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REDUCED VASORELAXANT RESPONSE TO VASOACTIVE INTESTINAL PEPTIDE IN EXPERIMENTAL HYPERTENSION

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Reduced Vasorelaxant Response to Vasoactive Intestinal Peptide in Experimental Hypertension" submitted by Janet Ann Petrillo in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

The responsiveness of several blood vessels to various vasoconstrictors and vasodilators was studied *in vitro* , in 12-14 week old Wistar rat, Wistar Kyoto (WKY) rat, and Spontaneously Hypertensive Rat (SHR). Preliminary studies were performed to determine the most suitable agent for use as an agonist, the most appropriate vessels to utilize, and to observe dose-response curves for vasoactive intestinal peptide (VIP) and forskolin. Blood pressures of the animals were monitored prior to tissue utilization. Tension changes in the vessels were determined isometrically using a force-displacement transducer.

The responsiveness to VIP and forskolin was studied in the thoracic aorta and superior mesenteric artery. Vessels were precontracted with norepinephrine before the addition of VIP or forskolin. The relaxant response to papaverine was utilized to determine the percent relaxation of VIP and forskolin. The vasorelaxant response to VIP was significantly lower in the superior mesenteric artery of the SHR as compared to Wistar and WKY animals. In thoracic aorta, VIP induced similar responses in the normotensive and hypertensive animals. Studies examining the relaxant response to forskolin did not permit a definitive conclusion regarding the significance of forskolin relaxation in the SHR. It is possible that the reduction of VIP vasorelaxation in the

superior mesenteric artery may contribute to increased peripheral resistance in experimental hypertension.

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I would like to express my appreciation of the support, understanding, and encouragement which my parents have provided throughout all my endeavors.

I am especially grateful to my husband Joe, for his constant support, encouragement, and his continued belief in my abilities.

DEDICATION

I would like to dedicate this thesis to my husband Joe, for his love and constant support, and to my parents for their help and encouragement always.

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LIST OF ABBREVIATIONS

AVP	Arginine Vasopressin
cAMP	Cyclic Adenosine Monophosphate
G_i	Inhibitory Guanosine 5'triphosphate-Binding Protein
G_s	Stimulatory Guanosine 5'triphosphate-Binding Protein
GTP	Guanosine 5'triphosphate
NPY	Neuropeptide Y
PHI	Peptide with N-terminal Histidine and C-terminal Isoleucine Amide
SHR	Spontaneously Hypertensive Rat
SHRSP	Stroke-Prone Spontaneously Hypertensive Rat
VIP	Vasoactive Intestinal Peptide
WKY	Wistar-Kyoto

I INTRODUCTION

A.Hypertension

The vasculature has three levels of regulation. The most rapid responses involve the nervous system, while hormonal responses are delayed somewhat. Lastly, long term changes may involve adaptations of structure, composition, and behaviour of tissues, organs, and/or systems (1).

The pressure necessary to move blood throughout the circulation (mean arterial pressure) is a function of the heart's pumping action (cardiac output) and the tone of the arteries (peripheral resistance). It is believed that a hypertensive state is initiated by an elevation of cardiac output. Subsequently, peripheral resistance may increase due to narrowing of arterioles, and this state of heightened peripheral resistance is maintained by permanent damage (vascular hypertrophy) to arteries and arterioles. In established hypertension, one typically finds elevated peripheral resistance and normal cardiac output (1,2).

The initial increase in cardiac output which precedes the sustained rise in blood pressure may be due to a variety of mechanisms. While heredity plays a role in the level of blood pressure, environmental factors such as stress, obesity, and sodium also contribute significantly. Stressors may cause the sympathetic nervous system to be overactive. A persistant stress may thus invoke an increased secretion of

catecholamines, such as the mobilization of epinephrine release from the adrenal medulla and norepinephrine release from adrenergic nerve endings. Both epinephrine and norepinephrine are agonists for α_1, α_2, and β_1 receptors, however, epinephrine has much greater potency on β_2 receptors. All β-adrenergic receptors stimulate adenylate cyclase via stimulation of a G protein (guanosine 5'triphosphate-binding protein). The α_2-adrenergic receptors inhibit adenylate cyclase by interacting with a different G protein, while stimulation of α_1-adrenergic receptors increase intracellular concentrations of Ca^{2+} by phospholipase C activation. The α- and β_1-adrenergic receptors are located close to adrenergic nerve terminals in peripheral target organs. The α_2 - and- β_2 adrenergic receptors are located in postjunctional regions that are remote from areas of norepinephrine release. Vascular smooth muscle possesses β_2 receptors (mediating relaxation) and α_1 and α_2 receptors (mediating contraction), while β_1 receptors are found primarily in the heart (increasing force and rate of the heart's contraction) (3).

The mechanism by which hypertension develops in the obese individual likely involves an increase in blood volume, stroke volume, and cardiac output. Additionally, insulin levels are increased in the obese, which may also play a significant role in hypertension. Conversely, individuals who are physically fit may develop less hypertension, and individuals with

hypertension may decrease blood pressure levels with exercise (2).

