numbers refer to articles in the List of References

choice of age-matching or weight-matching of controls and hypertensives nor in the sex of the animals chosen. To further complicate analysis, some authors have normalized their data to percentages of the maximum response recorded, while others have not. Consequently, the results of these studies do not conclusively resolve the question of the cause of increased reactivity in hypertension; there is evidence for both a structural cause and for an intrinsic increased responsiveness. This literature is reviewed in detail below with particular emphasis on studies of the SHR, although hyperreactivity to various excitatory stimuli has been reported in renal hypertension (Finch, 1971; Finch and Haeusler, 1974; Gordon and Nogueira, 1962; Greenberg and Bohr, 1975; Grollman and Krishnamurty, 1973; McGregor and Smirk, 1968; McQueen, 1961; Nolla-Panades, 1963) and in DOCA-induced hypertension (Bohr, 1974; Finch, 1971; Finch, 1975; Finch and Haeusler, 1974: Haeusler and Finch, 1972; Hinke, 1966; Vacek, 1970) as well.

Whether or not this reactivity change seen in various forms of hypertension is a function of structural changes in the arterial wall or represents a true innate supersensitivity is a question best answered by studying spontaneous hypertension in the SHR, since no experimental manipulations are necessary to produce hypertension in this animal. Folkow and co-workers (1970a) with the aid of mathematical models, concluded that the shift seen in the dose-response curve to noradrenaline in the SHR perfused hindquarters compared to normotensive hindquarters could be accounted for by a 30% increase in media thickness. In this preparation they found several parameters of reactivity were increased in the SHR; increased resistance to flow at maximum dilation, a steeper dose-response curve to noradrenaline and a higher maximum pressor response

were noted. However, one aspect of reactivity, the threshold of response was unchanged. This finding strongly supports Folkow's hypothesis since threshold is a parameter which should not change with hypertrophy. Finch and Haeusler (1974) also found the threshold to noradrenaline unchanged in isolated perfused mesenteric artery from the SHR compared to normotensive controls. However, both Haeusler and Finch, in an earlier study (1972), and Armstrong (1972) found a lower threshold to 5-hydroxytryptamine in isolated perfused femoral arteries from the SHR. Greenberg and Bohr (1975) also found the threshold to PGA_2 or PGB_2 was lower in isolated mesenteric artery from the SHR.

To apply the theoretical considerations outlined earlier, one parameter of vascular reactivity, the maximum response, should be dependent on the preparation used: In perfusion studies, if the ratio of wall-thickness to lumen diameter, an index of medial thickening (Folkow et al., 1973) is higher in hypertension one would expect to see an increased maximum resistance and a steeper slope to the dose-response curve in the hypertensive tissue, simply because the lumen of the artery perfused is narrower. But in isolated tissue preparations, hypertrophy of VSM might produce a decreased maximum tension development due to physical impedence.

In aortic strips from New Zealand GHR, Massingham and Shevde (1971) found the maximum tension development decreased to noradrenaline and to depolarizing K^+. This finding was confirmed in the Japanese SHR with both aortic strip (Shibata and Kurahashi, 1972) and aortic ring preparations (Field et al., 1973). Responsiveness to noradrenaline was depressed (Shibata et al., 1973; Shibata and Kurahashi, 1972; Spector et al., 1969) or unchanged (Clineschmidt et al., 1970; Hallback

et al., 1971) in SHR aortic strips in studies by several investigators, lending further support to Folkow's hypothesis. However, a study by Field and co-workers (1972a) on SHR aorta and the study by Massingham and Shevde (1971) on GHR aorta found a lower threshold to both noradrenaline and depolarizing K^+ in the SHR aorta, supporting the increased sensitivity hypothesis of altered activity.

Most studies of reactivity in perfused vascular beds in SHR have found the slope of the dose response curve shifted to the left (eg., Dupont and Sassard, 1974; Finch and Haeusler, 1974), as Folkow's hypothesis predicts. However, one study by Bhattacharya et al. (1977) reported data which contradicts this hypothesis: They used 2 controls; Wistar rats and normotensive SHR which had been isolated by inbreeding from the hypertensive SHR colony. They found the maximum resistance was greater in the hypertensive SHR than in the normotensive SHR, perhaps reflecting lumen narrowing due to hypertrophy. But the slope of the dose-response curve to noradrenaline and the threshold dose were similar in both normotensive and hypertensive SHR, although both curves were shifted to the left of the Wistar dose-response curve. This suggests that increased reactivity is not a function of the development of hypertension, but rather is a genetic characteristic of vascular tissue in the SHR.

Somlyo and Somlyo (1970) in a review of VSM pharmacology suggested that hypertension may be in part a disease of the VSM and that abnormal Ca^{2+} permeability or a disability of the muscle to relax may be responsible for the hyperreactivity seen. Studies of both VSM relaxation and Ca^{2+} probe studies in hypertensive VSM have born out these predictions.

Table IV shows that most studies of vascular relaxation of aortic rings found that SHR aorta relaxes more slowly than aorta from normotensive controls. This suggests either an impaired ability to remove intracellular Ca^{2+} or perhaps increased Ca^{2+} permeability may be associated with genetic hypertension, since relaxation seems to be hindered in a nonspecific way. The former possibility is supported by studies of cyclic nucleotides in SHR VSM: Cyclic AMP has been shown to relax blood vessels (Berti et al., 1970; Somlyo et al., 1972) and Amer (1975) has shown that the ratio of cyclic GMP to cyclic AMP is higher in SHR VSM. The possibility of increased Ca^{2+} permeability in spontaneous hypertension is supported by the observation that SHR VSM is more sensitive than normotensive VSM to inhibitiors of transmembrane Ca^{2+} flux such as diazoxide (Choma and Triggle, 1977; Janis and Triggle, 1973) and nifedipine (Pederson et al., 1978).

Indeed, several studies seem to indicate that the change in responsiveness of hypertensive VSM may be due to altered Ca^{2+} flux. Holloway et al. (1972) found the optimal concentration of calcium (the concentration which elicits the maximum contraction) was higher in SHR than in normotensive rats. A. W. Jones (1974) showed functional changes in Na^{+}-K^{+} transport in hypertensive rats, a finding which he suggested is consistent with increased vascular reactivity due to increased Ca^{2+} lability.

Several investigators have studied Ca^{2+} kinetics in subcellular membrane preparations from the SHR. Both microsomes and more discrete plasma membrane-enriched fractions or sarcoplasmic reticulum fractions of aorta from SHR show decreased $^{45}Ca^{2+}$ uptake compared to preparations from normotensive controls (Aoki et al., 1976; Moore et al., 1975;

Table IV: Relaxation Rate of Isolated Aortic Tissue from Hypertensive Rats*

Stimulus:	SHR	Renal
After K^+	↓ 41, 62	↓ 62
Isoproterenol	↓ 41, 211	↓ 41
	↓ 226	
Nitroglycerin	↓ 41, ≈ 211	↓ 41
Adenosine	↓ 41	↓ 41
C-AMP	↓ 41	↓ 41
Acetylcholine	↓ 211	
Papaverine	≈ 211	

↓ Slower relaxation rate in hypertensive rat aorta

≈ Similar relaxation rates in hypertensive and normotensive rat aorta

* Numbers refer to articles in the List of References

Webb and Bhalla, 1976; Wei et al., 1976a; Zsoter et al., 1977). Sarcoplasmic reticulum from cardiac muscle in SHR also shows decreased $^{45}Ca^{2+}$ uptake (Limas and Cohn, 1977). Shibata and co-workers (1975) found no difference in $^{45}Ca^{2+}$ uptake by microsomal fractions of aorta from SHR and Wistar rats. Wei et al. (1976b) reported an increase in $^{45}Ca^{2+}$ uptake in the presence of ATP and a decreased uptake in the absence of ATP by the plasma membrane-enriched fraction from mesenteric arteries of SHR and normotensive controls.

Using isolated pieces of aorta, Noon et al. (1977) reported that Ca^{2+} crosses the VSM membrane of aorta from SHR with greater ease than in aorta from WKY, as shown by the SHR aorta's ability to develop tension in the presence of raised extracellular Ca^{2+} without previous depolarization.

Thus, altered Ca^{2+} kinetics could play a role in reactivity changes that have been reported.

In studying the pharmacology of calcium in VSM many investigators have studied the actions of other divalent elements. Hudgins and Weiss (1969) found that Sr^{2+} and Ba^{2+} could exchange with bound Ca^{2+} in rabbit aorta, suggesting that these 2 ions compete with Ca^{2+} for binding sites. Ebashi et al. (1968) found that Sr^{2+} and Ba^{2+} can bind to troponin and activate myosin B. Bohr (1974) found that Sr^{2+} and Ba^{2+} could substitute for Ca^{2+} in supporting contraction of VSM in ca Ca^{2+}-free bath. Keene et al. (1972) showed that manganese, another divalent ion, decreased the Ca^{2+} permeability of VSM.

Another ion, La^{3+}, has received considerable attention (see:Weiss, 1974). In 1964, Lettvin and co-workers predicted that La^{3+}, by virtue of its similar ionic radius and higher valence will bind at superficial Ca^{2+} sites with a slower dissociation rate than Ca^{2+} itself. Fuchs (1971)

found Ca^{2+} bound to rabbit skeletal muscle troponin was partially exchangable with La^{3+} or Sr^{2+} and to a lesser degree with Mn^{2+}. van Breemen et al. (1972) found that La^{3+} could decrease Ca^{2+} permeability in rabbit aorta.

These studies indicate that La^{3+}, Sr^{2+} and Mn^{2+} may alter calcium movement in VSM by altering permeability to Ca^{2+}, displacement of Ca^{2+} from binding sites or perhaps, by direct activation of troponin.

The effects of these ions on tissue from genetically hypertensive animals has been studied by Shibata and Kurahashi (1972). They found that aorta strips from SHR but not from normotensive rats showed a contractile response to Mn^{2+}, Sr^{2+} and La^{3+}. This response was also seen in "prehypertensive" (30 to 35 day old) SHR (Shibata et al., 1973). Triggle (unpublished data) found that thulium ion (another Lanthanide) produced contractions in only SHR and not in CFN Wistar VSM. Bohr (1974) found a slight contractile response to La^{3+} and Mn^{2+} in carotid artery strips in both DOCA hypertensive rats and normotensives, however, the response in tissue from SHR was greatly exaggerated.

These findings suggest that spontaneous hypertension as seen in the SHR may be accompanied by changes in Ca^{2+} movement due to altered binding, permeability, exchange or differences in excitation-contraction properties. These changes would in part explain the differences in reactivity outlined above. However, whether or not these changes are involved in the pathogenesis of essential hypertension or occur secondary to the development of hypertension as is the case in DOCA and renal experimental models, is uncertain.

c. Vascular Reactivity in Prehypertensives and in Hypertensives Treated with Antihypertensive Drug Therapy.

There are clearly two ways to determine whether increased reactivity

preceeds to development of hypertension or occurs subsequent to the onset of elevated blood pressure: (1) Reactivity can be measured in prehypertensive subjects who have a high probability of developing high blood pressure later in life, or (2) prehypertensive subjects can be treated with antihypertensive drugs so that they remain normotensive; reactivity can then be measured in these normotensive, genetically hypertensive subjects. Both of these approaches have been attempted in the past.

Doyle and Fraser (1961) tested reactivity to noradrenaline in the forearm of young men whose parents were hypertensive. Although as a group the young men were normotensive, they had a significantly greater constrictor response to infused noradrenaline.

Reactivity studies in prehypertensive SHR have been reported: Dietz et al. (1978) found 5 week old SHR showed increased reactivity to noradrenaline in isolated perfused hindquarter preparations when compared to WKY control rats. This may not have been a true "prehypertensive" state, since Lais et al. (1977) found that blood pressure in the SHR starts to rise at 4 weeks of age. However, Lais and Brody, in a subsequent study (1978) found that even at 3 weeks of age, isolated perfused hindquarters from SHR showed increased reactivity to noradrenaline and barium chloride as well as on a pressure-flow curve when compared to normotensive WKY.

There have been studies reported in which SHR were treated with antihypertensive drugs and then after a period of time, reactivity was tested (e.g., Finch, 1974; Hamilton, 1975; Weiss et al., 1974). However, these studies do not provide a conclusive empirical test since treatment usually was initiated after 8 weeks of age at which time, according to

the findings of Lais et al. (1977) and Bohlen and co-workers (1977), blood pressure in the SHR is already significantly higher than in WKY, and structural alteration in the arterial vasculature may have already been initiated. An additional flaw in the studies of Finch and Hamilton was their choice of antihypertensive drug: hydrallazine, a smooth muscle relaxant which presumably alters Ca^{2+} kinetics, was included in the drug regimen. This may have confounded results of smooth muscle reactivity studies.

There was clearly a need for a controlled study of reactivity of SHR VSM from animals rendered normotensive by drug therapy initiated before the onset of hypertension.

C Aims of This Research

1. To further study the paradoxical action of La^{3+} previously reported (Bohr, 1974; Shibata et al., 1973) in VSM from SHR in an attempt to characterize the mechanism of altered reactivity to this ion.
2. To conduct a study to determine whether alterations in VSM reactivity preceed the development of hypertension in the SHR using antihypertensive drug treatment to prevent elevation of blood pressure and subsequent VSM hypertrophy.
3. To investigate the role of Ca^{2+} in the altered responsiveness of SHR VSM.

II METHODS

A. Blood Pressure Measurement and Assessment of Hypertension

Hypertension is usually defined in terms of blood pressures higher than a certain threshold level. In humans this threshold is often considered to be a diastolic blood pressure above 90 - 100 mm Hg and a systolic pressure above 140 mm Hg (Julius, 1977).

In this thesis the SHR, a strain of genetically hypertensive rats, was compared to 2 ancestral strains of rats which do not develop high blood pressure. Consequently, "normal blood pressure" was defined as the average systolic blood pressure of the WKY colony, the strain from which the SHR was derived. Average systolic blood pressures of other groups of rats were compared to this standard of normality and were considered to be normotensive only if their average systolic blood pressures were not significantly different from the average systolic blood pressure of the WKY colony. Systolic rather than diastolic pressures were used because they were measured with greater accuracy by the tail-cuff method. Rises in diastolic pressure measured by carotid artery cannulation as described below, correlated well to rises in systolic pressure in the SHR at various ages.

Blood pressure was measured with the use of an inflatable tail-cuff (model no. 2257), electrosphygmograph (model no. 2192), and a biograph (model 2120) all purchased from Harvard Apparatus.

In a randomly selected group of animals, pressures recorded using the tail-cuff method were verified by the experimentor by cannulation of the carotid artery just prior to sacrifice. In this procedure, animals were anesthetized with sodium pentobarbital (30mg/Kg) and the right carotid artery was cannulated a few centimeters proximal to the bifurcation. Pressure was recorded with the use of a Statham strain gauge (model no. P23AA) and a Beckman polygraph (model R411). Pressures recorded this way were consistantly 10-12 mm Hg higher than those recorded using the tail-cuff method. This discrepancy was considered to be due to the reflex effects of impeding flow to the right carotid baroceptor, and was considered acceptable.

B. Animals

Male, albino, SHR derived from the Japanese strain of Spontaneously Hypertensive Rats developed by Aoki and Okamoto (1963) were obtained from Charles River Laboratories through Canadian Breeding Laboratories, St. Constant, Quebec, or were bred from NIH-derived stock of SHR in the animal quarters of the Faculty of Medicine, Memorial University of Newfoundland.

In the initial experiments male, albino Wistar rats obtained from Canadian Breeding Laboratories were used as control animals. Subsequently, male, albino Kyoto Wistar Rats (WKY) were obtained from Charles River Laboratories through Canadian Breeding Laboratories and were used as control animals.

All animals were maintained in the animal quarters of the Faculty of Medicine, Memorial University of Newfoundland under a 12 hours light/12 hours dark photo period with controlled humidity and free access to Purina rat chow and water at all times.

C. Preparation of Drugs and Solutions.

The composition of the physiological solutions used is tabulated in Appendix A and a list of the drugs used is given in Appendix B. Solutions were prepared on the day of the experiment from stock solutions and were diluted with either double-distilled or Milli-Q (Millipore Corp., Bedford, Mass.) deonized, filtered water. The resistivity of water prepared by Milli-Q filtration was always greater than 10 megohm-cm. Ca^{2+}-free solutions refer to physiological solutions given in Appendix A from which the $CaCl_2.2H_2O$ was omitted.

Noradrenaline was solubilized with the addition of a minimal amount of 0.01 N HCl. Serial dilutions of noradrenaline were prepared on the day of the experiment from a 10^{-1} M stock solution.

Ouabain and D-600 were solubilized in a minimal amount of 95% ethanol and then diluted with double-distilled or Milli-Q filtered water.

Solutions of nifedipine, which is light-sensitive, were kept in covered vials.

D. Isolated Tissue Techniques

1. Tissue Preparation

Three to ten month old SHR and age-matched control rats were sacrificed by cervical dislocation. The thorax was opened along the midline and the thoracic aorta was carefully removed. The aorta was immediately placed in a dish of warmed, oxygenated physiological solution. It was then cleaned, removing loose fat and connective tissue. The aorta was then cut into helical strips (2-4 mm wide, 15 mm long) or rings (3-4 mm long) and suspended individually in a 10 ml double-jacketed organ bath, as shown in Figure 2, containing physiological solution at 37°C.

Isometric tension was recorded on a Beckman R-411 dynograph using Medical Systems (type 4151) or Grass (model FT 03C) force transducers. The transducers were mounted on geared, adjustable clamps, permitting fine adjustment of resting muscle tension without overstretching the tissue.

The aortic tissue was placed under 2 g tension and allowed to equilibrate for 90-120 minutes, during which time the solution was changed every 20 minutes and the tension readjusted to 2 g as necessary.

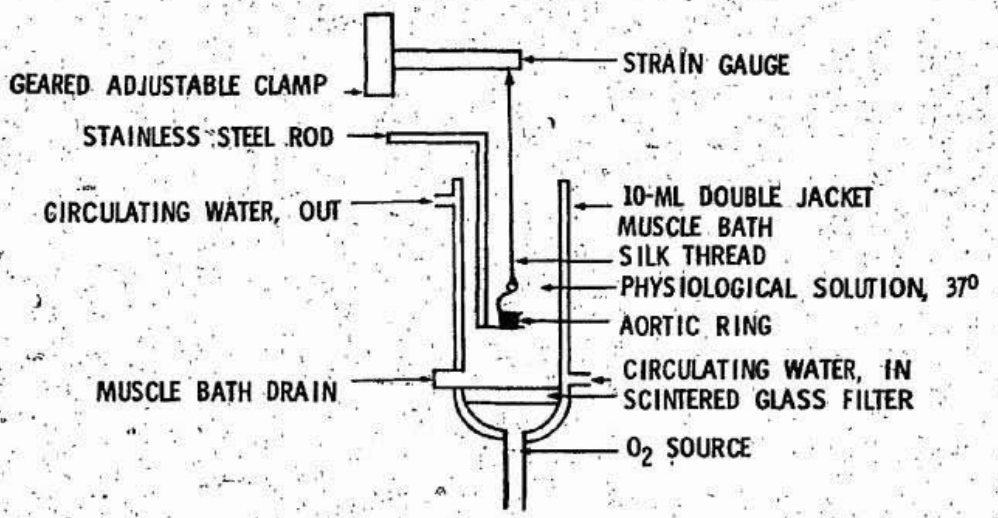
Figure 2: Isolated rat aorta apparatus

2. Reactivity Recordings

After the equilibration period, responses of the tissues to 80 mM KCl were observed. If a given tissue did not respond, it was discarded. Dose-response data was obtained by adding KCl, noradrenaline, $CaCl_2$, $LaCl_3$ or $La(NO_3)_3$ and/or hydrochloric, nitric or sulphuric acid. Agonists were added cumulatively with a 30-45 minute relaxation period and adequate rinsing with physiological solution between agonists to return the tension recording to a baseline of 2g.

For example, in one series of experiments (Figures 3-9), KCl was added cumulatively in seven doses from a concentration of 10 mM to 120 mM. Noradrenaline was added cumulatively in 11 doses from a concentration of 10^{-10}M to 10^{-5}M. La^{3+} was then added cumulatively as either $LaCl_3$ or $La(NO_3)_3$ in seven doses from a concentration of 10^{-4}M to 10^{-1}M. In some experiments in this series either HCl, HNO_3 or H_2SO_4 was substituted for La^{3+} and added cumulatively in seven doses from a concentration of 1 mM to 11 mM. To minimize the precipitation of insoluble lanthanum salts in Krebs solution some experiments were performed in phosphate- and bicarbonate-free HEPES-buffered solutions.

When responses to $CaCl_2$ were recorded, and in other experiments where the physiological solution was changed to a Ca^{2+}-free solution, a 60-90 minute equilibration period and several Ca^{2+}-free rinses preceded recording of responses in Ca^{2+}-free solution. Keatinge (1972) has shown that the omission of calcium from the physiological solution does not necessarily insure the absence of an extracellular source of Ca^{2+};


GEARED ADJUSTABLE CLAMP
STRAIN GAUGE
STAINLESS STEEL ROD
CIRCULATING WATER, OUT
10-ML DOUBLE JACKET MUSCLE BATH
SILK THREAD
PHYSIOLOGICAL SOLUTION, 37°
AORTIC RING
MUSCLE BATH DRAIN
CIRCULATING WATER, IN
SCINTERED GLASS FILTER
O_2 SOURCE

some superficially bound Ca^{2+} is apparently still present. Therefore, the following test was adopted: After the tissues had incubated in Ca^{2+}-free solution for 30 minutes, they were exposed to one or two additions, 30 minutes apart, of 10^{-6}M noradrenaline to ascertain whether the tissue would support a tonic response to this agonist: It has been demonstrated (Godfraind and Kaba, 1969; Krishnamurty and Grollman, 1976; Sitrin and Bohr, 1971; van Breemen 1969; van Breemen et al., 1973) that the tonic response of aortic tissue to noradrenaline is critically dependent on extracellular Ca^{2+} and thus the lack of a tonic component was interpreted as indicating that the extracellular source of Ca^{2+} had been eliminated.