Excess sodium present in the circulation and/or within cells may be a significant factor influencing the development of hypertension. Excess dietary sodium may be a factor contributing towards the pathogenesis of hypertension in individuals with an inherited defect in renal sodium excretion. Reduction of the filtration surface and ischemic nephrons contribute towards this condition. These individuals with sodium sensitivity have increased levels of plasma norepinephrine and an increase in cardiac output while on a high sodium diet (2).

Furthermore, abnormalities in sodium ion transport across membranes, causing an increase in the intracellular sodium concentration, may induce hypertension by increasing calcium concentrations in the vascular smooth muscle cells. A decrease in activity of the sodium pump and an increase in the permeability of cell membranes to sodium contribute to the elevation of intracellular sodium. The inhibition of the sodium pump may increase intracellular sodium resulting in inhibition of the sodium-calcium exchange, thereby, raising intracellular calcium levels by reducing calcium efflux. The sodium-calcium exchange, which is important for extrusion of calcium after contractile activity, is inhibited by as little as a 5% increase in intracellular sodium, resulting in an increase in intracellular calcium causing up to a 50%

increase in vascular smooth muscle resting tone. Thus, the increased concentrations of free calcium in the cytosol of the vascular smooth muscle cells may be responsible for the increased contractility of vessels, resulting in increased peripheral resistance in hypertension (1,2).

Another mechanism which may contribute to increased cardiac output is the redistribution of blood to the central or cardiopulmonary component because of increased peripheral vasoconstriction. Additionally, it was found that hypertensives had a relatively expanded blood volume when blood pressure was compared to total blood volume - that is, the plasma volume was inappropriately high for the level of blood pressure (2).

There are several hormones which are involved in cardiovascular regulation. Arginine vasopressin's (AVP) effects are mediated via at least two types of vasopressinergic receptors - the v_1 receptors in the vascular smooth muscle and liver cells, and v_2 receptors in the renal tubule. Stimulation of the v_1 receptors produces smooth muscle contraction, while stimulation of v_2 receptors produces an antidiuretic effect - the reabsorption of free water. There are also AVP receptors in the central nervous system which are distinct from the above mentioned receptors. The major role of AVP as a hypertensive factor is likely that of a neuromodulator acting through central nervous system receptors on vasopressinergic and catecholaminergic neurons

to affect baroreflex sensitivity and sympathetic outflow (4).

The renin-angiotensin-aldosterone system also contributes to cardiovascular regulation. Firstly, renin is a major factor in the control of blood pressure. Renin is stored and secreted from the renal juxtaglomerular cells and acts upon angiotensinogen to form angiotensin I. In the presence of converting enzyme, angiotensin I is cleaved to form angiotensin II. Major regulators of renin release include the following: renal sympathetic nervous system stimulation, decreasing pressure in the afferent arterioles, decreasing sodium concentration in the macula densa, and angiotensin II's short-loop feedback suppression. Additionally, the production of extrarenal renin in various peripheral tissues leads to angiotensin production locally (2).

Most of the actions of angiotensin II result in an elevation of blood pressure. Angiotensin II directly contracts vascular smooth muscle as well as indirectly causing vasoconstriction by stimulating the central nervous system vasomotor center and increasing catecholamine secretion. Angiotensin II also causes volume expansion by stimulating the central nervous system thirst center and stimulating aldosterone secretion (2).

Endothelial factors may also play a role in regulating vascular tone. Endothelium - derived relaxing factor (nitric oxide) relaxes vessels, while endothelial - derived vasoconstrictor (endothelin) is a potent vasoconstrictor which

stimulates vascular smooth muscle cell proliferation, aldosterone secretion, and inhibits renin release. The endothelium also liberates adenosine and prostacyclin which vasorelax but are distinct from endothelium - derived relaxing factor (2).

Neuropeptides, which are likely neurotransmitters in the central and peripheral nervous systems, form the peptidergic nervous system which contributes to normal vascular regulation. The regulatory influence by neuropeptides may be a direct action on the heart or blood vessels, or by interacting with systems such as the renin-angiotensin-system and adrenergic or cholinergic nervous systems (5).

B. Spontaneously Hypertensive Rat and the Wistar Rat

The origin of the Wistar rat dates back as far as 1900, when albino rats of European origin were brought to the U.S.A. by Dr. H.H. Donaldson, and where he then established a colony at the University of Chicago. In 1906-1907, Donaldson's colony was transferred to the Wistar Institute of Anatomy and Biology in Philadelphia, where he began to standardize the albino rat (Rattus norvegicus albinus). The original intent was to develop reliable strains to study the growth and development of the nervous system, however, his work also produced a broad foundation for the rat's use in many fields of study (6).