In some experiments, as described in the Results section, addition of agonists were repeated after treatment of the tissues with D-600, ouabain, nifedipine, procaine, or a low-Na^{+} solution for at least 15 minutes.

Agonists were always added to the 10 ml tissue baths in volumes of less than 200 µl and usually in volumes of less than 100 µl, so that addition of agonists did not significantly alter the volume of the bath solution.

When pH was monitored in an experiment, a Fisher (model 220) pH meter was used. A pencil electrode (Fisher model 13-639-92) permitted readings to be taken directly from the tissue bath.

E. Antihypertensive Drug Treatment

1. Propranolol study

Six week old SHR and Wistar rats were maintained in the animal quarters of the Health Science Centre at Memorial University. The animals were weighed weekly and water intake rates were recorded with the use of graduated drinking water tubes, purchased from Fisher Scientific. Propranolol (75 mg/Kg/day) was added to the drinking water. The dosage was prepared separately for SHR and Wistar rats based on the previous week's average water intake rate and average weight for each group. The animals' blood pressures were monitored every other week until 5 months of age, at which time the animals were sacrificed.

2. Timolol study

Litter-mate pairs of SHR derived from the NIH stock and Charles River Kyoto Wistar control rats (WKY) were bred in the animal quarters of the Health Science Centre at Memorial University. Breeding pairs were treated with timolol (2 mg/Kg/day), which was dissolved in the drinking water. Timolol maleate was kindly donated by Dr. Dorian of Merck Frosst Laboratories, Dorval Quebec. The animals' weights and water intake rates were recorded weekly and the dosage for each group was adjusted weekly, based on the previous week's records.

Offspring of these timolol-treated breeders were retained in the parental cage until weaned, at which time they were placed in separate

cages and treated with 6 mg/Kg/day of timolol. When these animals reached 4 to 5 months of age they were sacrificed for aortic reactivity studies.

"Some people use statistics the way a drunk uses a lamppost; more for support than illumination".

Pat Colgan

F. Data Presentation and Statistical Analysis

Data presented comparing hypertensive and control tissues are from paired experiments conducted simultaneously. Tension development reported has been normalized for each tissue in terms of the percentage of the maximum response recorded, usually in a precedingcontrol experiment.

The means and standard error of the means for each dose in the dose-response curves were calculated and are represented in the figures as points with error bars. The "N" reported in tables and figures refer to the number of animals, except as noted, where the results from more than one tissue from each animal are represented.

Student's t distribution was used to test for significance of differences between responses at one point on the dose-response curves; usually either the highest or lowest dose in a cumulative dose-response curve. Differences with p values of less than 0.05 as determined by a one-tailed t test (Walpole, 1972) were considered significant, and are signified so in figures with a star (★).

Calculations as well as data storage and analysis were performed on a PDP 11/10 computor (Digital Equipment).

III RESULTS

A. Effect of La^{3+} and H^{+}

Although La^{3+} is usually thought of as Ca^{2+} antagonist in vascular smooth muscle (van Breeman et al., 1973), both Shibata and coworkers (1973) and Bohr (1974) reported that La^{3+} induced tension development in vascular smooth muscle (VSM) from the SHR. These were strong pieces of evidence suggesting an ionic basis for altered reactivity in the SHR VSM, so it was important to confirm these findings.

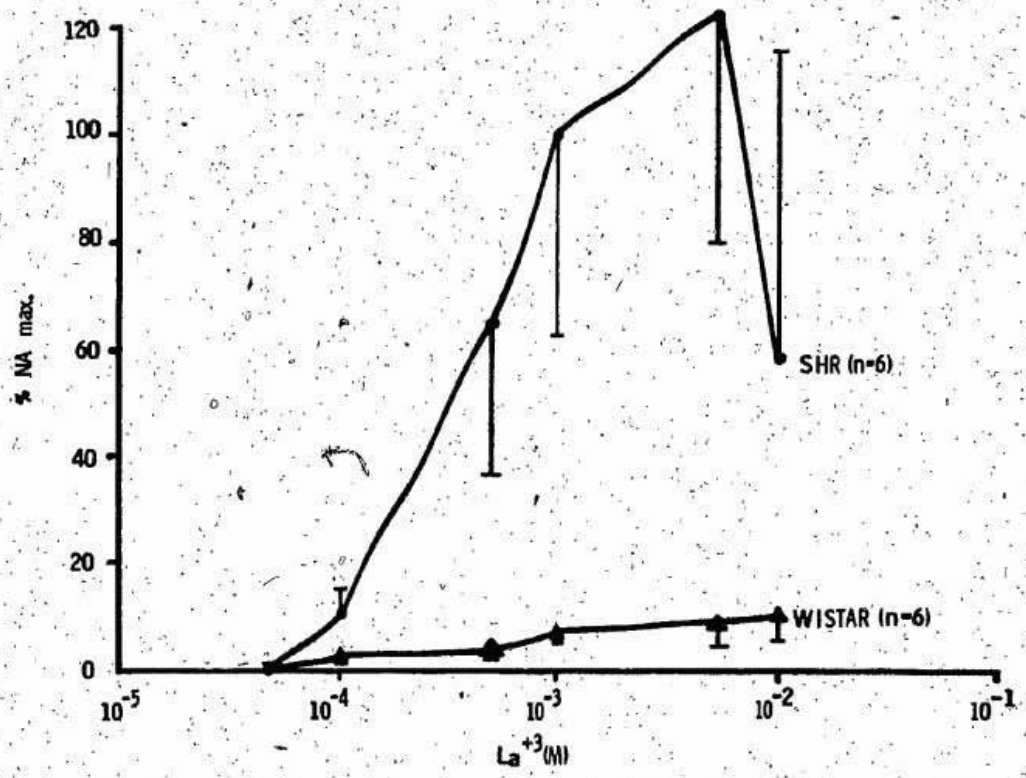
La^{3+} was added cummulatively to the Krebs bathing media and responses were recorded as tension development. This data is summarized in Figure 3, expressed as a percentage of the maximum response of SHR aortic strips to noradrenaline and in Figure 4, expressed as a percentage of the maximum response of SHR aortic strips to depolarizing K^{+}. The response of the SHR tissue to La^{3+} is significantly greater than that of the Wistar tissue, verifying the previous reports.

After equilibration in Ca^{2+}-free solution for 30 minutes, the response of the SHR aorta to La^{3+} was greatly diminished, but still significantly greater than the response of the Wistar tissue, as shown in Figure 5.

In the course of these initial experiments, the formation of a percipitate was observed on the addition of La^{3+}. Both $LaCl_3$ and $La(NO_3)_3$ showed the same effect, suggesting that La^{3+} was forming a

Figure 3: Shows cumulative La^{3+} dose-response curves expressed as a percentage of the maximum response to noradrenaline (NA) in aortic strips from SHR and Wistar rats recorded in Krebs solution.

Figure 4: Shows cumulative La^{3+} dose-response curves as in Figure 3, but expressed as a percentage of the maximum response to depolarizing K^{+} in aortic strips from SHR and Wistar rats in Krebs solution.


120
100
80
60
40
20
0
% NA max.
SHR (n=6)
WISTAR (n=6)
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
La^{+3}(M)

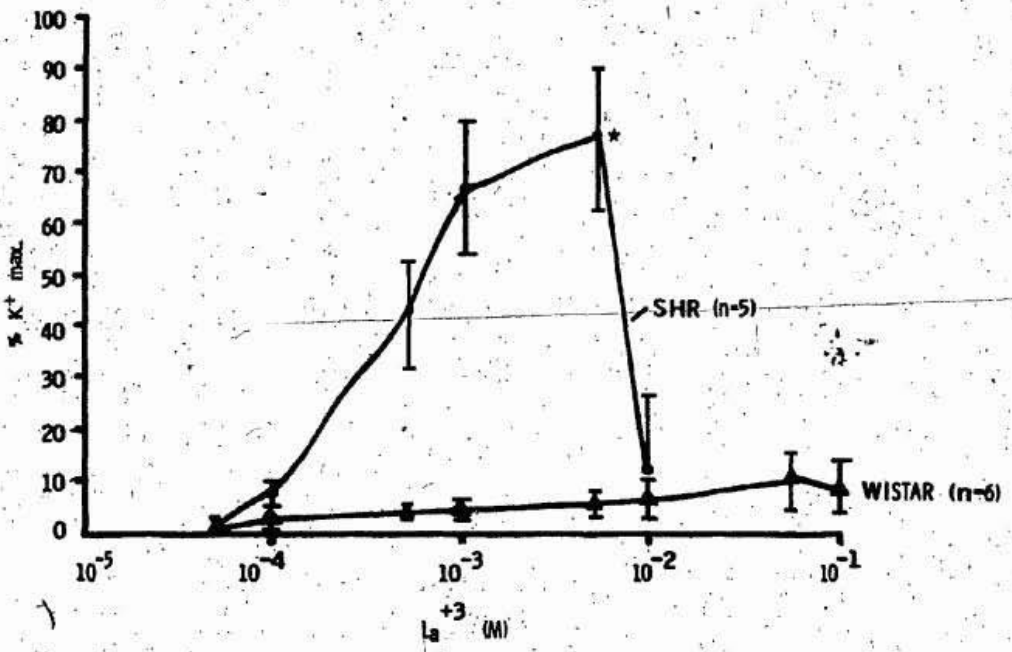
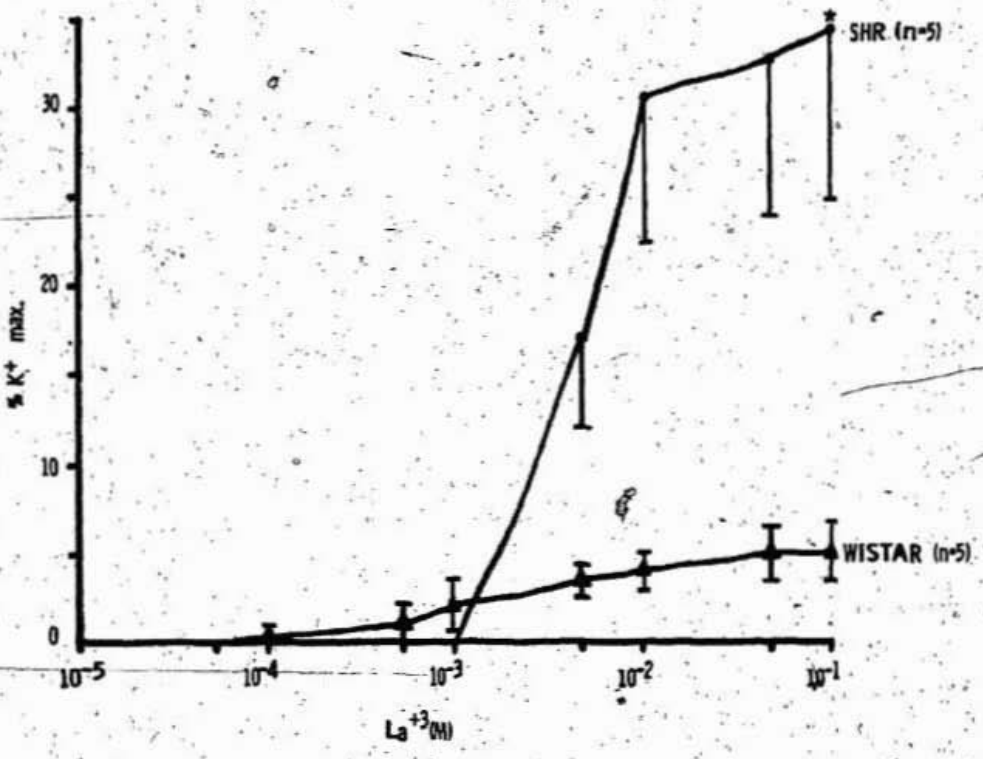

100
90
80
70
60
50
40
30
20
10
0
% K+ max.
SHR (n-5)
WISTAR (n-6)
10-5
10-4
10-3
10-2
10-1
La+3 (M)

Figure 5: Shows cumulative La^{3+} dose-response curves expressed as a percentage of the maximum response to K^{+} in aortic strips from SHR and Wistar rats recorded in Ca^{2+}-free Krebs solution.


SHR (n=5)
WISTAR (n=5)
% K+ max.
30
20
10
0
10-5
10-4
10-3
10-2
10-1
La+3(M)

salt with the bicarbonate and phosphate buffers in the Krebs solution. If the buffer was precipitating out of solution it was also suspected that the pH of the solution might have been altered by the addition of acidic $LaCl_3$. This was verified by measuring the pH in the tissue bath as La^{3+} was added stepwise. As shown in Table V, the addition of La^{3+} as $LaCl_3$ caused both Krebs solution and the low-bicarbonate Krebs solution of Hansen and Bohr (1975) to become acidic.

When Tris was substituted as the buffer, the tissue was unable to maintain a stable baseline tension. For this reason, and also because Gillespie and McKnight (1975) have reported that Tris alone can induce tension development in VSM, the use of Tris was abandoned. When HEPES was substituted as the buffer, the addition of even the highest dose of 10^{-1}M La^{3+} did not produce a shift from the normal pH range (Table V). Substitution of HEPES as the buffer did not significantly alter the dose response curve to K^+ in the SHR aorta (Figure 6).

When the ability of La^{3+} to produce tension development in VSM from the SHR bathed in HEPES-Krebs solution was tested it was found to be greatly diminished when compared to the results obtained with bicarbonate/phosphate-buffered Krebs, but still significantly larger in the SHR than in the Wistar aorta (Figure 7). This finding suggested that not all of the tension development seen on addition of La^{3+} to normal Krebs solution was due to the La^{3+} itself, and that perhaps the change in pH was partially responsible for the response observed. This hypothesis was tested by constructing a cummulative dose-response curve to hydrochloric acid (HCl) while simultaneously recording pH of the

Figure 6: Shows cumulative dose-response curves to K^+ in aortic rings from SHR recorded in normal and HEPES-buffered Krebs solution.

Table V: Change in pH on Addition of Lanthanum to Solutions

$LaCl_3$ added (M)	HEPES-Krebs	Krebs	Low Bicarbonate Krebs
0	7.4	7.4	7.4
10^{-4}	7.4	7.2	7.0
5×10^{-4}	7.4	7.0	6.6
10^{-3}	7.4	6.8	6.3
5×10^{-3}	7.4	5.5	5.6
10^{-2}	7.4	5.4	5.5
5×10^{-2}	7.4	5.2	5.3
10^{-1}	7.35	5.1	5.2

* Recorded directly from organ baths while solutions were being aerated.

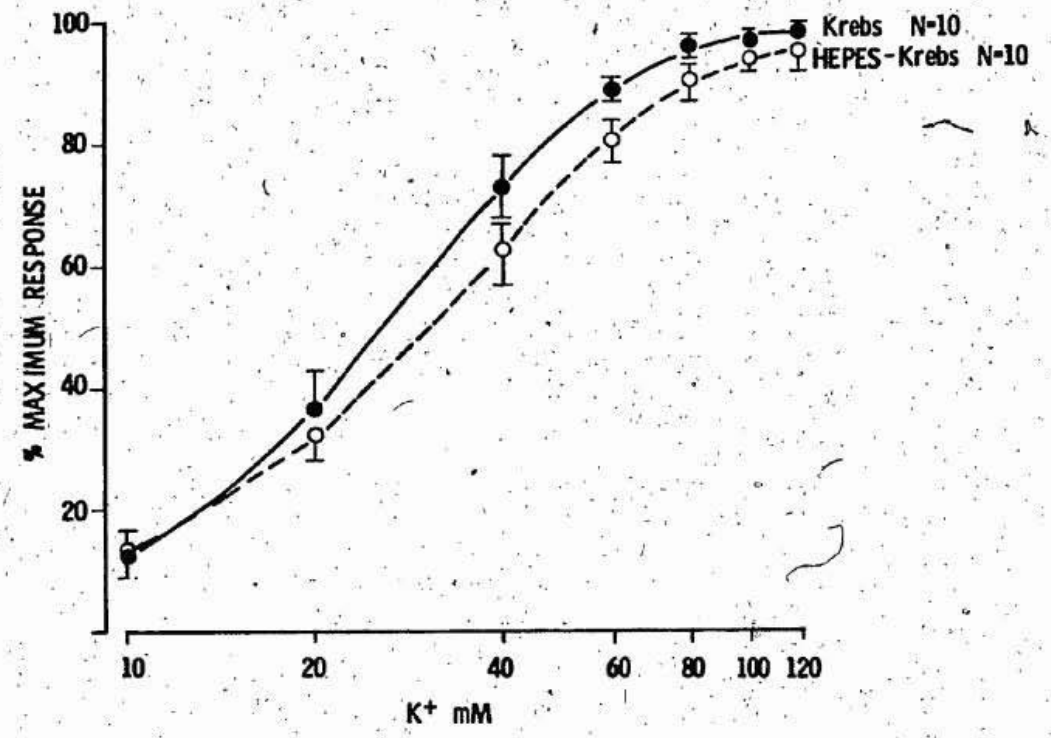
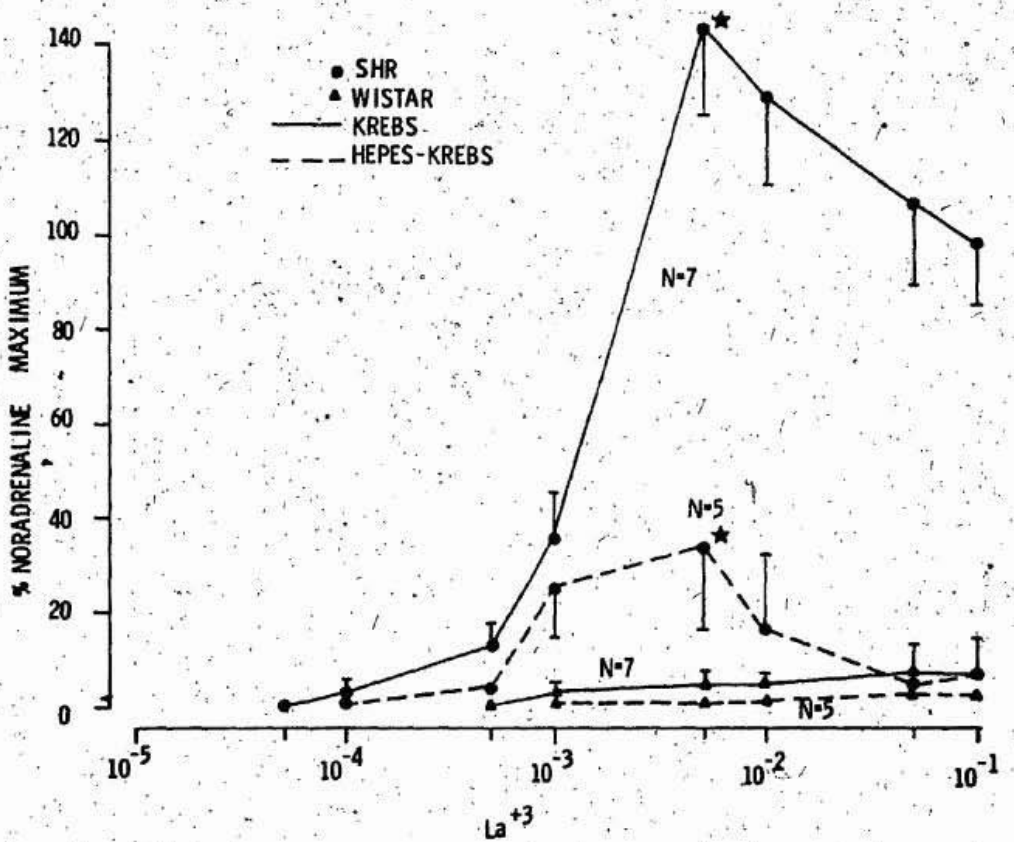

Krebs N=10
HEPES-Krebs N=10
% MAXIMUM RESPONSE
100
80
60
40
20
10
20
40
60
80
100
120
K+ mM

Figure 7: Shows cumulative La^{3+} dose-response curves expressed as a percentage of the maximum response to NA in aortic strips from SHR and Wistar rats in normal and HEPES-buffered Krebs solution.