Professor Okamoto and Kyuzo Aoki in 1963, developed a hypertension model from Wistar rats. The spontaneously hypertensive rats (SHR) were selected and established as an inbred strain in 1969 at the Department of Pathology, Faculty of Medicine, Kyoto University. The SHR were established by selectively inbreeding Wistar-Kyoto (WKY) rats, illustrating the significance of heredity in the aetiology of hypertension (7).

Initially a male Wistar rat whose systolic blood pressure ranged from 145-175 mm Hg was mated with a female rat having blood pressures of 130-140 mm Hg. Offspring which exhibited hypertension over 1 month were utilized for brother-sister matings. Blood pressures of subsequent generations progressed until systolic blood pressures greater than 180 mm Hg were obtained (8).

The use of the SHR as an experimental model for research in hypertension is widespread. Similarities between SHR and the patient with essential hypertension include the following: no specific aetiology, sodium sensitivity, multivariable control of blood pressure, genetic predisposition, sodium transport alterations, shortened lifespan, neural mechanisms (such as reduced sensitivity of the SHR baroflex with age and altered central catecholaminergic mechanisms), increased total peripheral resistance, arterial and venous constriction, hypertrophy of vascular smooth muscle and myocardium, lack of volume expansion, and similar responses to treatment (9). On

the other hand, there are also dissimilarities between the SHR and the hypertensive patient which may impose a few limitations on the model, and have led to some degree of controversy as to whether the SHR is a suitable model. The SHR does not become obese, has no enhanced atherogenesis, and does not have a potent prostaglandin system (9). However, because of the numerous similarities, the SHR appears to be the best model available for studying essential hypertension.

Many physiological differences exist between the SHR and the normotensive WKY. Such differences may lead to a more complete understanding of the clinical disorder. The major disturbances observed in the SHR are neurogenic and cellular ionic transport abnormalities (10,11). Hypertension (>150 mm Hg, systolic pressure) develops in the SHR by the seventh week of life (12,13), and the average systolic blood pressure ($\pm$SD) at ten weeks of age is 184 $\pm$ 17 mm Hg in males and 178 $\pm$ 14 mm Hg in females (8). Wistar rats, on the other hand, average 120-140 mm Hg as measured by tail plethysmography (8).

The autosomal additive inheritance of at least three to five major genes (8,14,15) appears to account for the genetic factor contributing towards the disease. This abnormality enhances sodium ion permeability across erythrocyte membranes, thereby, concentrating the ion in erythrocytes and vascular walls (16). As a result, sodium reabsorption is inhibited due to the inhibition of Na^+-K^+-ATPase in the renal tubules, vascular tissues,and erythrocytes. Thus, membrane potential

of excitable tissue such as vascular smooth-muscle cells is affected so that sensitivity to neuronal norepinephrine or humoral pressor agents increases, resulting in increased peripheral vascular resistance structurally (16). Also, sodium-calcium exchange is enhanced by the increase of intracellular sodium ions resulting in an increase of intracellular calcium ions which contract smooth-muscle cells and increase peripheral vascular resistance functionally (16). In short, hypertension in SHR may be brought about by genetic alterations of biomembrane permeability and transport mechanisms, and later maintained by structural and functional changes leading to increased peripheral vascular resistance.

It has been reported that the dense distribution of the adrenergic nerves in the SHR peripheral arterial walls may lead towards the development and maintenance of hypertension (17). Additionally, the neuropeptide Y-containing (NPY) nerve fibers are increased in the distal regions of mesenteric arteries of the SHR (17), and the large and small mesenteric arteries of the stroke- prone strain of SHR (SHRSP) (18). Thus, it has been suggested that the NPY-containing nerves as well as the adrenergic nerves could play a role in the development and maintenance of hypertension (18).

C.Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is a highly basic 28

amino acid peptide which was first isolated from porcine small intestine (Figure 1) (19-21). The structure of VIP is closely related to secretin, pancreatic glucagon, gastric inhibitory polypeptide, growth hormone-releasing factor, PHI (peptide with N-terminal histidine and C-terminal isoleucine amide), PHM (peptide with N-terminal histidine and C-terminal methionine amide), corticotropin-releasing factor, sauvagine, urotensin I, helodermin, and pituitary adenylate cyclase-activating polypeptide (PACAP) (22-30). Human VIP is cosynthesized with PHM from the same large precursor molecule (31). The amino acid sequence of VIP in pig, cow, rat, human, dog and goat are identical, but slight differences are found in the guinea pig, chicken, dogfish, and cod (25,31-38).

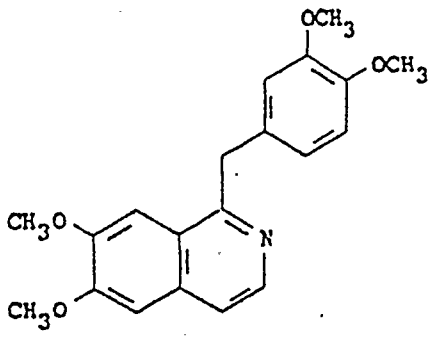
VIP has a broad range of biological actions: it stimulates hormonal secretions, inhibits smooth muscle activity, and is a potent vasodilator (22). The potent dilator action of VIP is observed in many arteries (39-41). The result of VIP's widespread vasodilator activity at pharmacological doses is systemic hypotension (42). VIP is localized in the brain, the spinal cord, sympathetic ganglia, adrenal gland, and peripheral neurons innervating most organ systems (43). The entire amino acid sequence of VIP is required for complete bioactivity (44).

FIGURE 1

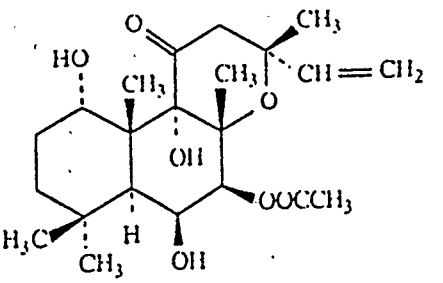
A. Amino Acid Sequence of Porcine VIP and Porcine PHI.

	1	2	3	4	5	6	7	8	9		
VIP	His	Ser	Asp	Ala	Val	Phe	Thr	Asp	Asn		
PHI	His	Ala	Asp	Gly	Val	Phe	Thr	Ser	Asp		
	10	11	12	13	14	15	16	17	18		
VIP	Tyr	Thr	Arg	Leu	Arg	Lys	Gln	Met	Ala		
PHI	Phe	Ser	Arg	Leu	Leu	Gly	Gln	Leu	Ser		
	19	20	21	22	23	24	25	26	27	28	
VIP	Val	Lys	Lys	Tyr	Leu	Asn	Ser	Ile	Leu	Asn	NH_2
PHI	Ala	Lys	Lys	Tyr	Leu	Glu	Ser	Leu	Ile	NH_2	

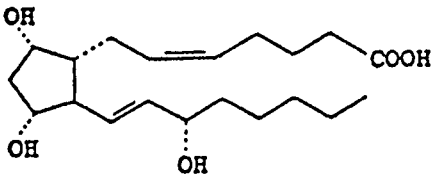
B. Structures of Non-Peptide Agents.



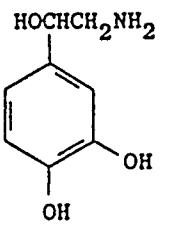
Papaverine



Forskolin



Prostaglandin $F_{2\alpha}$



Norepinephrine

The vascular effects of VIP are independent of adrenergic or cholinergic receptors, and with the exception of vasodilation of the pial vessels (45) are independent of prostaglandin release. The effects of VIP result from direct interaction with its own specific membrane-bound receptor, resulting in stimulation of adenylate cyclase and formation of cyclic adenosine monophosphate (cAMP) (39,46,47). There are two functional states existing for VIP receptors, one having a higher affinity than the other. Most often these receptors show high specificity for VIP, however, interaction also exists with VIP-related peptides (48). Furthermore, Morris and Murphy (1989) using VIP analogues found two distinct VIP receptors in guinea-pig uterine artery, one activated by guinea-pig VIP (1-28) which mediates relaxation, and the other activated by guinea-pig VIP (1-28) and porcine VIP (10-28) which mediates vasoconstriction (49). The vasodilation produced by guinea-pig VIP(1-28) predominates over the vasoconstriction (49).

The binding of VIP to its receptor requires a protein molecule, phospholipids, and disulfide bonds (50-52). Specific VIP-binding sites are localized in plasma membranes from blood vessels and many other tissues and organs (53-55). Immunoreactive VIP is localized in neuronal cell bodies, axons, dendrites, and in presynaptic nerve terminals, where it can be released as a neurotransmitter (56-58).

The distribution of VIP fibres is widespread. VIP nerves

are most dense in arteries of regional vascular beds including mesenteric arteries, uterine arteries, cerebral arteries, and erectile vessels (Figure 2) (59). Within the superior mesenteric artery, it was found that the proximal portion (conductance vessel) had greater VIP immunoreactivity, than the distal portion (resistance vessel); accordingly, the proximal segment was found to be much more responsive to VIP than the distal segment (60). VIP nerves are less prominent in the heart and around the aortic arch, subclavian artery, iliac artery, skeletal muscle arteries, and most veins (59).

D. Norepinephrine

Norepinephrine is a neurotransmitter synthesized from tyrosine at sympathetic neuroeffector junctions (Figure 1). Norepinephrine can activate β_1-adrenergic or α-adrenergic receptors. The adrenergic receptors are integral membrane glycoproteins with seven transmembrane spanning domains. When norepinephrine binds to the β_1-receptor, the receptor then interacts with a stimulatory GTP-binding protein G_s which stimulates cAMP synthesis by adenylate cyclase, activates cAMP-dependent protein kinase, and phosphorylates many cellular proteins (Figure 3). When norepinephrine binds to the α_2-adrenergic receptor, the receptor interacts with G_i, an inhibitory guanine nucleotide- binding protein which inhibits

FIGURE 2. VIP - like immunoreactivity in nerves associated with the cardiovascular system of the guinea-pig

Symbols refer to VIP-like immunoreactivity which is 3+, very dense; 2+, moderately dense; 1+, sparse; and 0, fibres absent or very rare.