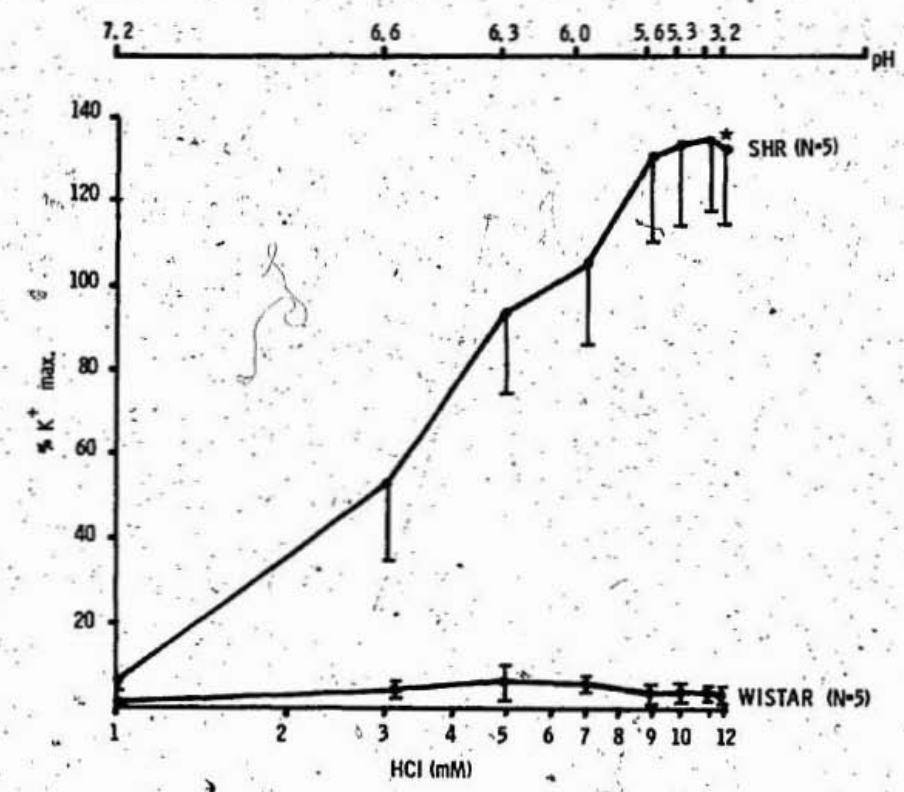

SHR
WISTAR
KREBS
HEPES-KREBS
% NORADRENALINE MAXIMUM
140
120
100
80
60
40
20
0
N=7
N=5
N=7
N=5
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
La^{+3}

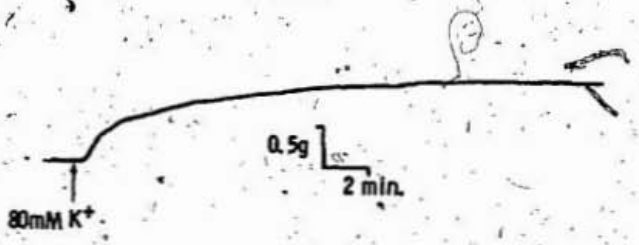
bathing medium (Krebs solution). This data is plotted in Figures 8 and 9, showing that SHR aortic tissue responds to HCl while Wistar tissue does not. This response was identical when either nitric (HNO_3) or sulphuric acid (H_2SO_4) was substituted for HCl, demonstrating that H^+ was the active agent.

The magnitude of the response to HCl at pH 5.6 closely corresponds to the pH resulting from the addition of 5 mM La^{3+} (the dose which elicited the maximal response) suggesting that the response to H^+ and not to La^{3+} itself accounts for most of the tension development recorded in Figures 3 and 4. This suggestion is substantiated by the data in Figure 10: Aortic tissue from SHR and Wistar rats was incubated in aerated tissue baths for 1 hour in Krebs solution titrated to pH 4, 6, 8, or 10 by the addition of HCl or sodium hydroxide (NaOH). Tension alteration at the end of 1 hour was recorded and expressed as a percentage of the maximum tension developed to depolarizing K^+ previously recorded in each tissue at physiological pH (7.4). The SHR tissue showed the greatest increase in tension at pH 4 while the normotensive Wistar tissue showed the greatest increase at pH 10.

The responsiveness of aortic tissue after exposure to HCl is greatly diminished, as shown in Table VI. The maximum response to K^+ in the SHR was only 42.36% of its previous maximum response after exposure to 11 mM HCl.

Figure 8: Shows cumulative HCl dose-response curves expressed as a percentage of the maximum response to depolarizing K^+ in aortic rings from SHR and Wistar rats in Krebs solution. The corresponding pH of the Krebs solution is recorded above. Below is shown the response of an SHR aortic ring to 80mM K^+ and the response of the same tissue to 10mM H^+.


7.2
6.6
6.3
6.0
5.6
5.3
3.2
pH
140
120
100
80
60
40
20
% K+ max.
SHR (N=5)
WISTAR (N=5)
1
2
3
4
5
6
7
8
9
10
12
HCl (mM)


0.5g
2 min.
80mM K+

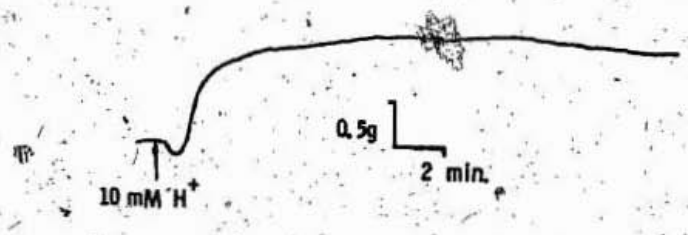

0.5g
2 min.
10 mM H+

Figure 9: Shows cumulative HCl dose-response curves as in Figure 8, but expressed as a percentage of the maximum response to noradrenaline.

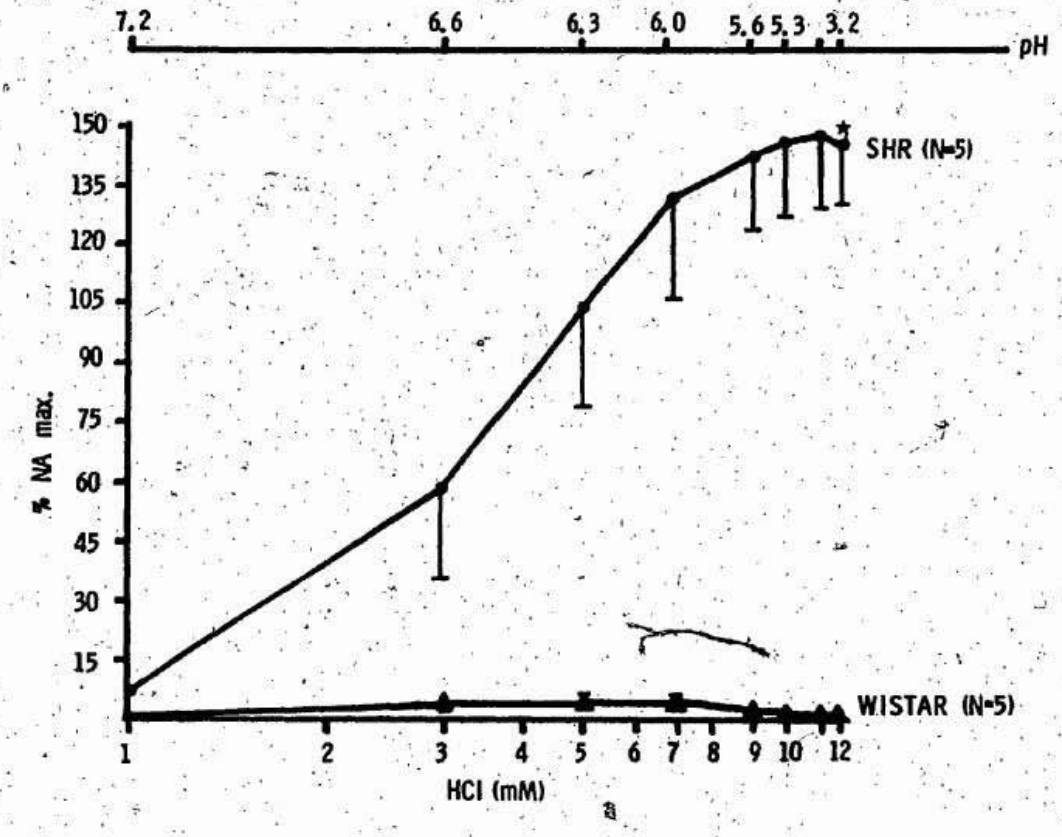

7.2
6.6
6.3
6.0
5.6
5.3
3.2
pH
150
135
120
105
90
75
60
45
30
15
% NA max.
SHR (N=5)
WISTAR (N=5)
1
2
3
4
5
6
7
8
9
10
12
HCl (mM)

Figure 10: Shows tension developed by aortic rings from SHR and Wistar rats after a 1 hour incubation in aerated Krebs solution titrated to the pH indicated with HCl or NaOH, expressed as a percentage of the maximum response to depolarizing K^+ at pH 7.4.

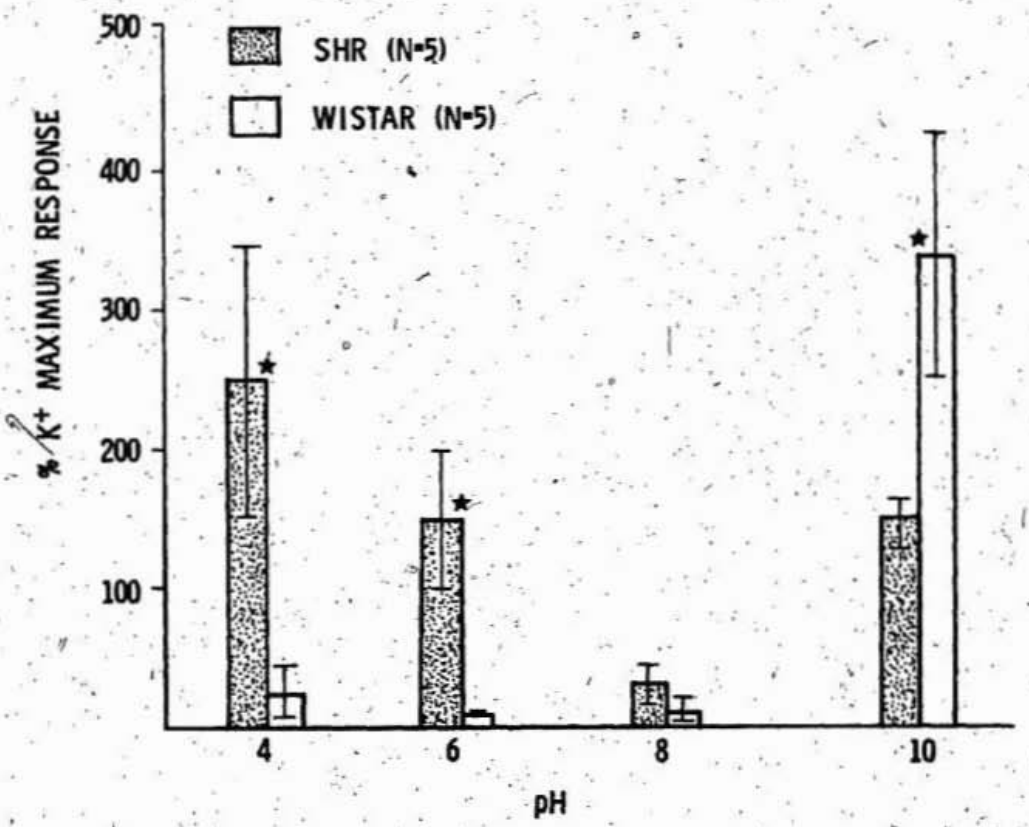

SHR (N=5)
WISTAR (N=5)
% K^+ MAXIMUM RESPONSE
500
400
300
200
100
4
6
8
10
pH

Table VI: Responsiveness After Exposure to HCl*

	SHR (n=8)	Wistar (n=4)
K^+ maximum response after HCl as a percentage of the previous K^+ maximum response	42 ±8 42 ±8	23 ±10 23 ±10

* Dose-response data to KCl were obtained, then HCl was added cumulatively to all tissues as in Figure 8. After a 10 minute wash out, during which tension returned to baseline levels, and a 30 minute equilibration period, KCl was again added in cumulative doses. The maximum response to this second KCl challenge was expressed as a percentage of the initial maximum response to KCl.

The role of extracellular Ca^{2+} in the La^{3+}- and the H^{+}-induced contractions was explored with the aid of D-600, the methoxy-derivative of the calcium channel antagonist verapamil. The effect of 1 mM D-600 on the response of depolarized SHR VSM to Ca^{2+} added to Ca^{2+}-free Krebs solution is shown in Figure 11. This same D-600 concentration appeared to antagonize the response to H^{+} when this tissue is bathed in Krebs solution (Figure 12). When the H^{+} response is recorded in Ca^{2+}-free Krebs solution, it is greatly diminished and not significantly antagonized by D-600 (Figure 13). The response to La^{3+} in Ca^{2+}-free HEPES-Krebs solution is also not significantly antagonized by 1 mM D-600 (Figure 14).

The studies reported in this section utilized aortic tissue from 5-9 month old SHR which originated from the NIH breeding stock and had an average systolic blood pressure of 226 $\pm$ 25 mm Hg.[1] The Wistar control colony had an average systolic blood pressure of 124 $\pm$ 14 mm Hg. These pressures were significantly different.

1. Means $\pm$ standard deviations are reported for blood pressure data

Figure 11: Shows cumulative dose-response curves to Ca^{2+} in SHR aortic rings. The open circle points were recorded subsequently in the presence of 10^{-3}M D-600 and are expressed as a percentage of the initial maximum response. (N = 6)

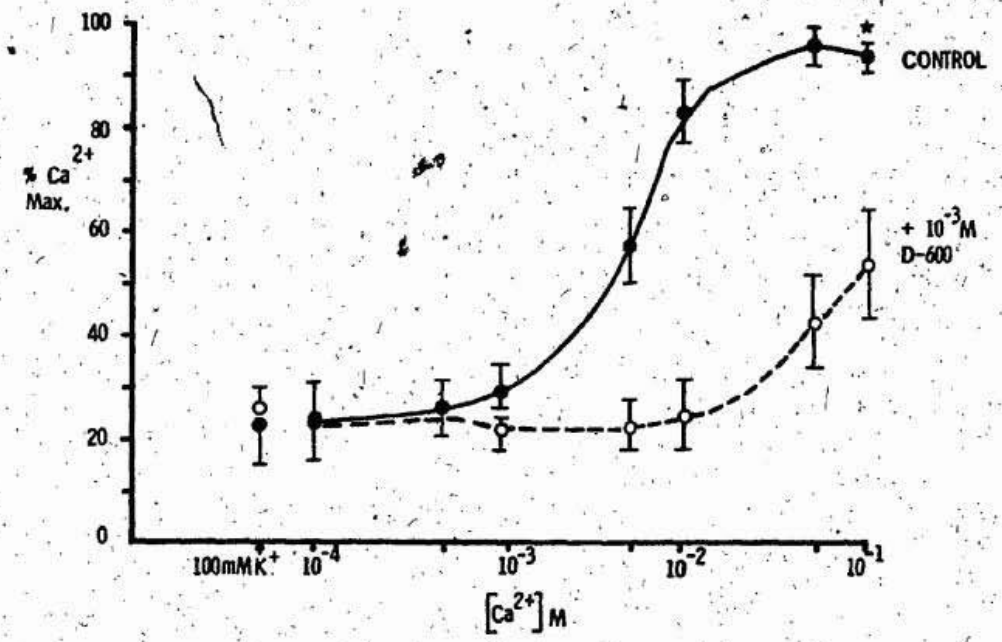

100
80
60
40
20
0
% Ca 2+ Max.
CONTROL
+ 10-3M D-600
100mM K+
10-4
10-3
10-2
10-1
[Ca2+] M

Figure 12: Shows cumulative HCl dose-response curves expressed as a percentage of the maximum response to NA recorded from aortic rings from SHR in normal Krebs solution in half of the tissues and after a 30 minute exposure to 10^{-3}M D-600 in the other half of the tissues. Each pair of tissues is from one animal.

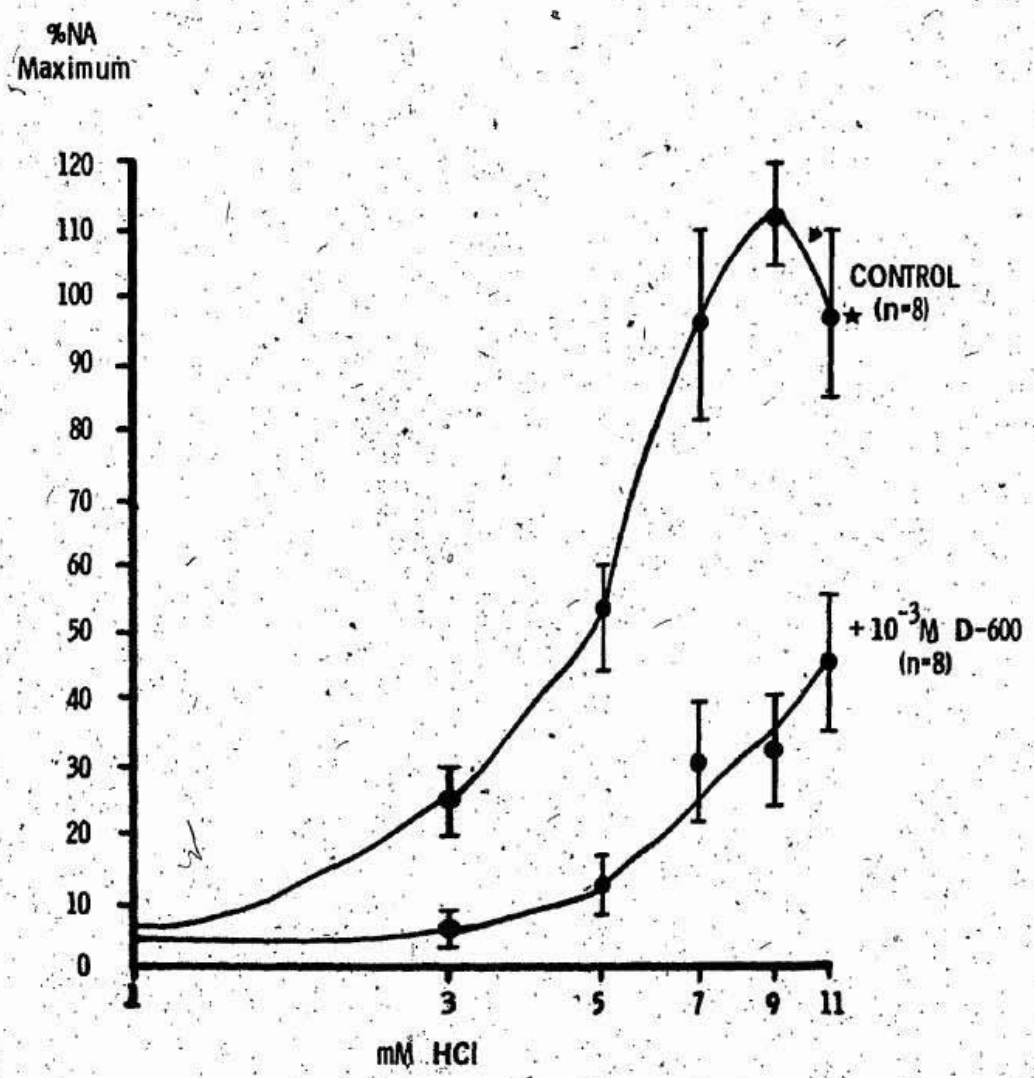

%NA
Maximum
120
110
100
90
80
70
60
50
40
30
20
10
0
3
5
7
9
11
mM HCl
CONTROL
(n=8)
+ 10⁻³M D-600
(n=8)

Figure 13: Shows cumulative HCl dose-response curves expressed as a percentage of the maximum response to NA as in Figure 12, but recorded in Ca^{2+}-free Krebs solution.