Furness J, Costa M, Papka R, Della N & Murphy R (1984). Clin Exp Hypertens [A] A6: p. 94.

VASOACTIVE INTESTINAL PEPTIDE

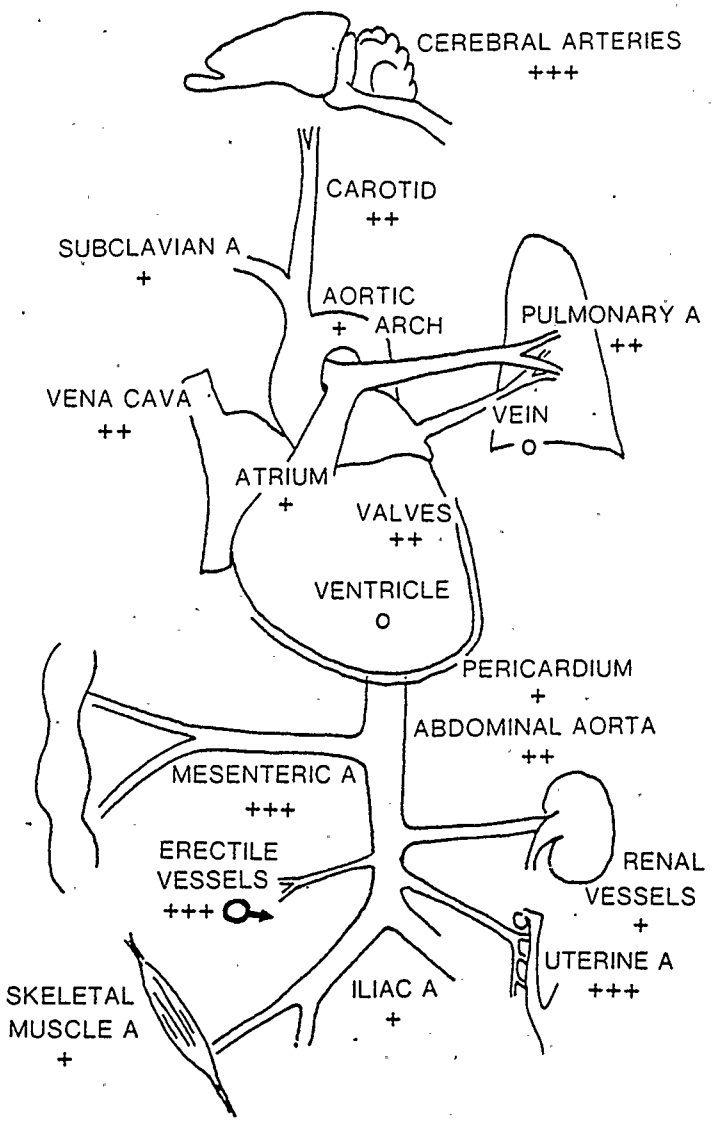
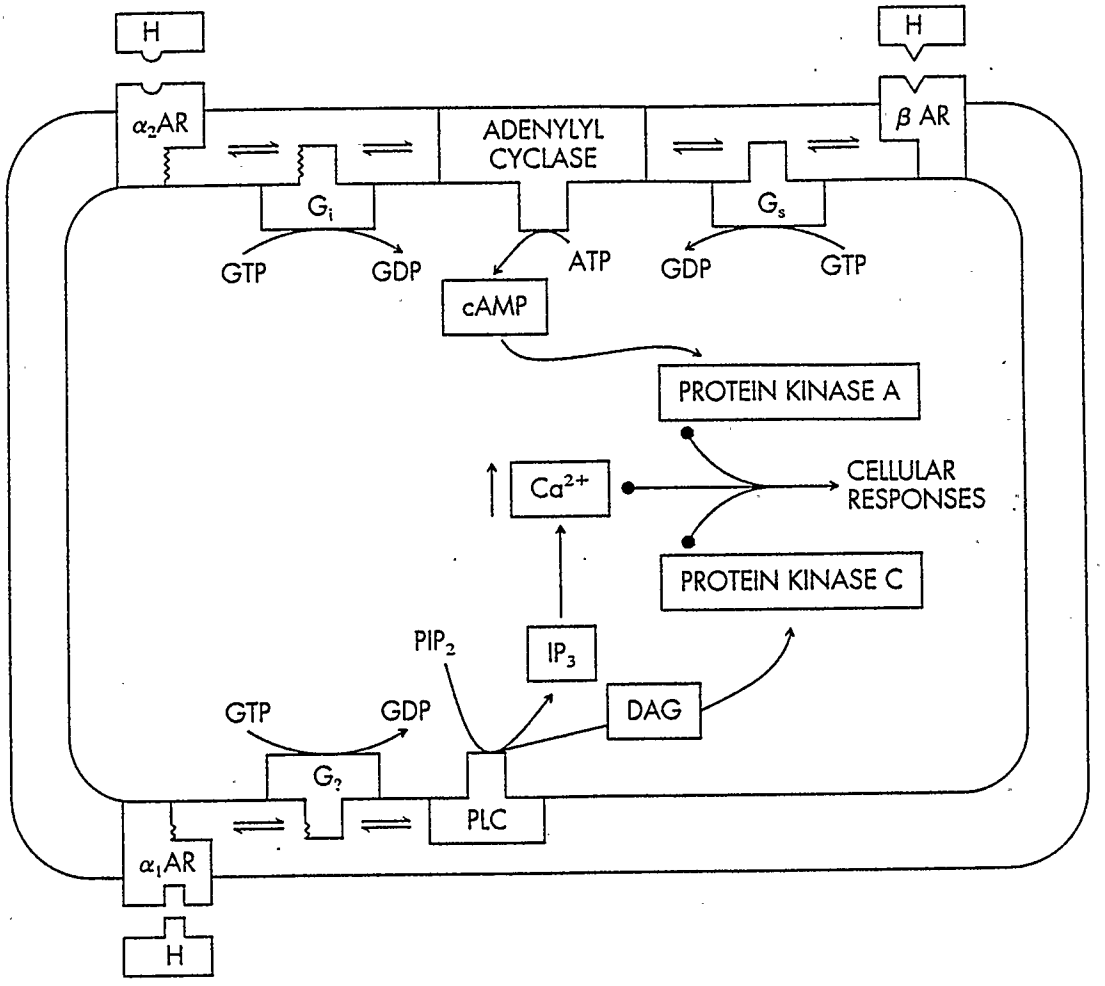


FIGURE 3. Adrenergic Transmission

The definitions of abbreviations are as follows: H, hormone; AR, adrenergic receptors; G_s, stimulatory guanine nucleotide-binding protein; G_i, inhibitory guanine nucleotide-binding protein; GTP, guanosine triphosphate; GDP, guanosine diphosphate; IP_3, inositol-1,4,5-triphosphate; PIP_2, phosphatidylinositol-4,5-biphosphate; DAG, diacylglycerol; $G_?$, unidentified G protein; and PLC, phospholipase C.

Gilman AG, Rall TW, Nies AS & Taylor P (1990). Goodman and Gilman's The Pharmacological Basis of Therapeutics Pergamon Press, Elmsford, New York, USA. p. 109.

ADRENERGIC TRANSMISSION



adenylate cyclase so that intracellular concentrations of cAMP are lowered. Norepinephrine can also interact with the α_1-adrenergic receptor which increase the concentration of intracellular Ca^{2+} by activating phospholipase C, mediated by an unidentified G protein. The cardiovascular effects of norepinephrine given intravenously include increased systolic and diastolic pressure, increased total peripheral resistance in most vascular beds, and slowing of the heart resulting in increased stroke volume (3).

E. Forskolin

The diterpene forskolin (Figure 1) activates adenylate cyclase not by interacting with the guanine nucleotide regulatory subunit, but rather by interacting with the catalytic subunit of adenylate cyclase (61). There have been several studies examining the effects of forskolin in the hypertensive rat. It appears that not only may there be a reduction in basal adenylate cyclase activity in the SHR, but also, in the myocardial sarcolemma and aorta of the SHR forskolin stimulation of adenylate cyclase , guanine nucleotide regulatory protein, and the catalytic subunit of the cyclase system is diminished (62). Also, it has been observed that forskolin relaxation is diminished in the SHR (63). On the other hand, a few studies have also found no significant difference between forskolin relaxation in SHR and

WKY rats in mesenteric and femoral arteries (64,65), as well as renal and carotid arteries (66).

F. Papaverine

Papaverine, one of the alkaloids of opium, is a nonspecific smooth muscle relaxant, relaxing all types of smooth muscle in vitro (Figure 1) (67). The term nonspecific implies papaverine likely does not interact with a specific type of membrane receptor (68). Papaverine, an inhibitor of cyclic nucleotide phosphodiesterase in many tissues, is involved in the breakdown of cAMP (69). As a result, the concentration of cAMP can increase (67). It has been suggested that the mechanism of action of papaverine may be through its ability to inhibit phosphodiesterase, resulting in a reduction of free myoplasmic Ca^{2+}, a variable to trigger contraction (68). The increase in cAMP stimulates a Ca^{2+} binding process in smooth muscle. Papaverine also stimulates some dopamine receptors which may contribute to the smooth muscle relaxant effect (68). Papaverine relaxes smooth muscle of larger blood vessels and decreases peripheral resistance primarily by dilating arterioles (68).