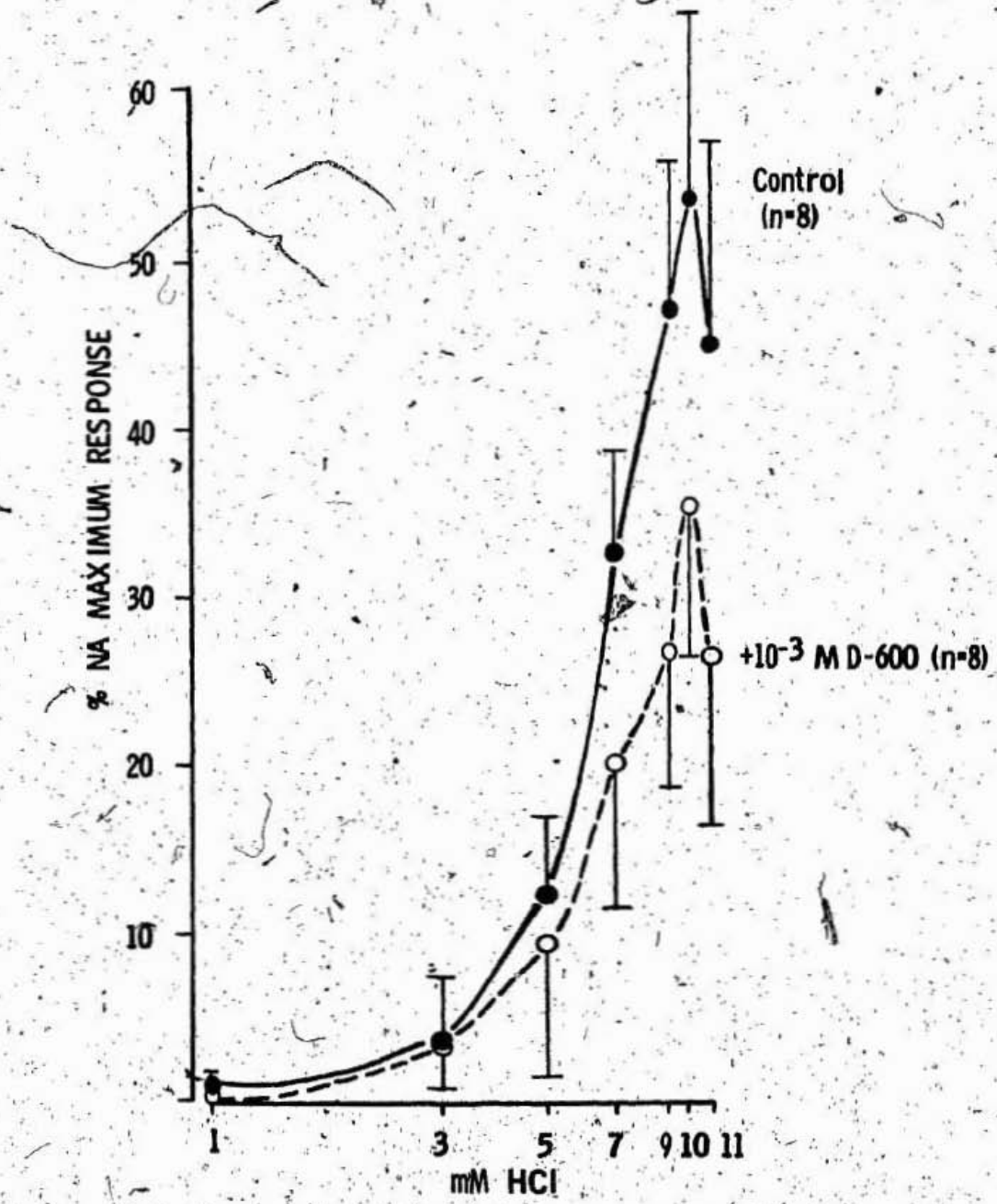
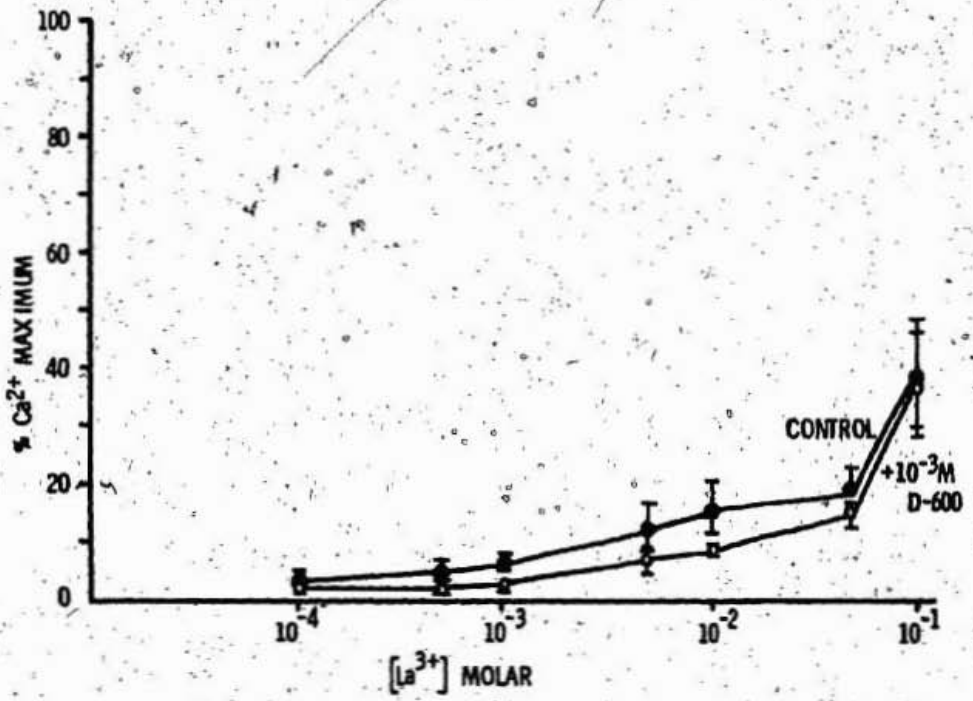

60
50
40
30
20
10
% NA MAXIMUM RESPONSE
Control
(n=8)
+10⁻³ M D-600 (n=8)
1
3
5
7
9
10
11
mM HCl

Figure 14: Shows cumulative La^{3+} dose-response curves expressed as a percentage of the maximum response to Ca^{2+} (as shown in Figure 11) of aortic rings from SHR in Ca^{2+}-free HEPES-Krebs solution in half of the tissues and after 30 minute exposure to 10^{-3}M D-600 in the other half of the tissues. Each pair of tissues is from one SHR. (N = 10.)


100
80
60
40
20
0
% Ca^{2+} MAXIMUM
CONTROL
$+10^{-3}$M
D-600
10^{-4}
10^{-3}
10^{-2}
10^{-1}
$[La^{3+}]$ MOLAR

B. Antihypertensive Drug Studies

The preceeding studies suggested that one aspect of altered vascular reactivity in the SHR was the paradoxical response to La^{3+} and also to H^{+}. The following studies were done in an attempt to separate effects that could be recorded in the absence of hypertension from those present subsequent to the onset of hypertension and perhaps, developed subsequent to VSM hypertrophy.

SHR and Wistar rats were treated with 75 mg/Kg/day of propranolol, administered in the drinking water from the age of 6 weeks until the animals were 5 months old. Both SHR and Wistar treated groups experienced a high mortality rate (about 50%). At age 5 months surviving SHR (n=3) had a significantly lower systolic blood pressure (148.3 $\pm$ 5.8) than untreated SHR (226 $\pm$ 25) while systolic blood pressures of treated SHR and treated Wistar rats (136.7 $\pm$ 7.6, n=3) were not significantly different.

This pilot study demonstrated that a β-adrenergic blocker could be an effective antihypertensive agent in the treatment of SHR. However, the high mortality rate suggested that propranolol was not an appropriate choice of β-blocking drug for a chronic study of this nature.

In the subsequent study, SHR and WKY breeding pairs were treated with 2 mg/Kg/day timolol. When new-born offspring of these treated breeders were weaned, they were treated with 6 mg/Kg/day timolol. At 4-5 months of age, these animals were sacrificed for aortic reactivity

studies. Systolic blood pressures are recorded in Table VII. Treated SHR had a mean systolic blood pressure that was significantly lower than untreated SHR, but not significantly different from untreated WKY rats. Treatment did not significantly lower systolic blood pressure of WKY rats when compared to untreated WKY rats.

Aortic rings from timolol-treated SHR showed increased reactivity to K^+_o when the lower end of the dose-response curve is compared to the response from timolol-treated WKY VSM (Figure 15). Timolol treatment did not appear to alter the reactivity of SHR aorta as there was no significant difference between treated and untreated SHR aorta in their response to 10 mM KCl. The dose-response curve to noradrenaline showed no significant differences (Figure 16). The response to H^+ was present in timolol-treated SHR aorta, but not in aorta from treated WKY rats (Figure 17). When Ca^{2+} was added stepwise to tissues bathed in normal Krebs solution (i.e., non-depolarizing) contractile responses were recorded from treated SHR VSM but not from treated WKY rat VSM (Figure 18).

Experiments reported in this section used aortic rings from NIH-derived SHR. The WKY rats used were purchased from the Charles River Breeding Labs.

Table VII: Systolic Blood Pressures at Sacrifice

	Untreated	Timolol Treated
SHR	170±21.1 (9)	135±6.1 (5)*†
WKY	129±10.7 (10)	116±5.5 (5)†

* Significantly different from untreated SHR group, $p < 0.05$

† Not significantly different from untreated WKY group

NOTE: Mean systolic blood pressures ± standard deviations are shown: number of animals in parentheses.

Figure 15: Shows cumulative dose-response curves to K^+ (added as KCl) in aortic rings from SHR (●, n=8), Wistar rats (▲, n=8) and from timolol-treated SHR (○, n=15 tissues from 5 animals) and Kyoto Wistar (WKY) rats (□, n=15 tissues from 5 animals). There was a significant difference in the response of both SHR groups to the initial dose compared to both control groups. There was no significant difference between treated and untreated SHR, nor between treated WKY and untreated Wistar tissues in their responses to the initial dose.

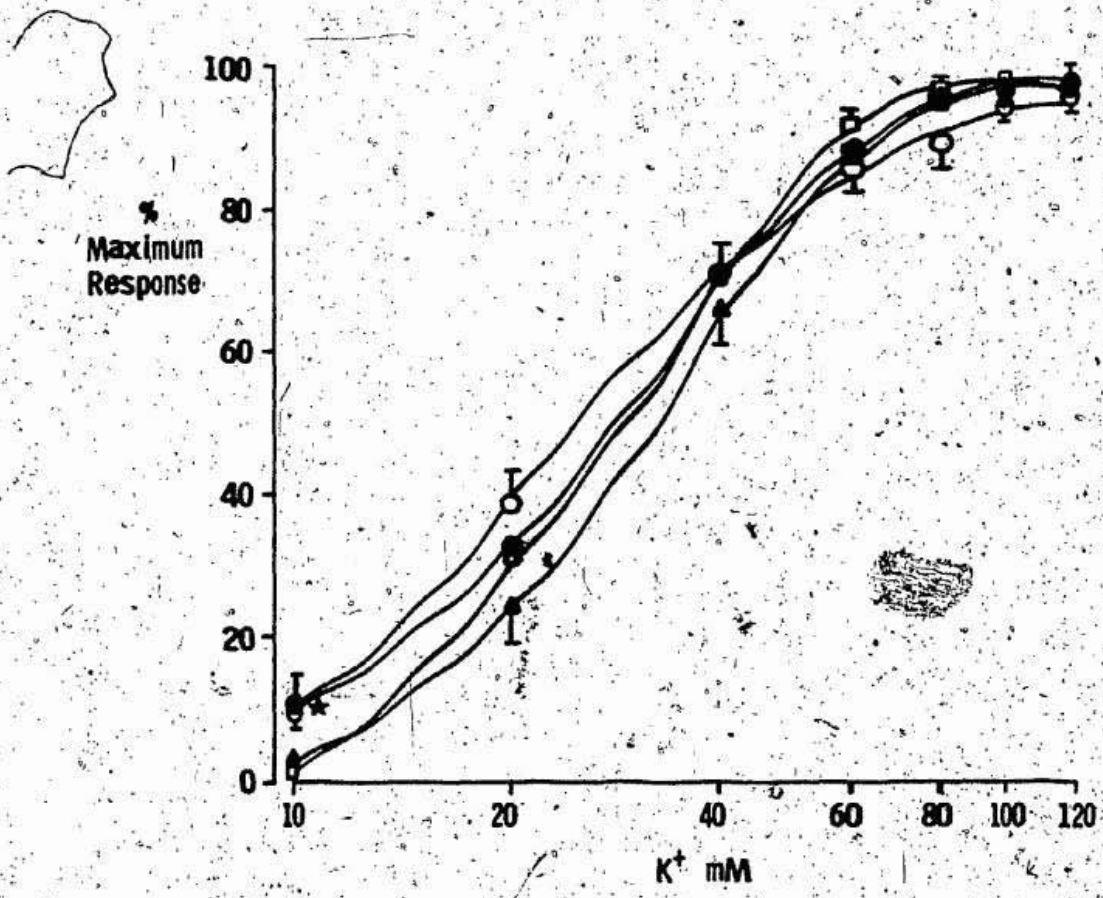

100
80
60
40
20
0
% Maximum Response
10
20
40
60
80
100
120
K+ mM

Figure 16: Shows cumulative dose-response curves to noradrenaline in aortic rings from timolol-treated SHR (●) and timolol-treated WKY (O). N = 15 tissues from 5 animals per curve.

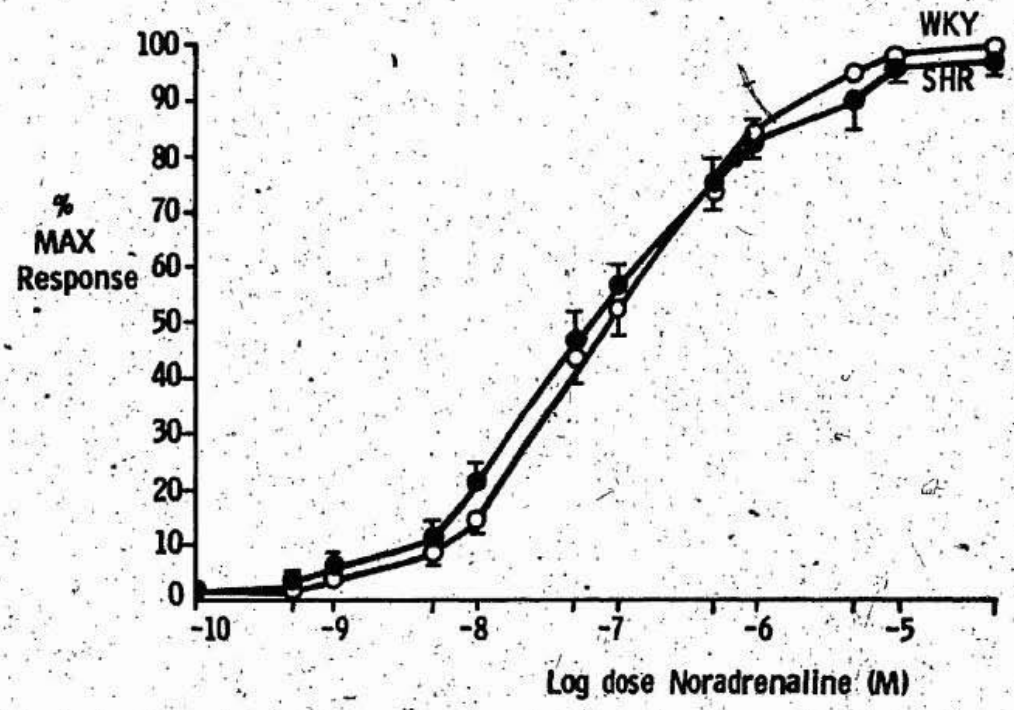

WKY
SHR
% MAX Response
100
90
80
70
60
50
40
30
20
10
0
-10
-9
-8
-7
-6
-5
Log dose Noradrenaline (M)

Figure 17: Shows cumulative dose-response curves to HCl in aortic rings from timolol-treated SHR (●) and timolol-treated WKY (O) expressed as a percentage of the maximum response to noradrenaline. N = 9 tissues from 5 animals per curve.

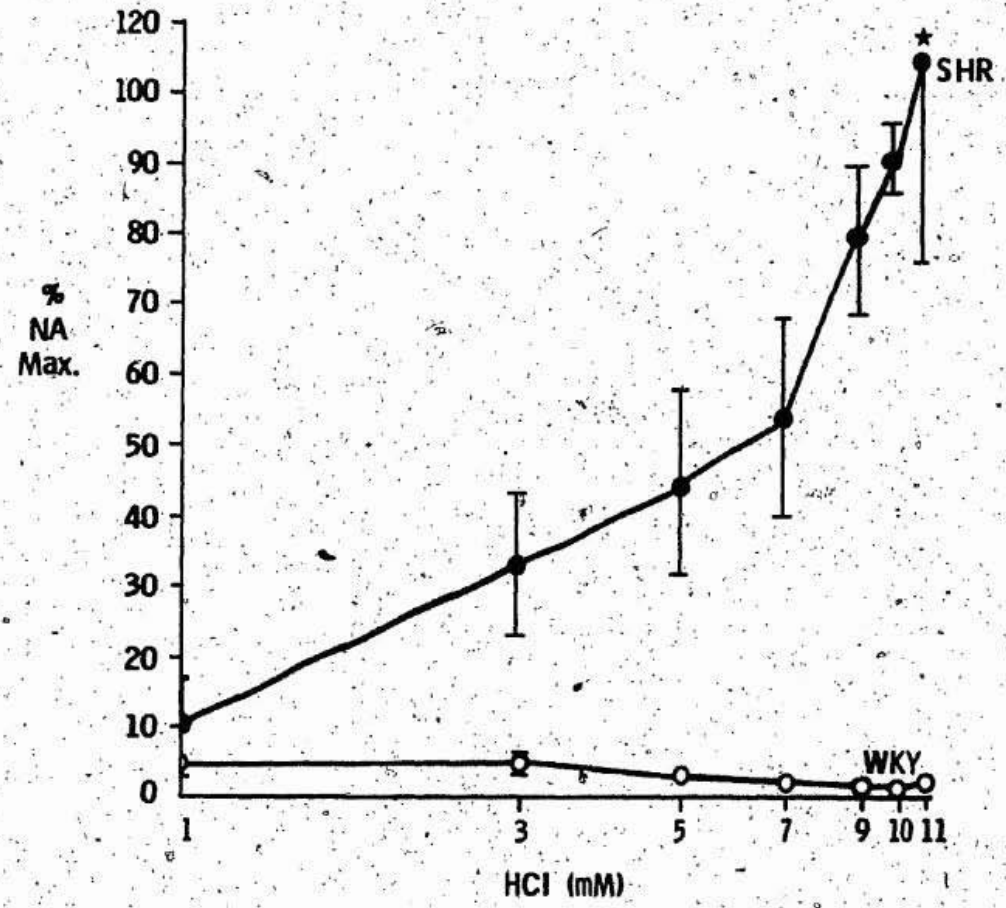
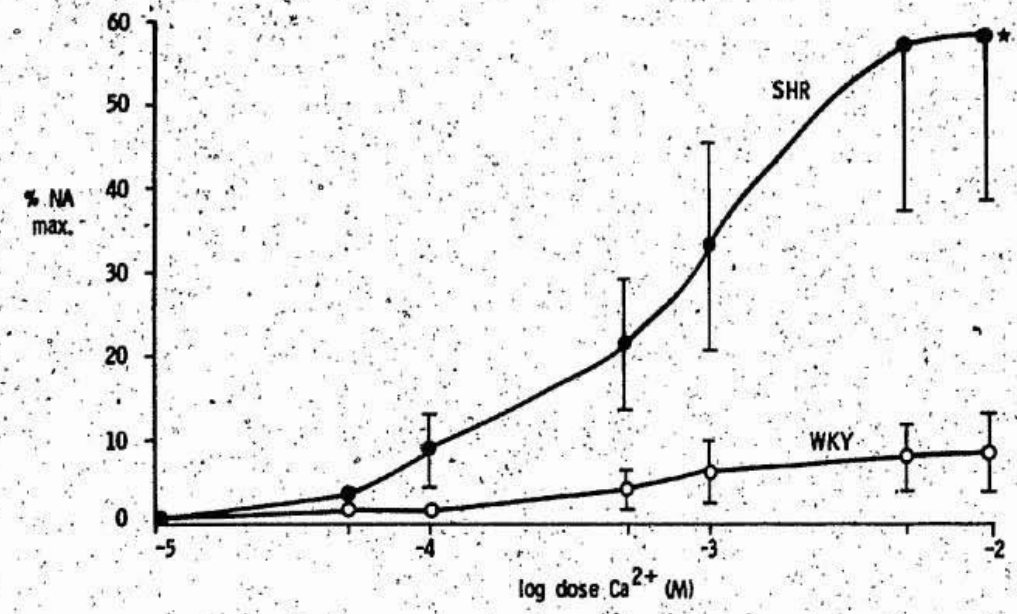

120
100
90
80
70
60
50
40
30
20
10
0
% NA Max.
SHR
WKY
1
3
5
7
9
10
11
HCl (mM)

Figure 18: Shows cumulative dose-responsecurves to Ca^{2+} (added as $CaCl_2$) in aortic rings from timolol-treated SHR (●) and timolol-treated WKY (O), recorded in normal Krebs solution <u>without</u> previous addition of KCl (as in Figure 11). Results have been expressed as a percentage of the maximum response to noradrenaline. N = 7 tissues from 5 animals per curve.


60
50
40
30
20
10
0
% NA max.
SHR
WKY
★
-5
-4
-3
-2
log dose Ca^{2+} (M)

C. The Effects of Ouabain and Low-Na^+ Solution

In this series of experiments the effects of ouabain and of low-Na^+ solution on the contractility of SHR and WKY VSM were examined. Bohr et al. (1969), first raised the possibility that Na^+ and Ca^{2+} are exchanged across the membrane of vascular smooth muscle. They found that both Na^+-free solution and 10^{-5}M ouabain potentiated contraction in rabbit VSM. They suggested that poisoning the Na^+-K^+ pump with ouabain allowed Na^+ to accumulate inside the cell and led to decreased exchange of extracellular Na^+ for intracellular Ca^{2+}. The ultimate result of ouabain treatment, they suggested, was an increased intracellular Ca^{2+} concentration and thus, greater contractility. The inotropic action of cardiac glycosides on the myocardium was reviewed by Akera and Brodie (1977). These authors favoured an action based on the Na^+-Ca^{2+} exchange hypothesis, as originally proposed by Langer (1971). The role of Na^+-Ca^{2+} exchange in smooth muscle was recently reviewed by van Breemen et al. (1979), but remains controversial.

Charles River SHR were used for reactivity studies in this section. Figure 19 shows that the dose-response curve to K^+ recorded from Charles River SHR was not significantly different from the K^+ dose-response curve recorded from NIH-derived SHR.

Smooth muscle from the rat is known to be relatively insensitive to ouabain (Daniel, 1964). Shibata et al. (1973) used a concentration of 10^{-4}M ouabain in their study of SHR VSM. Daniel (1964) found that even this comparatively high concentration did not sufficiently inhibit the Na^+-K^+ pump in rat myometrium, and used a concentration of 10^{-3}M.

Figure 19: Shows cumulative dose-response curves to K^+ in aortic rings from Charles River SHR (SHR-CR, O) and from NIH-derived SHR (SHR-NIH, ●), recorded in Krebs solution.

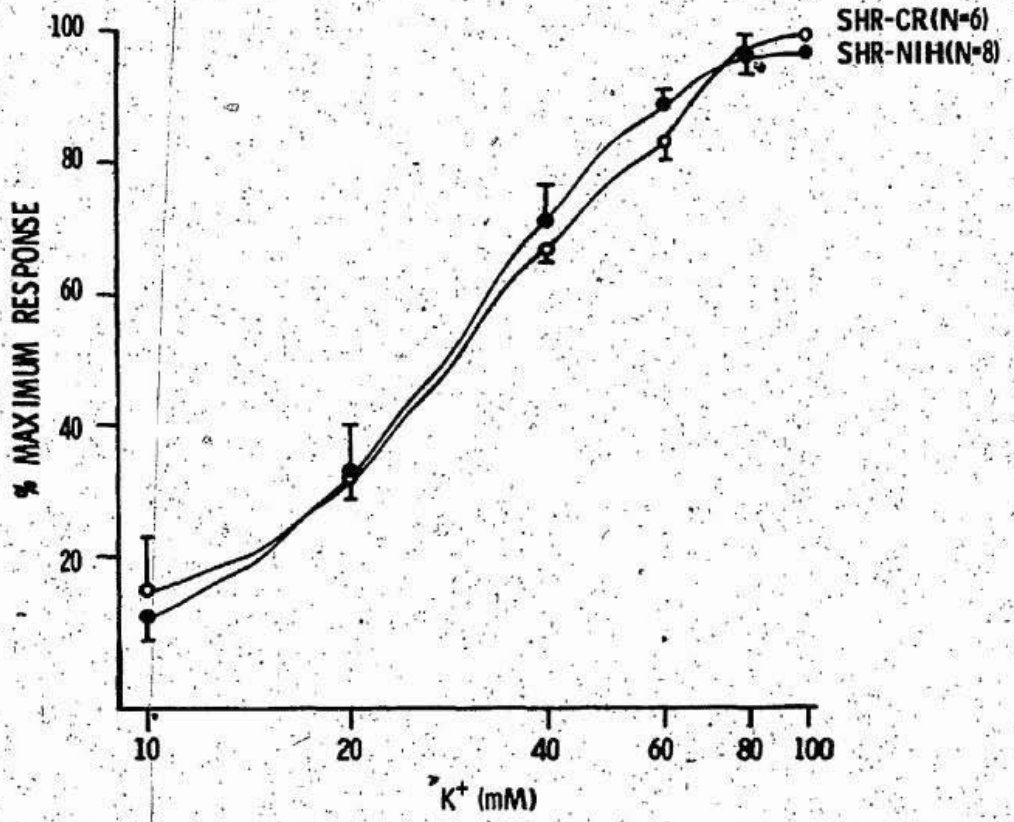

SHR-CR(N=6)
SHR-NIH(N=8)
% MAXIMUM RESPONSE
100
80
60
40
20
10
20
40
60
80
100
K+ (mM)

Table VIII shows that there was no significant difference in the response of SHR and WKY aorta to 10mM K^+ when the ouabain concentration was raised 10 fold from 10^{-4}M to 10^{-3}M.

Table VIII: Effect of 10^{-4}M and 10^{-3} ouabain on aortic response to 10 mM K^+

Animal	Control Response (no ouabain)	Response in presence of 10^{-4}M ouabain	Response in presence of 10^{-3}M ouabain	t value and degrees of freedom comparing responses in the presence of 10^{-3}M and 10^{-4}M ouabain
SHR	16.9 ± 6.4% (n=6)	15.9 ± 5.6% (n=6)	29.4 ± 4.3% (n=4)	t = 1.73, d.f. = 8 n.s.
WKY	4.0 ± 1.6% (n=6)	16.05 ± 8.2% (n=6)	14.2 ± 6.5% (n=4)	t = 0.16, d.f. = 8 n.s.

Tabulated above are the responses to 10mM K^+ expressed as a percentage of the maximum response to K^+, as in Figure 20. This Table shows that there was no significant difference between the effects of 10^{-4}M and 10^{-3}M ouabain on the response of aortic tissues from SHR or WKY.

Figure 20 shows the dose-response to K^+ is shifted slightly, although not significantly, to the left after pretreatment with 10^{-4}M ouabain for 15 minutes in both the SHR and WKY aortic tissues. Figure 21 shows the effect on the K^+ dose-response curves of the removal of most of the Na^+ from the Krebs bathing media and replacement of the NaCl removed with equimolar choline chloride. This treatment shifts both the SHR and WKY curves to the left and significantly increasing the response to the lowest dose of K^+.

Occasionally, changing to a low-Na^+ solution or addition of ouabain alone would result in tension development, which usually faded to base-line levels during the 15 minute incubation period.

In Figures 22 and 23, the K^+ dose-response curves recorded before and after addition of ouabain and before and after changing to a low-Na^+ solution, respectively, were replotted comparing SHR and WKY aortic tissue. Both ouabain and replacement of most of the Na^+ eliminated the significant differences between hypertensive and control tissues seen at the lower end of the K^+ dose-response curve.

The effect of 10^{-4}M ouabain on the Ca^{2+} dose-response curve is shown in Figure 24 for SHR aorta and Figure 25 for WKY aorta. The Ca^{2+} dose-response curve in the ouabain pretreated SHR aorta is shifted to the left, although not significantly. The response to Ca^{2+} in the WKY aorta without previous addition of depolarizing K^+ is less than 10% of the maximum response to K^+ in the presence and absence of ouabain. Substitution of choline chloride for most of the NaCl in Krebs solution significantly increased the response of SHR aorta to the lowest dose of Ca^{2+} added, as seen in Figure 26.

Figure 20: Shows cumulative dose-response curves to K^+ in aortic rings from WKY (left) and Charles River SHR (right) in Krebs solution alone (●) and after 15 minute pretreatment with 10^{-4} M ouabain (O).

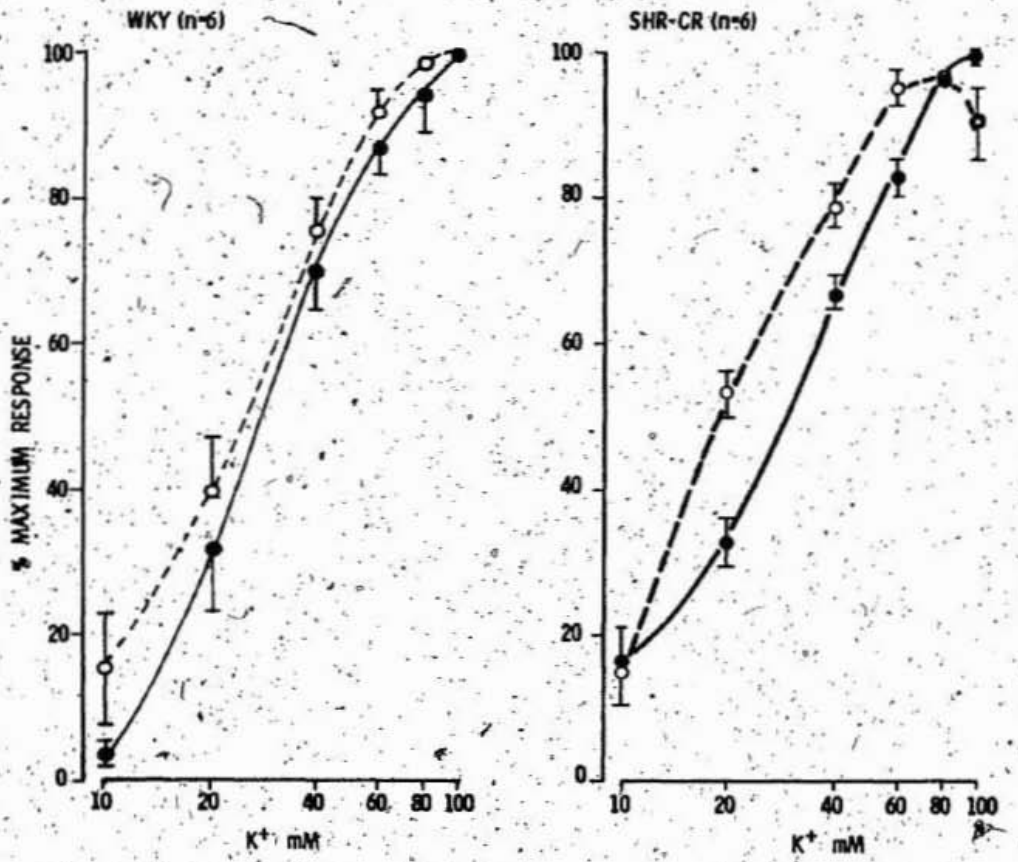

WKY (n=6)
SHR-CR (n=6)
% MAXIMUM RESPONSE
100
80
60
40
20
0
10
20
40
60
80
100
K+ mM

Figure 21: Shows cumulative dose-response curves to K^+ in aortic rings from WKY (left) and Charles River SHR (right) in Krebs solution (●) and in low-Na^+ Krebs after a 15 minute incubation in low-Na^+ Krebs solution (○).

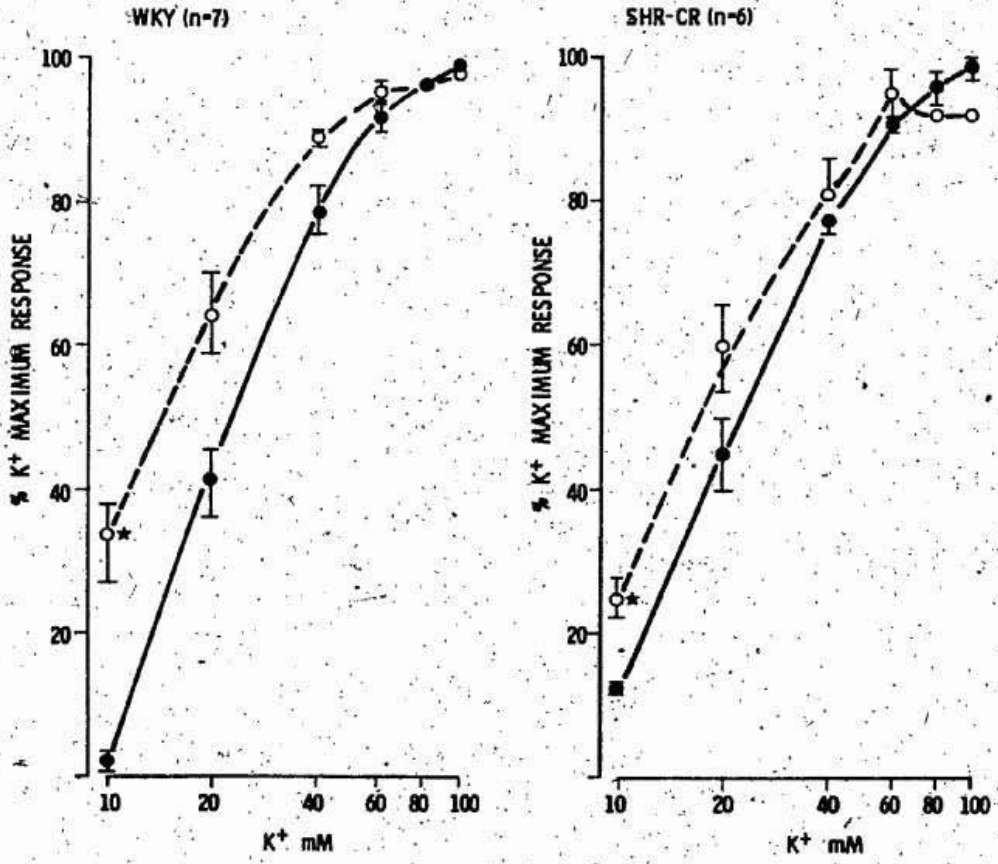
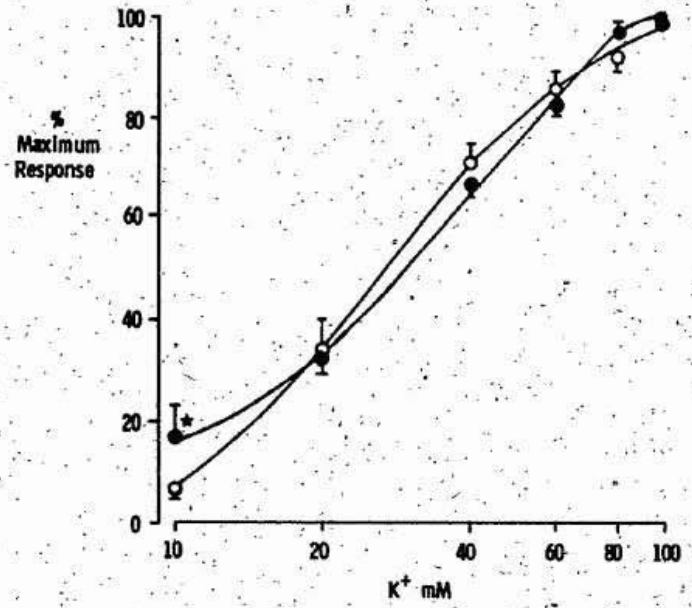

WKY (n-7)
SHR-CR (n-6)
% K+ MAXIMUM RESPONSE
100
80
60
40
20
10
20
40
60
80
100
K+ mM

Figure 22: Shows cumulative K^+ dose-response curves replotted from Figure 20, comparing responses of SHR (●) and WKY (○) aortic rings in Krebs solution alone (left) and after a 15 minute pretreatment with 10^{-4}M ouabain (right).


% Maximum Response
100
80
60
40
20
0
10
20
40
60
80
100
K+ mM

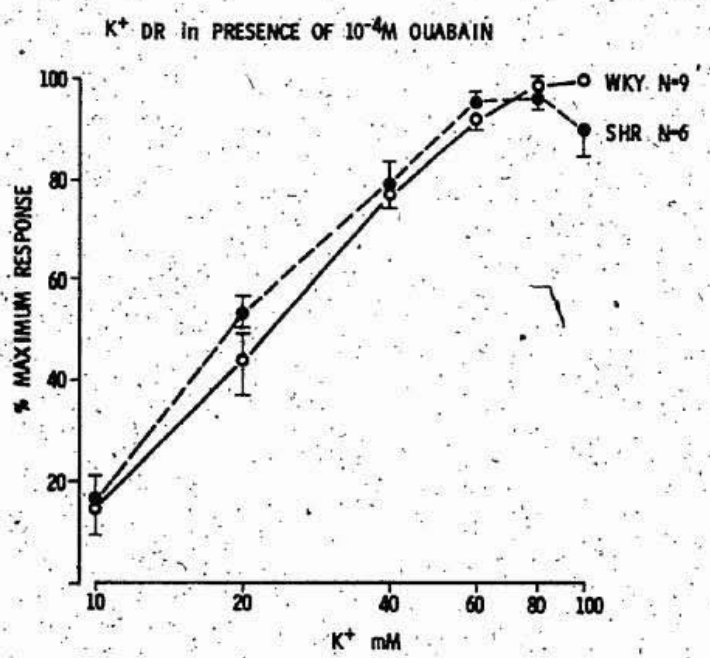
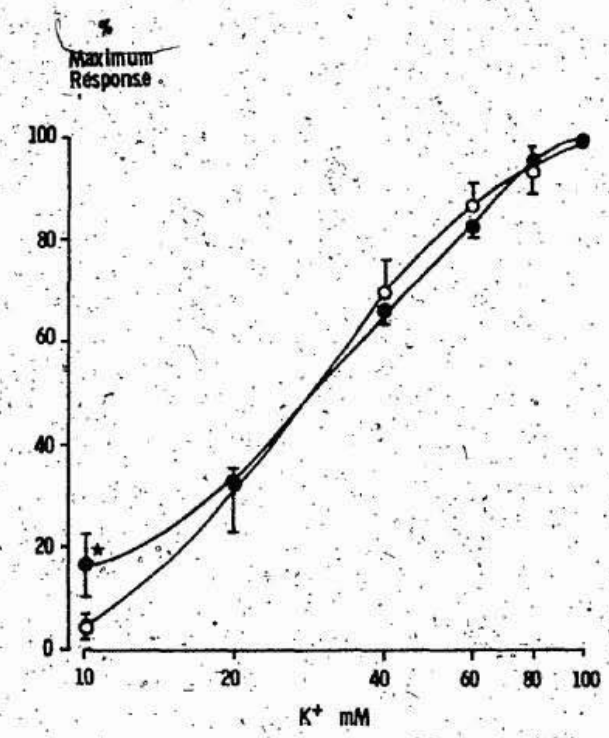

K+ DR in PRESENCE OF 10-4M OUABAIN
% MAXIMUM RESPONSE
WKY N=9
SHR N=6
100
80
60
40
20
10
20
40
60
80
100
K+ mM

Figure 23: Shows cumulative K^+ dose-response curves replotted from Figure 21, comparing responses of SHR (●) and WKY (⊘) aortic rings in Krebs solution (left) and in low-Na^+ Krebs after a 15 minute incubation in low-Na^+ Krebs solution (right).


%
Maximum
Réponse
100
80
60
40
20
0
10
20
40
60
80
100
K+ mM

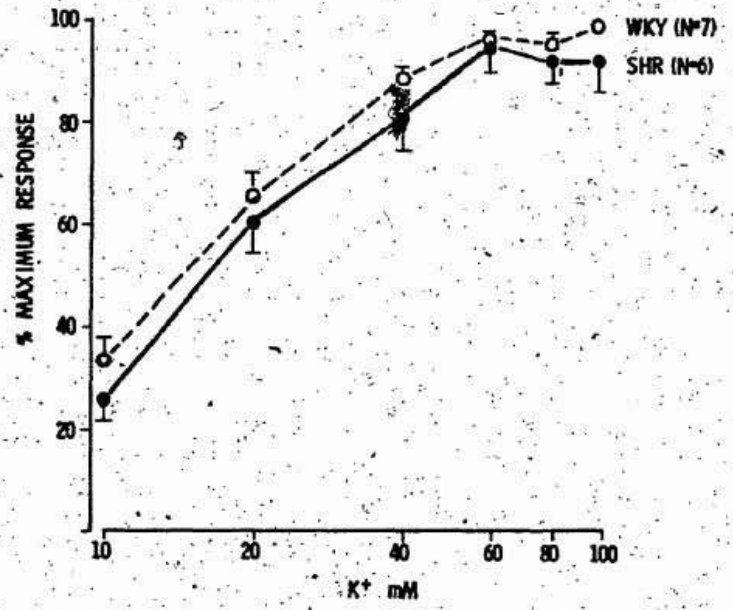

% MAXIMUM RESPONSE
WKY (N=7)
SHR (N=6)
100
80
60
40
20
10
20
40
60
80
100
K+ mM

Figure 24: Shows cumulative dose-response curves to Ca^{2+} expressed as a percentage of the maximum response to K^{+} in aortic rings from Charles River SHR in Ca^{2+}-free Krebs solution alone (●) and after 15 minutes pretreatment with 10^{-4} M ouabain (O).

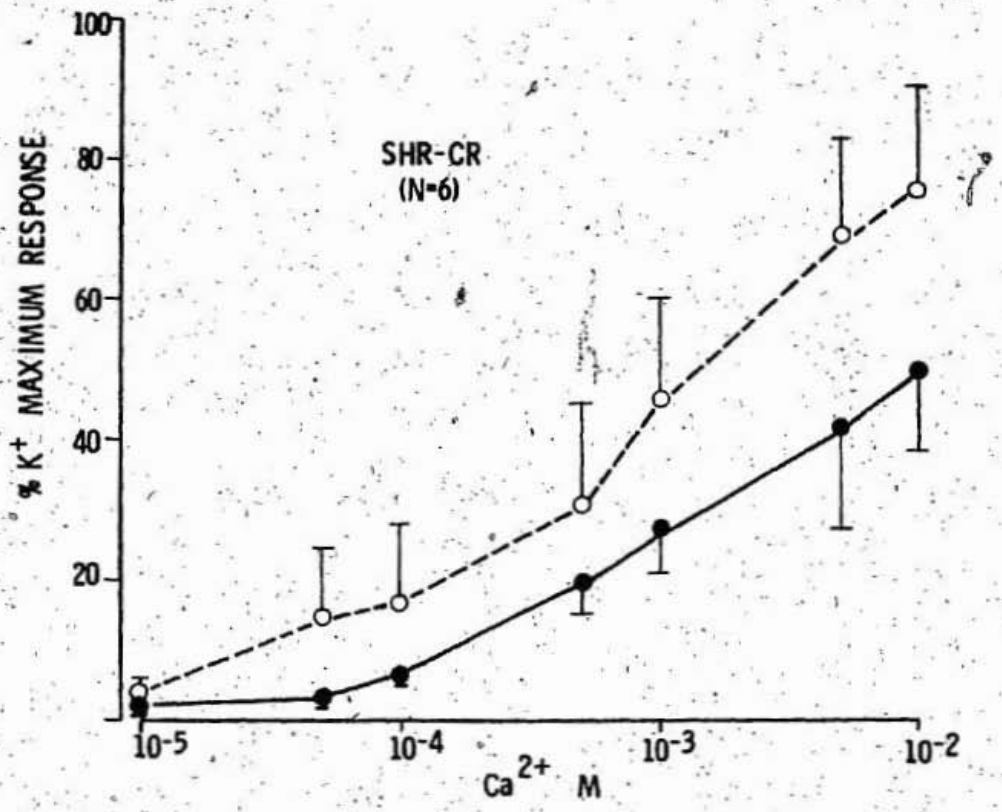

100
80
60
40
20
% K+ MAXIMUM RESPONSE
SHR-CR
(N=6)
10-5
10-4
10-3
10-2
Ca2+ M

Figure 25: Shows cumulative dose-response curves to Ca^{2+} as in Figure 24, but recorded from WKY aortic rings.