G. Morphology of Normal Rat Aorta and Mesenteric Artery

Vascular smooth muscle has a wide spectrum of

ultrastructural and physiological properties depending on the part of the vascular bed that is being considered. All mammalian vessels share a basic three-layered structural pattern - tunica intima, tunica media, and tunica adventitia separated by two elastic laminae. The variation of ultrastructural and physiological properties observed in different anatomical regions and in different vascular beds depends upon the arrangement of these layers. In "elastic" blood vessels such as the aorta and pulmonary artery where the wall tension is high, the circularly oriented smooth muscle cells are arranged in lamellae separated from each other by elastic tissue and the smooth muscle cells are mainly limited to the elastic laminae. Unlike the circumferentially oriented cells in elastic arteries, muscular arteries tend to have cells that are arranged helically or spirally (70).

When muscular arteries (tail artery), musculoelastic arteries (femoral artery), elastic arteries (superior mesenteric artery), and large veins (portal vein) were compared in terms of smooth muscle cell length, smooth muscle cells from the superior mesenteric artery were significantly shorter than those from the tail and femoral arteries. On the other hand, when cell size was measured in terms of cell volume, tail artery cells were 2-3 times larger than cells from the femoral and mesenteric arteries and 6 times larger than those from the portal vein. The extracellular material in the smooth muscle cells of muscular arteries is relatively

sparse, however, amounts increase to a maximum in elastic arteries. Conversely, the wall-to-lumen proportions decrease from muscular to elastic arteries (71).

1. Structure of the rat mesenteric arteries

a) Tunica Intima

The tunica intima consists of a single layer of endothelial cells lying on a basement membrane. Functions of the endothelium include the following: maintaining blood in a fluid state, allowing exchange of materials in capillaries between blood and tissue, secretions of plasma proteins, antigen-antibody reactions, and defence reactions to tissue damage and infection. Most endothelial cells have small foot processes which project into the lumen of vessels. These processes have been observed in rat mesenteric arteries and at endothelial cell junctions in the rat aorta. It has been suggested that they may be involved in a gross type of pinocytosis (70).

The basement membrane lies close to the internal elastic lamina. This internal elastic lamina separates the tunica intima from the media. Beyond the basement membrane and between the internal elastic lamina collagen is usually present. Because the internal elastic lamina is impermeable, the movement of material occurs only through pores. In rat mesenteric arteries, projections of the endothelium penetrate the pores and contact the smooth muscle cells of the media

(70).

In the rat mesenteric arteries, endothelial cell junctions are edge-to-edge, however, interdigitating membranes may also be present. Endothelial cells in the rat mesenteric artery contain the usual cell organelles. Amounts of endoplasmic reticulum and Golgi apparatus are relatively small, suggesting metabolism in these cells is low. Lastly, dense bodies whose composition and function is unknown, have been observed in rat mesenteric arterial and rat aortic endothelial cells.

b) Tunica Media

The tunica media is the thickest compartment of the wall of mesenteric arteries. The number of muscle cells in the media increases with the size of the vessel. Medial muscle cells contain many more mitochondria and rough-surface endoplasmic reticulum than in endothelial cells, suggesting that in the smooth muscle the metabolism is very high (70).

The myofilaments of arterial smooth muscle are actomyosin. The filaments are thought to enter dense bodies. In the rat mesenteric arteries, these dense bodies are usually oriented in the direction of the myofilaments. Large arteries may be separated by a thin elastic lamina, while in smaller arteries such as the mesenteric arteries, thin elastic laminae are often found between smooth muscle cells. Amounts of elastic tissue is greatest in vessels which are exposed to the most variation in circumference during each pulsation (70).

c) Tunica Adventitia

The adventitia acts as a supportive framework for the arterial wall. The adventitia is separated from the media by an external elastic lamina, which is much thinner than the internal elastic lamina. As the size of the artery decreases, so does the external elastic lamina. The tunica adventitia adjoins loose connective tissue which surrounds the vessel. Adventitia is composed of fibroblasts, collagen fibers and nerve fibers along with Schwann cells. The autonomic nerves are situated between the collagen fibers and fibroblasts. Nerves consist of Schwann cells with unmyelinated axons. Each axon in a mesenteric arterial nerve lies in a grove of the Schwann cell membrane outside the cytoplasm (70).

2. Structure of the rat aorta

a) Tunica Intima

The endothelium is usually a single layer of flattened cells resting directly on the internal elastic lamina. No basement membrane underlies the endothelium. Endothelial cells have large nuclei and well-defined cell borders. The fine structure of these cells is similar to those in the smaller vessels. Although tissue may occasionally occur in the subendothelial area, the endothelium clearly coats the luminal surface of each vessel (72)

b) Tunica Media

Aortic tunica media consists of muscle cells which spiral around the long axis of the vessel, and are mostly encompassed by spaces containing collagen fibrils and elastic tissue fragments. The adjoinment of a smooth muscle cell to an elastic fibre is indicated by an accumulation of dense material at the cell membrane and the edge of the elastic tissue. Collagen fibrils are found in intercellular spaces, and do not attach directly to cells (72).