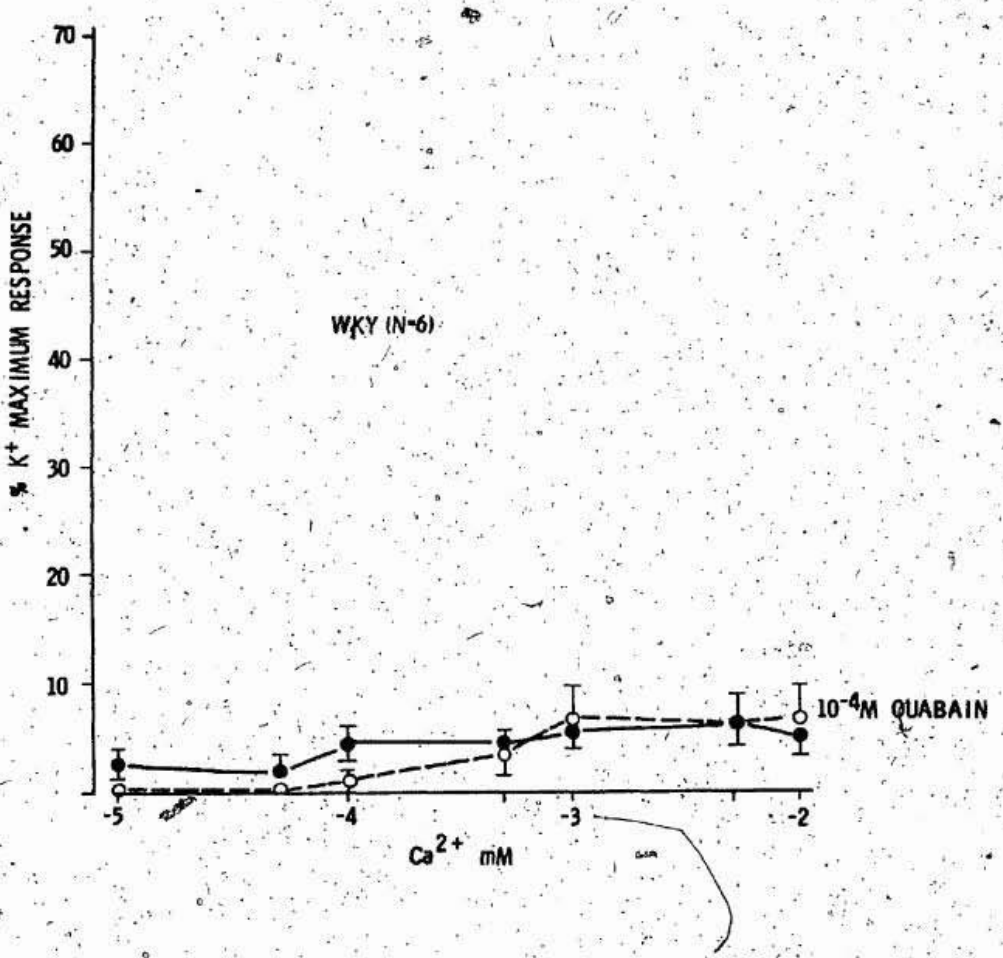
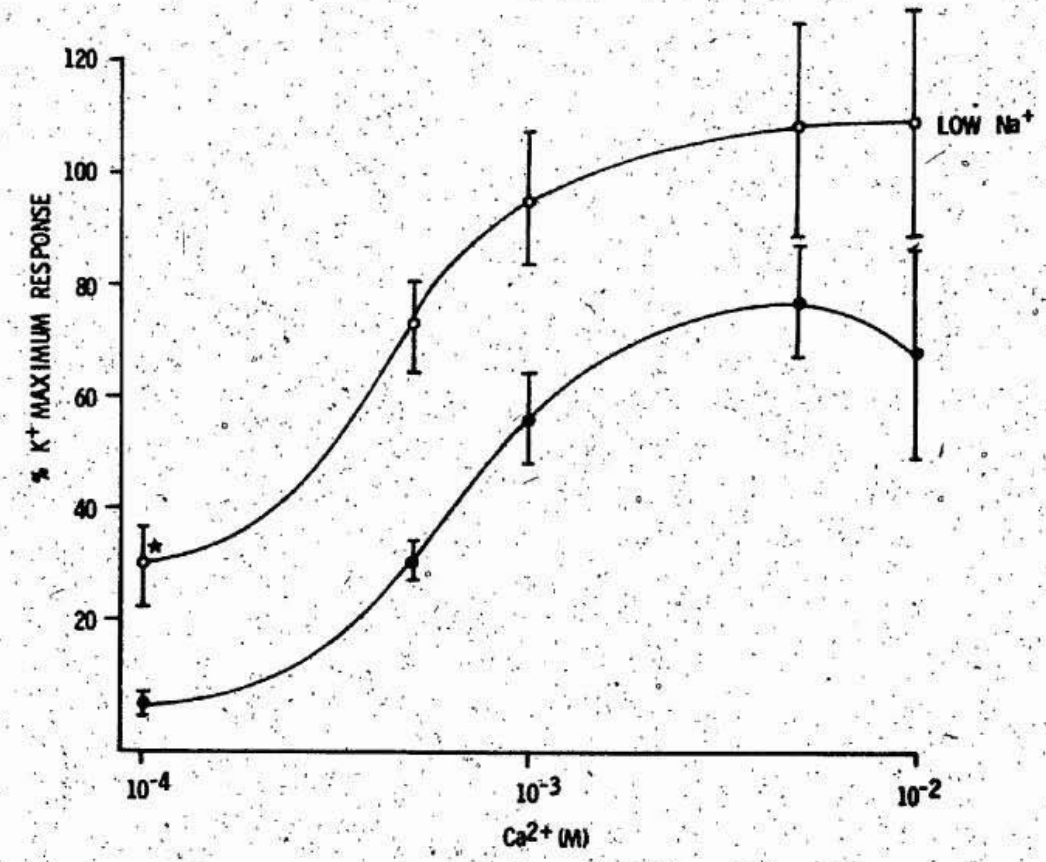

70
60
50
40
30
20
10
% K+ MAXIMUM RESPONSE
WKY (N=6)
10-4M OUABAIN
-5
-4
-3
-2
Ca2+ mM

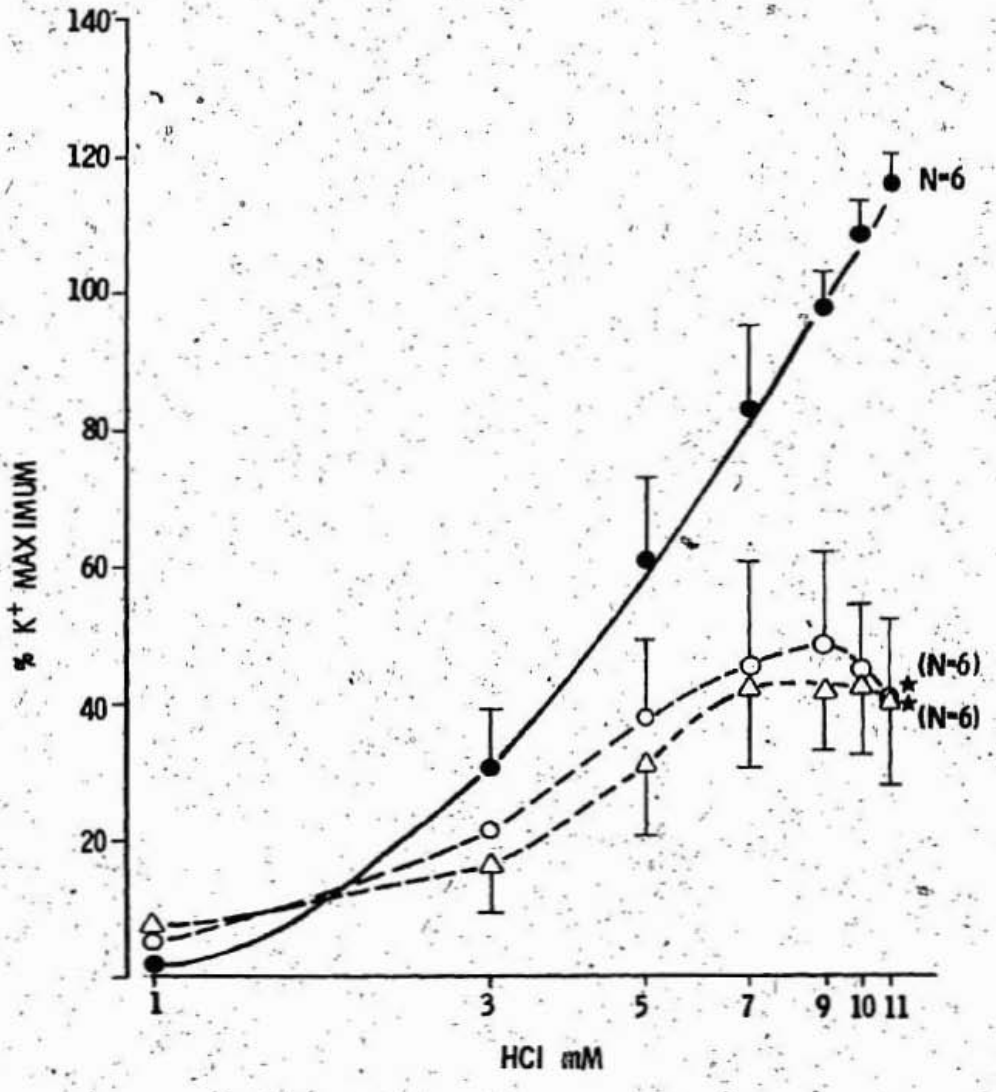
Figure 26: Shows cumulative dose-response curves to Ca^{2+} expressed as a percentage of the maximum response to K^{+} in aortic rings from SHR in Ca^{2+}-free Krebs solution (●) and in Ca^{2+}-free low-Na^{+} Krebs solution after a 15 minute incubation period in low-Na^{+} Krebs solution (O). N = 4


120
100
80
60
40
20
% K+ MAXIMUM RESPONSE
LOW Na+
10-4
10-3
10-2
Ca2+ (M)

The effects of ouabain and low-Na^+ solution on the response to H^+ in SHR VSM is seen in Figure 27. Each of these treatments significantly diminished the response to H^+ in this tissue.

Experiments reported in this section used aortic rings from 5 - 10 month old SHR and WKY rats, all obtained from the Charles River Breeding Labs. The SHR had an average systolic blood pressure of 182$\pm$ 12.1 mm Hg and WKY rats had an average systolic blood pressure of 129 $\pm$ 10.7 mm Hg. These blood pressures were significantly different.

Figure 27: Shows cumulative dose-response curves to HCl in SHR aortic rings expressed as a percentage of the maximum response to K^+ in Krebs solution (●) and after a 15 minute incubation period in 10^{-4} ouabain (Δ) or in low-Na^+ Krebs solution (○).


140
120
100
80
60
40
20
% K+ MAXIMUM
N=6
(N=6)
(N=6)
1
3
5
7
9
10
11
HCl mM

D. Further Studies on the Action of H^+ in SHR VSM

In light of the diminished responsiveness of SHR aorta to H^+ in low-Na^+ solution and in the presence of 10^{-4}M ouabain, the action of this ion was studied further with use of D-600 and nifedipine, both Ca^{2+} antagonists, and the local anaesthetic procaine.

Figure 28 shows the H^+ response is diminished as the concentration of D-600 is increased. Figure 29 shows that both 3 mM procaine and 10^{-7}M nifedipine significantly diminished the response of SHR aorta to H^+. The structures of these compounds are illustrated in Figure 30.

The responses in this section were recorded from 3 to 5 month old Charles River SHR with an average blood pressure of 181 $\pm$ 12.1 mm Hg.

Figure 28: Shows cumulative dose-response curves to HCl in SHR aortic rings expressed as a percentage of the maximum response to K^+ recorded in Krebs solution alone (●) and in the presence of 10^{-4}M (O), 5×10^{-4}M (Δ) or 10^{-3}M D-600 (□).

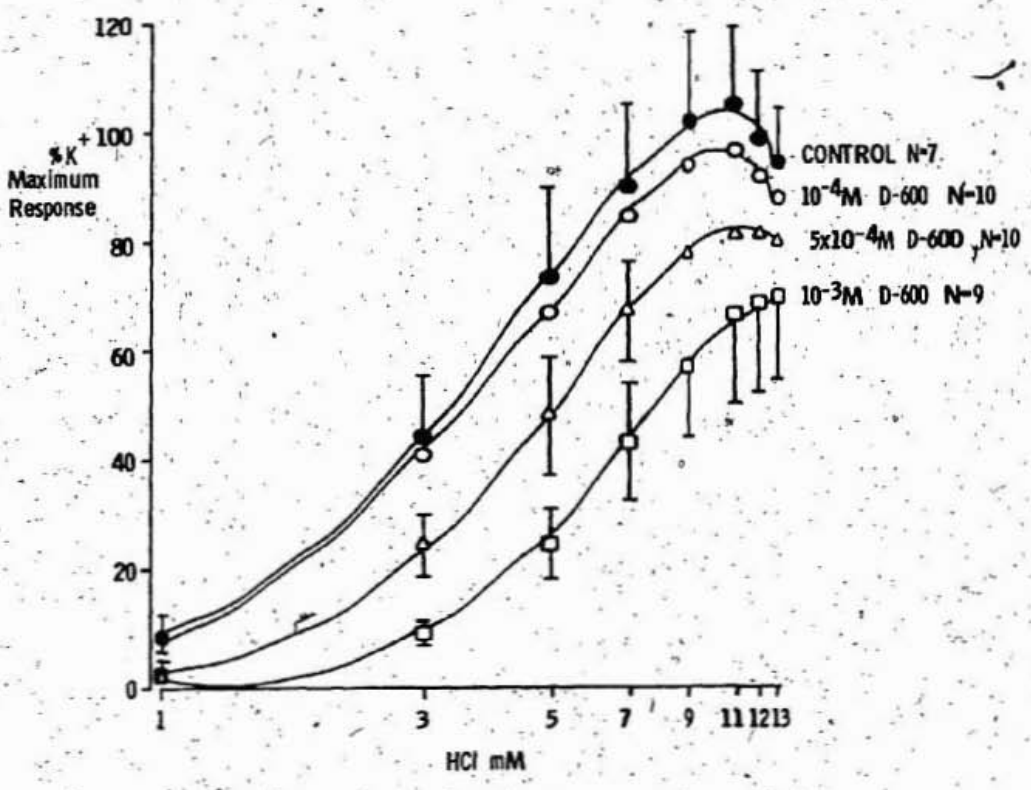

120
100
80
60
40
20
0
% K^+ Maximum Response
1
3
5
7
9
11
12
13
HCl mM
CONTROL N=7
10^{-4}M D-600 N=10
$5x10^{-4}$M D-600 N=10
10^{-3}M D-600 N=9

Figure 29: Shows cumulative dose-response curves to HCl in SHR aortic rings expressed as a percentage of the maximum response to K^+ recorded in Krebs solution alone (●) and in the presence of 10^{-8} M nifedipine (○), 10^{-7} M nifedipine (□) or 3mM procaine (Δ).

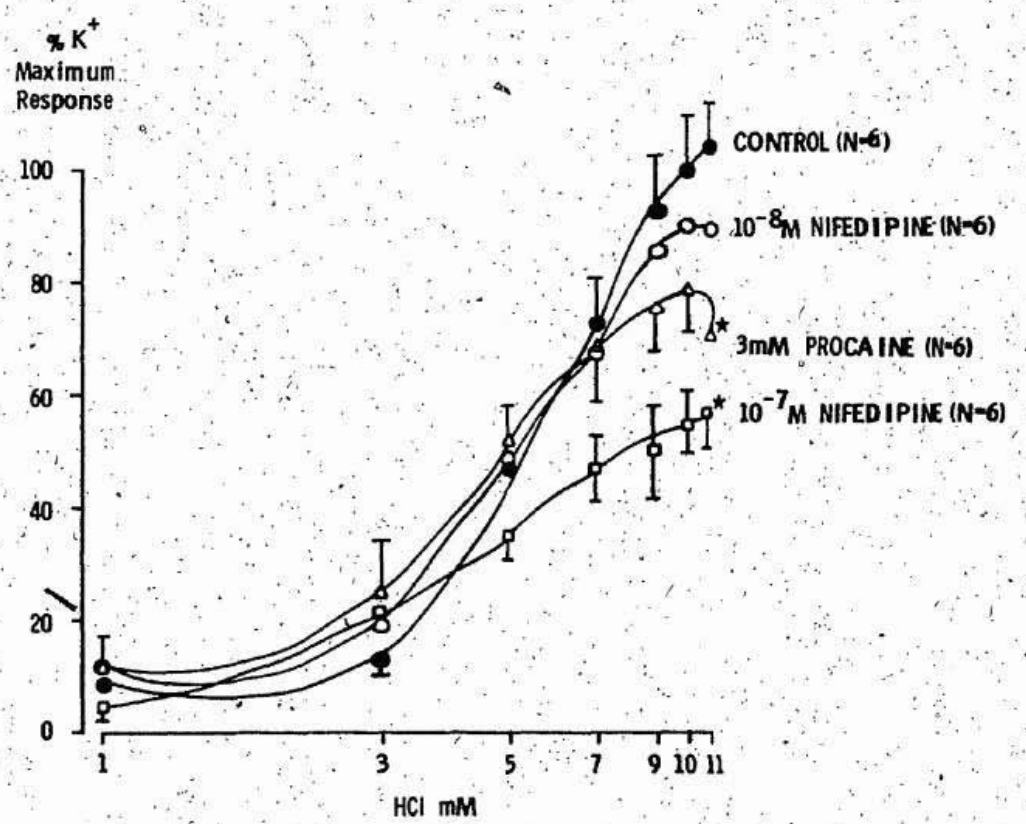
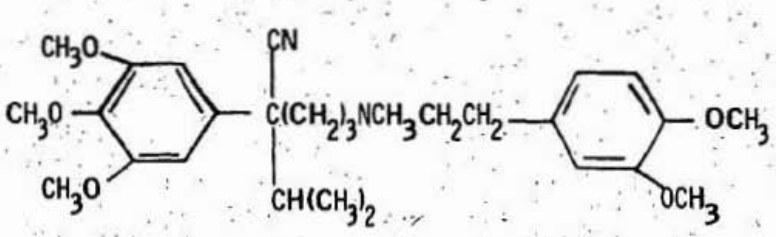
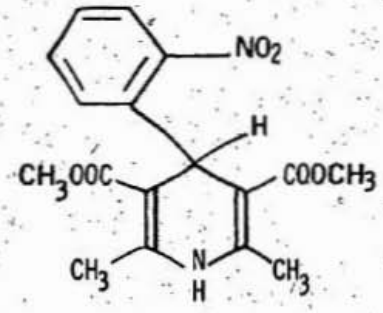

% K+
Maximum
Response
100
80
60
40
20
0
1
3
5
7
9
10
11
HCl mM
CONTROL (N=6)
10-8M NIFEDIPINE (N=6)
3mM PROCAINE (N=6)
10-7M NIFEDIPINE (N=6)

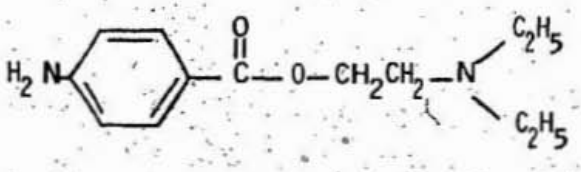
Figure 30: The structural configurations of D-600, nifedipine, procaine and lidocaine.



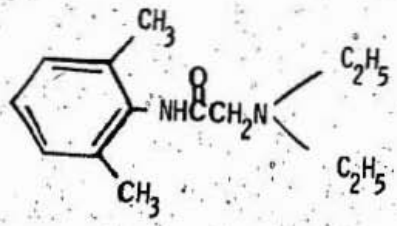
D-600



NIFEDIPINE (BAY 1040)



PROCAINE



LIDOCAINE

IV DISCUSSION

A. General Considerations

1. Methodology

The experiments presented here demonstrate pharmacologically increased vascular reactivity in the Spontaneously Hypertensive Rat aorta and address the question of the mechanism by which the reactivity becomes altered. In interpreting these studies certain considerations should be kept in mind:

First, here the problem has been studied using the isolated aortic tissue preparation. The drawbacks to this approach are (1) that isolated preparations of tissues in artificial solution may not reflect in vivo situations, and (2) that aorta is a highly distensible vessel and therefore is not representative of the small arterioles that truly determine vascular resistance (Folkow and Neil, 1971).

Secondly, there are several ways to define and measure vascular reactivity. Here, comparisons are made between responses to either the lowest dose of agonist or the dose of agonist that yields the greatest response. Therefore, vascular reactivity has been approximated to threshold sensitivity or maximum response, in terms of Kalsner's (1974) analysis of supersensitive phenomenum, while ED_{50} and the slope of the dose-response curve were rarely considered.

These reservations are appropriate, but not without explanations: The advantage of the isolated tissue technique is that experimental manipulations can be performed accurately, since most important variables can be controlled. Thus, VSM tension development can be recorded in response to known concentrations of agonists while minimizing the

influence of nerves, circulating hormones and metabolites. Aortic tissue was chosen for these studies initially to duplicate and extend the findings of Shibata et al. (1973) and Bohr (1974). Rat thoracic aorta is poorly innervated (Patil et al., 1972), another factor which makes this tissue suitable for a study of this nature.

As for the actual measurement of vascular reactivity, one of the aims of this study was to differentiate the possibility of truly increased reactivity (hypersensitivity) in SHR VSM from that of secondary structural changes producing apparent reactivity increases. Muscular hypertrophy might have significant effects on maximum tension development. However, it should have little effect on threshold (Folkow et al., 1973), since as muscle mass increases, the receptor population may also increase, but the sensitivity of individual receptors should remain unaltered. Accordingly, threshold sensitivity seemed to be the most appropriate measure of reactivity in this case.

2. Control and experimental animals

In these experiments, SHR from two sources and two strains of normotensive control rats were studied. The vascular responsiveness of the two types of SHR was not different (Figure 19) and both had significantly higher blood pressures than the WKY or Wistar controls.

The choice of controls is crucial in a study of this nature. Cline-schmidt et al. (1970) found a significant difference in maximum tension development between two normotensive strains of rats. Strain differences of this nature were not seen in the present study, perhaps because the two control strains used here were closely related. There was no difference observed in reactivity to K^+ between normalized responses of Wistar and timolol-treated WKY rats (Figure 15).

The NIH-derived SHR was initially used for these studies. However, when the animal facilities were moved to the new Health Science Centre, where construction was ongoing, it became difficult to breed SHR in the quantities necessary for completion of this work. The Charles River SHR was then purchased and used as the experimental animal.

B. Antihypertensive Drug Studies

The initial study with propranolol indicated that a β-adrenergic blocker could be an effective antihypertensive agent in the treatment of hypertension as seen in the SHR. This finding confirmed the reports of Weiss et al. (1974) who used 100 mg/Kg oral propranolol or H93/26 (a cardioselective β-receptor antagonist), of Kubo et al. (1977) who used 64 mg/Kg oral propranolol, of Vavra et al. (1973) who used 5 mg/Kg/day oral propranolol in weanlings, and of Conway et al. (1975), who used 20, 40 or 100 mg/Kg propranolol daily, administered orally.

These reports are contrasted by reports by Nishiyama et al. (1978) and Pfeffer et al. (1977) from the same laboratory who were unable to significantly lower the blood pressure in SHR using either 60 mg/Kg propranolol or 65 mg/kg timolol daily in the former study and at least 100 mg/Kg propranolol or 66 mg/Kg timolol administered from conception in the later study. Nishiyama et al. suggested inconsistancy in the characteristics of SHR bred in various places around the world may account for the lack of antihypertensive activity of β-blockers in their study.

Timolol was chosen as the antihypertensive drug for this study because (1) it was potent when administered orally (Sweet et al., 1975) (2) it was thought to act centrally (Sweet et al., 1975) and (3) it was considered unlikely to have prominent direct effects on Ca^{2+} homeostasis in VSM. This latter suggestion is supported by the findings that timolol is 5-6 times less potent than propranolol as a negative inotropic agent (Hall et al., 1975) and 15 times less potent as an inhibitor of

sarcoplasmic reticulum calcium uptake (Messineo and Katz, 1979), inspite of the fact that timolol is 5 to 10 times more potent than propranolol as a β-adrenergic blocker (Sweet et al., 1975).

Timolol was effective in significantly lowering blood pressures of SHR animals when compared to untreated SHR, to the extent that they were not significantly different from untreated WKY animals. Thus, the drug protocol was considered to have been successful in producing normotensive SHR. Blood pressures of treated WKY were not significantly different from untreated WKY, indicating the appropriateness of this group as a normotensive control for the effects of drug treatment. Figure 15 shows that timolol treatment did not alter the responsiveness of SHR aorta to K^+, nor did normotensive timolol treated WKY rats show altered aortic responsiveness compared to untreated Wistar rats; both indications that treatment alone had no effect on reactivity.

The contractile response to high Ca^{2+} in the non-depolarized SHR aorta (Figure 18) may indicate the VSM membrane has an abnormally high permeability to Ca^{2+}. Noon et al., (1978) also found tension development in SHR aorta when Ca^{2+} was reintroduced after an incubation period in Ca^{2+} free solution. These authors concluded that the VSM membrane of the SHR leaky to Ca^{2+}.

A similar conclusion may be reached from the data presented in Figure 17. The contractile response to H^+ is seen in Figure 9 in the hypertensive SHR. This response can be partially antagonized by D-600 (Figure 12), suggesting that the H^+ response may be partly mediated through D-600-sensitive Ca^{2+} channels (as will be further

discussed later in this chapter). The response to H^+ is seen in aorta from normotensive, timolol treated SHR and not in tissue from normotensive, similarly treated WKY controls. This could be interpreted as a further indication of a possible membrane defect in the handling of Ca^{2+} in the SHR aorta.

This defective membrane hypothesis is supported by the data in Figures 15 and 16: The K^+ dose-response curve shows that aorta from timolol-treated SHR is significantly more sensitive than timolol-treated WKY aorta to the lowest concentration (10mM) of K^+. But there is no significant difference between the noradrenaline dose-response curves (Figure 16).

A possible explanation for the lack of a significant difference between SHR and WKY aorta in their response to noradrenaline, and the existance of significant difference in their responses to K^+ rests in the manner in which Ca^{2+} pools are utilized for these contractions: Both noradrenaline- and K^+-induced contractions in VSM are biphasic; Bohr (1964) described an initial, fast response (the phasic component) which is followed by a slower, further increase in tension (the tonic component). In rabbit aorta, the phasic component of the K^+-induced response is very dependant on extracellular Ca^{2+} (van Breemen et al., 1973). In contrast, the phasic component of the noradrenaline response is not dependant on extracellular Ca^{2+} (Deth and van Breemen, 1974) but rather utilizes intracellularly stored Ca^{2+} (Hudgins and Weiss, 1968). Swamy and Triggle (1980) found that when Ca^{2+} is removed from the physiological solution bathing isolated rat carotid artery strips the phasic response to noradrenaline remains while the phasic response

C. The Pharmacological Reactivity of SHR VSM

1. Reactivity to K^+

The data presented in Figure 15 shows a greater response to the lowest concentration of K^+ in VSM from normotensive and hypertensive SHR than in VSM from Wistar and WKY controls. Since the response to K^+ is initially dependent on extracellular Ca^{2+} and due to membrane depolarization rather than mediated through a receptor (van Breemen et al., 1973), there are two possible explanations for the cellular mechanism of the alteration in sensitivity in Figure 15: (1) Depolarization occurs to a greater extent to the low dose of K^+ (10mM) in the SHR aorta; or (2), more extracellular Ca^{2+} is made available to the contractile proteins in SHR aorta for a given depolarization.

The data of Kuriyama and Suzuki (1978) tend to rule out the first alternative. They measured membrane potential as K^+ was added to isolated SHR pulmonary artery and portal vein, two VSM tissues that are not exposed to elevated pressures. They found that K^+ added step-wise produced almost identical depolarizations in SHR and WKY tissues (their Figures 6 and 7).

The second possibility implies that more Ca^{2+} enters or perhaps that more stored Ca^{2+} is mobilized for a given depolarization. These possibilities will be dealt with further in following sections of this Discussion, but to conclude this section, increased reactivity to K^+ recorded in SHR VSM is consistant with a more accessable extracellular Ca^{2+} pool.

to K^+ is lost. Yamashita et al. (1977) reported similar findings in rat aorta.

The responses reported in this thesis were recorded cumulatively; that is an initial dose was added and as soon as the response reached a plateau, a second dose was added. This methodology, when applied to noradrenaline- and K^+-induced responses tends to record only the phasic responses, especially at the low end of the dose response curve. Consequently, if the plasma membrane of VSM in the SHR responds in an exaggerated fashion to agonists due to facilitation of the entry of extracellular Ca^{2+}, then a hyperresponsiveness to K^+ rather than to noradrenaline may be expected in this tissue. Perhaps this expains why hypersensitivity to K^+, but not to noradrenaline was seen in this study.

It has been suggested, as discussed in the Introduction, that the increased reactivity to various agonists seen in vascular smooth muscle from hypertensive animals is due solely to hypertrophy of the muscle secondary to the establishment of hypertension. However, the present study has shown that; (1) increased reactivity to K^+ persists in SHR treated from conception with timolol and rendered normotensive, (2) the previously demonstrated reactivity to low pH in SHR VSM persists in normotensive SHR, and (3) the normotensive SHR aorta develops significant tension when extracellular Ca^{2+} levels are raised, while the WKY tissue does not. These reactivity differences are apparent even in the absence of an elevated blood pressure in the SHR: an indication that reactivity changes are not a consequence of the development of hypertension in this animal. There are also indications that altered reactivity in the SHR aorta may be attributable to a defect in the control of Ca^{2+} homeostasis.

2. Reactivity to La^{3+}

The initial investigations (Figures 3 and 4) confirmed the findings of Shibata et al. (1973) and Bohr (1974), who reported that La^{3+} induced a contraction in VSM from SHR. Hermsmeyer and Walton (1977) were unable to demonstrate this response in isolated caudal artery from the SHR and suggested that not all parts of the vascular tree are homogeneous in their reactivities. The La^{3+}-induced contraction in SHR VSM was considered paradoxical, because La^{3+} inhibited contraction in rabbit aorta (van Breemen et al., 1973) and was reported to block Ca^{2+} entry into VSM (Godfraind, 1976); thus La^{3+} might be expected to have a relaxant action.

The observation that La^{3+} formed a precipitate in the bicarbonate-buffered solution was first reported by Sanborn and Langer (1970) in studies on cardiac muscle. The upper two curves of Figure 17 illustrate that most of the La^{3+}-induced contraction in the SHR aorta is a consequence of the change in pH (refer to Table V) that results when La^{3+} forms an insoluble complex with a buffer. When La^{3+} was added to HEPES-buffered solution no change in pH occured, but a contraction was recorded. This presumably was not simply the effect of the HEPES-buffer itself, since there was no significant difference between responses to K^{+} in SHR VSM recorded in HEPES- and bicarbonate-buffered Krebs (Figure 6). This residual contraction due to addition of La^{3+} in HEPES-buffered Krebs solution, where the pH remains stable, was greatly diminished but still significantly greater in the SHR VSM tissue (Figure 7), indicating that La^{3+} did have some direct action.

In Figure 14, the La^{3+} response recorded in HEPES-buffered Krebs was shifted to the right and was greatly diminished in the absence of Ca^{2+} (compared to Figure 7), but was not antagonized by D-600. Keatinge (1972), has shown that even after prolonged incubation in Ca^{2+}-free media, residual supplies of bound extracellular Ca^{2+} may still be present. On this basis, the mechanism of action of La^{3+} in VSM from the SHR may be partially accounted for by an inward displacement of extracellularly-bound Ca^{2+}.

There are several precidents in the literature of Ca^{2+}-mediated events where La^{3+} appears to have a facilatory action rather than its usual inhibitory effect: La^{3+} has been reported to release catecholamines from the bovine adrenal medulla (Borowitz, 1972) and histamine from mast cells (Foreman and Mongar, 1972). In frog neuromuscular junction preparations La^{3+} caused an increase in the frequency of miniature end-plate potentials, even in a Ca^{2+}-free solution (DeBassio et al., 1971). Heuser and Miledi (1971) reported similar findings and suggested La^{3+} was acting either by an inward displacement of Ca^{2+} from bound sites or by a direct intracellular action. In the present study, in the absence of Ca^{2+}, the peak response occurred at 10^{-1} M La^{3+} (Figure 5), while in the presence of extracellular Ca^{2+}, the peak response occurred at 5×10^{-3} M La^{3+} (Figure 3), suggesting a reliance on extracellular Ca^{2+}. However, in Ca^{2+}-free solution, the La^{3+} response was not antagonized by D-600. This suggests that a component of the La^{3+}-induced contraction may be a direct action on the VSM membrane to release intracellular Ca^{2+}. Alternatively, La^{3+} may enter the cell and displace bound intracellular Ca^{2+} or act directly on the contractile proteins once inside the cell.

La^{3+} has been reported to cross the rat uterine muscle membrane and enter the cell (Hodgson and Daniel, 1973) and La^{3+} entry into intestinal smooth muscle has been implied from the studies of Triggle and Triggle (1976a). Since La^{3+} has a greater affinity for calcium binding sites than Ca^{2+} (Lettvin et al., 1964), it is likely that the intracellular entry of La^{3+} would displace Ca^{2+} from intracellular bound pools and increase the free intracellular Ca^{2+} concentration. La^{3+} has also been shown to cross the mitochondria membrane and enter the matrix in isolated mitochondria (Piccinini et al., 1975) where it could displace bound Ca^{2+}. La^{3+} has been shown to displace Ca^{2+} from skeletal muscle troponin (Fuchs, 1971), although it should be noted that there is no direct evidence that indicates that La^{3+} can act like Ca^{2+} and mediate in actin-myosin interactions in skeletal or smooth muscle.

The paradoxical contraction to La^{3+} occurs only in SHR VSM to any significant degree and might reflect a functional alteration in the VSM membrane. If the VSM membrane in SHR aorta is leaky to Ca^{2+} then La^{3+}, an ion of similar unhydrated ionic radius, may be able to cross the membrane through those Ca^{2+} leakage sites. However, the residual contraction to La^{3+} in HEPES-Krebs solution was small compared to that recorded in Krebs solution, implying that the major portion of the contractile response to SHR aorta to La^{3+}, as reported by Bohr (1974) and Shibata et al., (1973) was mediated through H^{+}.

3. Reactivity to Ca^{2+}

a) Response in non-depolarized aorta

The SHR aorta (Figures 18 and 24) responds to Ca^{2+} added stepwise without previous depolarization, while the WKY aorta does not(Figure 25). This finding was also reported by Noon et al. (1978) and suggests the SHR VSM is unable to regulate Ca^{2+} permeability as well as the WKY VSM. Raised extracellular Ca^{2+} levels is generally thought to promote stability of the membrane (see Triggle and Triggle 1976b), but in SHR VSM tissue, tension develops on the addition of Ca^{2+} to the extracellular fluid.

There are several explanations for this response: (1) There may be more sites for Ca^{2+} diffusion in the SHR VSM membrane. (2) The potential-sensitive Ca^{2+} channels (see: Bolton, 1979) in the SHR may be open more often on a probabilistic basis at a given membrane potential than are similar Ca^{2+} channels in the WKY VSM membrane. (3) Ca^{2+} removal mechanisms may be impaired in the SHR tissue. Table IV shows that aorta from SHR relaxed more slowly in response to vasodilators and after removal of agonists. This data suggests that removal of Ca^{2+} occurs with greater difficulty in SHR VSM. This hypothesis will be discussed in the context of the discussion of the ouabain and low-Na^{+} experiments.

b) Effects of Ouabain and Low-Na^+ Solution

i. Na^+-Ca^{2+} exchange

Passive Na^+ influx has been shown to be coupled to Ca^{2+} efflux in a number of nerve and muscle preparations (see: Blaustein, 1974). The coupling ratio may vary from tissue to tissue, but it is generally believed that three or four Na^+ ions entering the cell, down the Na^+ concentration gradient supply the energy for the carrier-mediated removal of one Ca^{2+} ion from the cell against its concentration gradient (Blaustein and Ector, 1976). This exchange system is a particularly attractive hypothesis for Ca^{2+} removal from smooth muscle (Blaustein, 1976); since this tissue has very little sarcoplasmic reticulum, it is difficult to explain how removal of Ca^{2+} after tension development for the maintenance of a high inward Ca^{2+} gradient is accomplished without a highly active ATP-dependant Ca^{2+} extrusion pump, of the type characterized in red blood cells by Schatzmann (1966).

van Breemen et al. (1979) reviewed the evidence for a passive Na^+-Ca^{2+} exchange in mamalian smooth muscle. He reasoned that if Na^+ influx was coupled to Ca^{2+} efflux, then there should be a hypothetical relationship between the degree of contraction of the tissue and the ratio of extracellular to intracellular Na^+ concentration. The slope of this relationship when plotted should be predictable from the ratio of the number of Na^+ ions exchanged for one Ca^{2+}. He found the available data in the literature on various smooth muscles did not fit any of the predicted curves. He concluded Na^+-coupled Ca^{2+} exchange

was not the sole mechanism responsible for removal of Ca^{2+} from smooth muscle cells.

However, Na^+-dependant Ca^{2+} efflux has been demonstrated in smooth muscle (Brading and Widdicombe, 1976; Reuter et al., 1973). The former authors also found an intracellular Na^+-dependant Ca^{2+} uptake, using radioactive isotopes. In further support of a Na^+ coupled Ca^{2+} exchange mechanism in smooth muscle are the findings that relaxation following contraction is impaired in the absence of extracellular Na^+ in guinea pig ileum (Judah and Willoughby, 1974), guinea pig taenia coli (Katase and Tomita, 1972; Ma and Bose, 1977), rat uterus (Ma and Bose, 1977) arterial (Bohr, Seidel and Sobieski, 1969; Reuter, Blaustein and Haeusler, 1973) and venous (Biamino and Johansson, 1970) smooth muscle.

In contrast to these findings, Droogmans and Casteels (1977) found that La^{3+} induced a relaxation after K^+-induced contraction in Na^+-free solution. These authors suggested that Na^+ and Ca^{2+} may compete for the same channel, but that tightly coupled exchange was unlikely. van Breemen (1976) found no decrease in exchangeable cellular $^{45}Ca^{2+}$ in rabbit aorta when Li^+ was substituted for Na^+ in the extracellular solution, producing relaxation after a K^+-induced contraction. He concluded that Na^+-Ca^{2+} exchange was not important in the relaxation process for VSM.

ii. The action of ouabain and low-Na^+ solution

One possible method of resolving the importance of Na^+-Ca^{2+} exchange is to study the action of ouabain on smooth muscle contractility. Ouabain,

and other cardiac glycosides, prevent ATP from binding to Na^+-K^+ ATPase (Hansen et al., 1971), prohibiting the active extrusion of Na^+ against its concentration gradient. If extracellular Na^+ is exchanged for intracellular Ca^{2+}, the inhibition of the Na^+-K^+ pump by ouabain should allow Na^+ to accumulate in the cell, decreasing the inward Na^+ driving force and consequently slowing the removal of Ca^{2+} via a Na^+-Ca^{2+} exchange mechanism. In smooth muscle accumulation of intracellular Ca^{2+} will result in a contraction.

However, studies of the action of ouabain alone have not resolved the question of whether or not Na^+-Ca^{2+} exchange occurs in smooth muscle: Matthews and Sutter (1967) reported a ouabain-induced contraction in guinea pig taenia coli and rabbit anterior mesenteric vein, but van Breemen (1976) suggested the contraction that he and previous investigators found to either removal of K_o^+ or after treatment with ouabain was due to the inhibition of the electrogenic Na^+-K^+ pump (Hendrickx and Casteels, 1974). He suggested subsequent depolarization probably accounted for the tension development. This conclusion was also reached by Ozaki et al. (1978) from similar experiments in guinea pig taenia coli.

Ouabain does depolarize smooth muscle. This was demonstrated in guinea pig taenia coli and mesenteric vein by Matthews and Sutter (1967). However, the data of Droogman and Casteel also suggests that a large depolarization (in the order of 15-20mV) must occur before contraction is initiated.

In contrast, based on $^{42}K^+$ flux studies, Brading and Widdicombe (1974) calculated that the electrogenic Na^+-K^+ pump in taenia coli might generate a potential of 3 to 5 mV; a value which is in the order of magnitude of the depolarization characterized in squid giant axon

when the electrogenic Na^+-K^+ pump is inhibited (DeWeer and Geduldig, 1973). This suggests that the contraction reported on the addition of ouabain may not be due totally to the inhibition of the Na^+-K^+ pump.

The alternative explanation for the contractile action of ouabain in smooth muscle, that inhibition of the Na^+-K^+ pump allows the Na^+ gradient to run down, slowing the removal of Ca^{2+}, has been difficult to test. The present study provided an opportunity to test the involvement of Na^+-Ca^{2+} exchange. It has been postulated here that the increased reactivity seen to K^+ in the SHR aorta is due to increased availability of Ca^{2+} in this tissue, such that a small depolarization produces a relatively large contraction. When treated with ouabain or low-Na^+ solution, the sensitivity of WKY tissue to 10mM K^+ was increased, eliminating the significant difference between SHR and WKY aortic tissue at the low end of the dose-response curve (Figures 22 and 23). Ouabain will inhibit the electrogenic Na^+-K^+ pump and indeed will initiate a depolarization. However, low-Na^+ solution should <u>stimulate</u> the Na^+-K^+ pump since Na^+ and K^+ compete for the K^+ binding site on the outside of the membrane (Sjodin, 1971) and increased K^+ availability to extracellular sites stimulates the pump (Haddy, 1979) in smooth muscle. Consequently, low-Na^+ media should result in a hyperpolarization. It is therefore difficult to reconcile the suggestion that ouabain induces a contraction through its depolarizing effects on the membrane, since low-Na^+ solution and ouabain have similar, not opposite effects on K^+, H^+- and Ca^{2+}-induced contractions in this study.

It is reasonable to consider an alternative explanation that centres around a Na^+-Ca^{2+} exchange mechanism: Ouabain, by inhibiting the Na^+-K^+

pump, permits the Na^+ gradient to be reduced. The net result is that the inward driving force for Na^+ is reduced. This also occurs when the external Na^+ is reduced by an order of magnitude, through substitution of choline chloride for most of the NaCl in the low-Na^+ solution. If one postulates a Na^+-Ca^{2+} exchange in which external Na^+ diffusing down its concentration gradient is the driving force for the removal of internal Ca^{2+}, when the inward Na^+ driving force is reduced, Ca^{2+} will accumulate in the cell passively by diffusing down its concentration gradient. This could be the basis for increased contractility seen when the VSM in this study is treated with ouabain or when a low-Na^+ external solution is applied.

This explanation of the contractile effects of ouabain is in agreement with the observations of Fleckenstein et al. (1975) that verapamil, a Ca^{2+} antagonist, inhibits the contraction induced by cardiac glycosides in pig coronary artery strips, but that this inhibition can be overcome by increasing the extracellular Ca^{2+} concentration. Soares de Moura et al. (1979) found that strophantin-induced contraction in guinea pig ileum could be inhibited by verapamil, a calcium channel anatgonist, or by removal of the extracellular Ca^{2+}.

Changing the physiological solution to a low-Na^+ solution will immediately lower the Na^+ inward driving force, whereas the action of ouabain is slower, poisoning the Na^+-K^+ pump, allowing the Na^+ gradient to run down. The difference in the time course of these events may be reflected in the differences in their effects on the Ca^{2+} dose-response curves (Figures 24 and 26) where the enhancement of the Ca^{2+}-induced tension development was much more striking in low-Na^+ solution than in

the presence of ouabain.

4. Reactivity to H^+

a) The action of D-600

D-600, or methoxy-verapamil, is considered to be a specific antagonist of the tetrodotoxin-insensitive Ca^{2+} current, on the basis of electrophysiological data obtained from cardiac muscle preparations and consequently, has been labelled a Ca^{2+}-channel antagonist (Fleckenstein, 1977; Rosenberger and Triggle, 1978).

This agent is usually effective in the range of micromolar concentrations. For instance, D-600 was maximally effective in shifting the mechanical activation threshold in frog sartorius muscle at a concentration of $4x10^{-5}$M (Dorrscheidt-Kafer, 1977) and in cardiac muscle at a concentration of less than 10^{-6}M, it greatly reduced Ca^{2+} conductance (Kohlhardt et al., 1972).

Reports of its potency in smooth muscle vary: In rabbit ear artery the ID-50 to noradrenaline-induced contractions was greater than 10^{-4}M (Golenhofen and Weston, 1976), while in rat aorta, 10^{-8}M was the ID-50 for the spasmolytic effect of D-600 on noradrenaline-induced contractions (Massingham, 1973). Bilek et al. (1974) found $5x10^{-5}$M verapamil was the ID-50 for noradrenaline-induced contractions in rat aorta.

In Figure 28, increasing the concentrations of D-600 shifted the dose-response curve to H^+ to the right with a similar slope, while decreasing the maximum response. A significantly decreased maximum response is only achieved at a concentration of 10^{-3}M D-600. The parallel shift implies a competitive antagonism, however, decreasing

maximum responses with higher concentrations is atypical of competitive anatagonism (Goldstein et al., 1968).

This raised the possibility that D-600 may have a local anesthetic action on SHR VSM at the relatively high concentrations necessary to antagonize the H^+ response. Procaine, a local anesthetic, antagonized the response of rabbit aorta to noradrenaline at a concentration of 1mM (Hudgins and Weiss, 1968). D-600 has a local anesthetic-like structure: Figure 30 shows that D-600 bears a strong structural resemblance to another local anesthetic, lidocaine, with an amine bond between the aromatic group and the intermediate sidechain.

In an attempt to differentiate between the effects of a Ca^{2+} channel antagonist and the effects of a local anesthetic on the H^+ dose-response curve, the experiment recorded in Figure 29 was carried out. Procaine, at a concentration of 3mM significantly lowered the maximum response to H^+ in SHR aorta, as did the Ca^{2+} channel antagonist nifedipine (Bay 1040) at a concentration of 10^{-7}M. Thus, it was not possible to distinguish a local anesthetic-type inhibition from one that is clearly the action of a Ca^{2+} channel antagonist, working at a concentration 4 orders of magnitude lower. Therefore, the mechanism action of D-600 at a concentration of 10^{-3}M remains unresolved.

The nature of this dose-response must also be taken into consideration: H^+, as well as causing tension development, also has effects on the efficacy of antagonists by virtue of the changes in pH that occur on its addition to Krebs solution (as documented in Table V). Stepwise addition of HCl will gradually lower pH and consequently ionize

D-600, nifedipine and procaine, which are weak bases. Local anesthetics become less effective in inhibiting smooth muscle responses as the pH is lowered (Antonio et al., 1970). Data on the effectiveness of the two Ca^{2+} channel antagonists at low pH is not available. Thus, Figure 29 is difficult to interpret, since the antagonists might become more or less effective with each additional dose of H^{+}.

b) H^+ dose-response curves

Acidosis is usually associated with vasodilation (Folkow and Neil, 1971c; van Breemen et al., 1973). It has been suggested that raising the extracellular H^+ concentration results in protonation of the cell membrane, screening of cation binding sites and a consequent interference with Ca^{2+} binding at the cell membrane and influx of Ca^{2+} into the cell (D'Arrigo, 1974; Landau and Nachshen, 1975).

Lowering the H^+ concentration of the extracellular fluid has been shown to enhance calcium influx in ventricular muscle (Kohlhardt and Haap, 1976). Raising the H^+ concentration results in a negative chronotropic (Hughes and Coret, 1975), and a negative inotropic action (Lorkovic, 1966; Pannier and Brutsaert, 1968) in cardiac muscle. High H^+ concentration reduced neurotransmitter release (Landau and Nachshen, 1975) and decreased Ca^{2+} influx in rabbit aorta (van Breemen et al., 1973).

The surprising finding that increasing extracellular H^+ concentration caused a strong contraction in SHR aorta but not in Wistar (Figures 8 and 9) or WKY (Figure 17) aorta could prove to be a useful tool in determining the cause of the increased reactivity seen in this model of hypertension. It seems that the action of H^+ on the SHR aorta is partially dependant on extracellular Ca^{2+} since the H^+-induced contraction appears to be antagonized by D-600 (Figure 12) and by nifedipine (Figure 29). Also, the contractile response to H^+ is greatly diminished in a Ca^{2+}-free media (compare the responses in Figures 12 and 13). Since H^+ can displace Ca^{2+} from binding sites (see: Bass and Moore, 1966), these findings suggest that H^+ might

displace extracellular membrane-bound Ca^{2+} inward, through the membrane sites that leak Ca^{2+}, as previously discussed for the mode of action of La^{3+}.

If this inward displacement of extracellular Ca^{2+} alone were the mechanism of action of H^+, one would expect low-Na^+ solution or ouabain, manipulations that increase contractility, to have an additive effect on the H^+-induced contraction. Instead, the opposite occurs: Figure 27 shows that both low-Na^+ solution and ouabain treatment will antagonize the effect of H^+ in the SHR aorta.

A more plausible hypothesis is that H^+ also has an intracellular action. It could enter the SHR VSM cell via the same sites in the membrane through which Ca^{2+} appears to leak. Lea and Ashley (1978) reported that raising the internal H^+ concentration of barnacle muscle resulted in an increase in free intracellular Ca^{2+}, as measured by luminescence of aequorin, a Ca^{2+}-sensitive photoprotein. Since this does not occur in squid giant axon (Baker and Honerjager, 1978), these authors suggested that H^+ may act by displacing Ca^{2+} from an intracellular source which is absent in nerve axon. Increasing H^+ concentration has been shown to promote mitochondrial Ca^{2+} release (Addanki et al., 1978; Akerman, 1978; Schwartz, 1974). H^+ may act on the SHR VSM to increase free intracellular Ca^{2+} concentration through this mechanism.

Once inside the cell, H^+ probably alters enzyme activity as well. Murphy (1969) using dog carotid arteries found the optimal pH for arterial actomyosin ATPase activity was 5.2 (his Figure 2). Mrwa et al.

(1974) confirmed that the optimal ATPase activity of natural actomyosin in the same tissue occurred at pH 5.6. These values correspond well to the pH range at which peak tension development occurs in the SHR VSM in this study (Figure 8). This line of reasoning suggests that if H^+ is allowed free access to the intracellular space via Ca^{2+} leakage sites, VSM tension could develop just as a result of increasing the intracellular H^+ concentration.

It remains difficult to account for the apparent antagonism between H^+ and Ca^{2+}, assuming as explained in the preceeding discussion, that the effect of ouabain and low-Na^+ solution seen in Figure 27 are mediated through an increase in intracellular Ca^{2+}. Figure 27 seems to represent an intracellular antagonism between H^+ and Ca^{2+}. Fuchs et al. (1970) has shown that H^+ inhibits binding of Ca^{2+} to troponin. Perhaps Ca^{2+} and H^+ are competing for access to the contractile proteins in the experiment recorded in Figure 27.

The possibility that H^+ may act by denaturing the contractile or membrane proteins can not be ruled out, as the tissue loses responsiveness after a prolonged exposure to high concentration of H^+ (Table VI).

D. Conclusions

1. The mechanism of altered reactivity in SHR aorta

The altered responsiveness of SHR aorta appears to stem from an impaired ability of the tissue to regulate Ca^{2+} permeability. The data presented here with respect to the actions of La^{3+}, H^+ and K^+ on this tissue are consistant with this conclusion.

This conclusion is also consistant with data reported by other authors who have studied the reactivity of SHR VSM to Ca^{2+}: Noon et al. (1978) found that SHR aorta but not aorta from Wistar rats contracted when calcium was reintroduced after a 30 minute incubation period in calcium-free physiological solution. Field et al. (1972) found SHR aorta in Ca^{2+}-free solution containing 80mM K^+ showed an exaggerated maximum response when Ca^{2+} was added to the solution.

This conclusion is also compatible with the findings reported in Table IV that indicate relaxation of the SHR aorta to various vasodilators is impaired. Pederson et al. (1978) showed that relaxation of the SHR aorta is more complete than in Wistar rat aorta in response to nifedipine or a Ca^{2+}-free medium; suggesting that tension development in this tissue is much more dependant on variations in the extracellular Ca^{2+} concentration.

A. W. Jones (1974) reported that the rates of turnover of $^{42}K^+$, $^{36}Cl^-$ and $^{24}Na^+$ were higher in SHR aorta than in WKY or Wistar rat aorta. It is conceivable that in this tissue, Na^+ and Ca^{2+} compete for the leakage channels that have been attributed here to the SHR VSM membrane. In this case, the turnover of Na^+ would be higher due to leakage. The

Na^+-K^+ ATPase pump would have to be more active to maintain a stable membrane potential by removal of accumulating intracellular Na^+. K^+ turnover would therefore be higher as would Cl^- turnover if this anion passively follows the cations. This hypothesis is supported by several reports of increased Na^+-K^+ ATPase activity in SHR VSM as determined by the rate of K^+-induced relaxation in isolated tail artery strips (Webb and Bohr, 1979), the rate of liberation of inorganic phosphate by subcellular fractions from mesenteric arteries (Wei et al., 1976a), temperature dependant differences in membrane potential measurements in isolated tail artery (Hermsmeyer, 1976) and by measurement of Na^+ and K^+ in isolated tail artery VSM cells using ion-specific electrodes (Friedman and Friedman, 1976).

2. The relationship between hypertension and vascular reactivity

As outlined in the first chapter there are 2 opposing hypotheses to account for differences in vascular reactivity observed in hypertension. One proposes that these differences are the consequence of VSM hypertrophy developing secondary to the onset of hypertension. The other suggests that the changes in vascular reactivity observed in hypertension reflect true differences in sensitivity and that increased vascular reactivity may be a causative factor in hypertension.

The data presented here shows that differences in reactivity are evident in SHR VSM that has never been subjected to high blood pressure and consequently, it must be concluded that altered reactivity preceeds the onset of hypertension and therefore is not a consequence of hypertrophy.

This conclusion is supported by the observations of others who have found altered reactivity or differences in ion permeabilities in non-vascular tissue from the SHR: Corbett et al. (1980) have demonstrated that vas deferens from the SHR shows significantly higher reactivity to K^+, Ca^{2+}, La^{3+} and H^+. Caulfield et al. (1977) reported increased responsiveness to noradrenaline in SHR vas deferens. Altman, Da Ponte and Worcel (1977) found strips of smooth muscle from the fundus of the SHR stomach showed enhanced reactivity to Ca^{2+}. These studies lend support to the conclusion that altered reactivity in the SHR aorta is not a consequence of hypertension and hypertrophy. Furthermore, they imply that the membrane defect responsible for the apparent leakage of Ca^{2+} may not be limited to <u>vascular</u> smooth muscle.

Limas and Cohn (1977) reported a defect in Ca^{2+} transport in cardiac muscle from the SHR; implying that the impaired control of Ca^{2+} permeability may not be limited to smooth muscle. Other evidence suggests a similar defect may be present in red blood cells from SHR: Using ion-specific electrodes (Friedman et al., 1977) and radioactive isotopes (Postnov et al., 1976) it has been shown that SHR erythrocytes are more permeable to Na^{+} and K^{+} than are erythrocytes from Sprague-Dawley and Wistar rats (in the latter study) or from WKY and Woodlyn rats (in the former study). These data imply the SHR may have a generalized membrane defect.

It should be stressed that the causal link between increased ion permeability and hypertension in the SHR remains to be established. However, increased permeability to Ca^{2+} and perhaps to other ions such as Na^{+} in SHR VSM might result in higher free intracellular Ca^{2+} levels by direct inward diffusion of Ca^{2+} or by impairment of an extracellular Na^{+}-dependant Ca^{2+} removal system. This in turn would result in a higher resting tension or a reduced capacity for relaxation. If this process is not limited to aorta, but also occurs in the resistance vessels of SHR, blood pressure might rise slowly as high peripheral resistance results in a gradual resetting of baroreceptors (see: Sapru and Wang, 1976) and eventually to VSM hypertrophy, further increasing peripheral resistance and the blood pressure in a positive feedback manner.

In support of this hypothesis of the etiology of hypertension in the SHR is the study of Lais et al. (1977) that shows that as early as 3 weeks of age blood pressures recorded from the femoral artery of preweanling SHR were higher than those recorded from WKY rats.

To lend more support to this hypothesis, it would be interesting to treat these animals from conception with nifedipine and determine whether or nor reactivity differences are present. If nifedipine can antagonize Ca^{2+} leakage, perhaps both hypertension and reactivity alterations can be prevented in the SHR. Another approach to testing this hypothesis might be through backcrossing SHR to WKY (see: Judy et al., 1979). Assuming more than one gene is involved in producing this membrane defect, it may be possible to produce offspring with a degree of increased reactivity that corresponds with the severity of their blood pressure elevation.

3. Implications of this research for human hypertension

The SHR is probably the best animal model of human essential hypertension. The implications of the findings of studies of the SHR for the treatment and prevention of essential hypertension will depend on just how well human hypertension is modelled by the SHR. However, there is some evidence that a membrane defect of the type described here may be present in human essential hypertension: Postnov et al. (1977) reported that red blood cells from hypertensive patients show higher passive permeability to Na^+. Garay and Meyer (1979) found erythrocytes from patients with essential hypertension showed a higher K^+ influx and a lower Na^+ efflux. This might be interpreted as evidence of higher Na^+-K^+ pump activity. Interestingly, these authors found ion fluxes were normal in erythrocytes from renal hypertensive patients but were altered in erythrocytes from normotensive offspring of parents with essential hypertension; suggesting that a membrane defect is inherited.

Should studies of this nature verify the existance of an inherited membrane defect in hypertensive humans, this line of research may prove to be a useful diagnostic tool. Prehypertensives could be identified on the basis of the ion flux properties of their red blood cells. Once diagnosed, prophylatic proceedures may be initiated. But to date, only preliminary evidence of a membrane defect in human hypertension exists. It must be proven and characterized before treatment based on this hypothesis is initiated. Hopefully, the findings of this thesis may prove to be applicable to the diagnosis and prevention of human essential hypertension.

V. SUMMARY

1. Reactivity of SHR aorta to La^{3+} was found to be primarily due to a drop in pH that occurred on the addition of La^{3+} to bicarbonate-buffered Krebs solution. La^{3+} had some direct action when a HEPES-buffered solution was used to keep the pH stable, but the magnitude of the La^{3+}-induced contraction was greatly diminished.

2. H^+ induced a contraction in SHR aorta from WKY or Wistar rats. The action of H^+ appears to have both extracellular and intracellular components.

3. Ca^{2+} in a non-depolarizing media induced a contraction in SHR VSM. It was postulated that there is a flaw in the control of Ca^{2+} permeability in the SHR VSM, such that this ion leaks across the cell membrane. This apparent membrane defect was proposed as the underlying mechanism for the reactivity to La^{3+}, H^+, and for the increased sensitivity seen to K^+.

4. The reactivity alterations were present in SHR that had never been hypertensive, having been treated from conception with a β-adrenergic blocking agent, timolol. It was concluded that alterations in vascular reactivity in the SHR aorta preceed the onset of high blood pressure, and therefore, may be a causative factor rather than a consequence of hypertension.

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"90% of what I know is what I read in the papers"
Will Rogers

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APPENDIX A

COMPOSITION OF PHYSIOLOGICAL SOLUTIONS

Solutions*	Krebs,† mM	Low Bicarbonate Krebs ‡ mM	HEPES-Krebs § mM	Low-Na+ Krebs mM
NaCl	118	154	107	
KCl	4.7	5.4	4.7	4.7
$NaHCO_3$	12.5	6.0		12.5
$CaCl_2 2H_2O$	2.5	2.5	2.5	2.5
KH_2PO_4	1.2			1.2
$MgSO_4$	1.2			1.2
Dextrose	11.1	11.0	11.1	11.1
$MgCl_2$			1.2	
HEPES			5.8	
Choline Cl				118
O_2 Source	O_2, 95% CO_2, 5%	O_2, 95% CO_2, 5%	Air	O_2 95% CO_2 5%

* Prepared in double-distilled or Milli-Q filtered H_2O warmed to 37°C and adjusted to pH 7.2–7.4

† Adapted from Janis and Triggle (1973)

‡ Adapted from Hansen and Bohr (1975)

§ Adapted from Mayer et al. (1972)

Grind up cabbages or grind up kings, the
chemicals found are the very same things

Anonymous

APPENDIX B

List of Drugs and Chemicals and Suppliers

Calcium chloride dihydrate ($CaCl_2$-$2H_2O$) J. T. Baker, Phillipsburg, N.J.

Choline chloride, B.D.H. Chemicals Ltd., Poole, England

D-600, Knoll AG, West Germany

Dextrose (anhydrous) Natheson, Coleman and Bell, Norwood, Ohio

HEPES (N-2-hydroxyethylpiperazine-N'-2'ethane sulfonic acid)
Calbiochem, San Diego, C.A.

Hydrochloric acid (HCl) J. T. Baker, Phillipsburg, N.J.

Lanthanum chloride ($LaCl_3.7H_2O$), B.D.H. Chemicals Ltd., Poole, England

Lanthanun nitrate ($La(NO_3)_3.6H_2O$), B.D.H. Chemicals Ltd., Poole, England

Magnesium chloride ($MgCl_2.6H_2O$), J. T. Baker, Phillipsburg, N.J.

Magnesium sulphate ($MgSO_4.7H_2O$), J. T. Baker, Phillipsburg, N.J.

Nifedipine (Bay 1040) Bayer AG, West Germany

Nitric acid (HNO_3), J. T. Baker, Phillipsburg, N.J.

Noradrenaline (L-Arterenol HCl), Sigma Chemicals, St. Louis, Mo.

Ouabain octahydrate (Strophanthin-G), Sigma Chemicals, St. Louis, Mo.

Potassium chloride (KCl), J. T. Baker, Phillipsburg, N.J.

Potassium phosphate, monobasic (KH_2PO_4), J. T. Baker, Phillipsburg, N.J.

Procaine hydrochloride, Sigma Chemicals, St. Louis, Mo.

Propranolol (DL-Propranolol HCl) Sigma Chemicals, St. Louis, Mo.

Sodium bicarbonate ($NaHCO_3$), J. T. Baker, Phillipsburg, N.J.

Sodium chloride (NaCl), J. T. Baker, Phillipsburg, N.J.

Sodium hydroxide (NaOH), Zigher Scientific, Fort Lawn, M.J.

Sodium pentobarbital, M.T.C. Pharmaceuticals, Hamilton, Ontario

Sulphuric acid (H_2SO_4), J. T. Baker, Phillipsburg, N.J.

Timolol maleate, Merck-Frosst, Dorval, P.Q.