Like the mesenteric arteries, there are no blood vessels or nerves observed in the media of the rat. The aortic media contains only one cell type, the smooth muscle cell. Elastic laminae are surrounded by collagen sheaths. Lamellar units are composed of an elastic lamella and the content of the adjacent interlamellar space. Adult rats possess 7 elastic lamellar units in the thoracic aorta and 5 in the abdominal aorta. A helical structure is formed from the lamellae, to distribute stresses throughout the vessel wall (73).

In short, the aortic media of the rat has two important structural components which complement each other. First, the elastic tissue and muscle cells provide structural integrity and radial/longitudinal elasticity. Second, the collagen provides mechanical strength while limiting elastic expansion (72).

Aortic muscle cells have fewer myofilaments and greater numbers of cytoplasmic organelles than smooth muscle from the uterus and the bladder. Complex junctions are not observed between aortic muscle cells (72).

c) Tunica Adventitia

Like the rat mesenteric arteries, the aortic tunica adventitia consists mainly of collagen bundles encircling the artery and lying longitudinally. The tunica adventitia also consists of blood vessels, nerves, fibroblasts, mast cells, a few elastic fibers and the occasional smooth muscle cell (73).

H. Morphology of SHR Aorta and Mesenteric artery

There are many vascular changes evident in the SHR as compared to the WKY rat. Weber et al (1986) found that the development of intimal lesions occurs at an earlier age in the aortic and carotid arteries than the cerebral arteries of the SHR (74). No lesions were found in any of the vessels at the pre-hypertensive stage. Another study found some morphological differences between the SHR and WKY rat which occured first at 10 weeks of age and increased in severity with time (75). Medial thickening was observed in both aorta and peripheral arteries, and peripheral arteries and smaller luminal diameters, both of which were suggested to contribute

to maintaining high blood pressure (75). Intimal lesions containing acid mucopolysaccharides were found primarily in the aorta, suggesting a possible means of developing atheromatous lesions in hypertensive individuals capable of developing atherosclerosis (75). Also, intimal and medial lesions showed continuous progession with age, indicating the significance of the duration of elevated arterial pressure. Furthermore, another study relates aortic abnormalities in the SHR to predisposition for the development of atherosclerosis. In mature hypertensive SHR, it was found that the cholesterol content in the aorta was significantly higher than in WKY rat aortae (76). The same study examined 1,2-diacylglycerol content of thoracic aorta in pre-hypertensive rats and discovered an increase in 1,2-diacylglycerol in the SHR after pretreatment with norepinephrine, as compared to the WKY rats (76). It has also been shown that phospholipase C activity is increased in SHR aortae as compared to WKY rats, in both the pre-hypertensive and hypertensive condition (77).

A different approach was taken to observe the effects of hypertension in normotensive rats. In normotensive rats which were surgically rendered hypertensive, it was found that prolonged hypertension caused increased diameter, wall thickness, tangential tension, wall stress, medial area, and increased levels of elastin, collagen, and alkali-soluble protein in the aorta in both males and females (78). Furthermore, a study involving both pre- and postnatal SHR and

WKY rats reveals an increase of elastin and an increase in wall thickness involving both lamellar unit size and quantity in SHR aorta at all ages, suggesting arterial wall structure differences in the SHR are significant early in life (79).

In both pre-hypertensive and hypertensive SHR it was observed that aortic endothelial cells had tight junctions which were greater in area and more complex than those of WKY rats, indicating the SHR aortic endothelium can anticipate and respond to rising arterial pressure (80). Additionally, SHR aortic smooth muscle cells studied in culture proliferate faster than those from WKY rats (81). This finding indicates that arterial differences are prevalent between the SHR and normotensive rats even in the absence of hypertension (81).

In mesenteric resistance arteries of mature SHR, the content of the contactile proteins actin and myosin increases proportionately, thus, increasing contractile potential; however, there was less contractile protein in the media of SHR cerebral resistance arteries than in those from WKY arteries (82). The amounts of the connective tissue proteins elastin and collagen were equal in the SHR and WKY mesenteric resistance arteries, while total collagen was elevated in the SHR cerebral arteries (82). Additionally, the intrinsic contractile force or active smooth muscle cell stress of only the SHR cerebral arteries was much lower than that of the WKY rat vessels, suggesting a diminishment of brain vessel resistance to the effects of elevated arterial pressure (82).

Another study which examined mesenteric arteries found both SHR and WKY rats increased frequency of polyploid cells at all levels of the mesenteric arterial tree with age (83). Furthermore, the smallest mesenteric vessels penetrating the small intestine and the superior mesenteric arteries had lower levels of polyploidy than the intermediate mesenteric arteries, suggesting the effects of age and elevated arterial pressure are most prevalent in the middle of the mesenteric arterial tree (83).

I. VIP and Hypertension

There have been several physiological differences involving VIP abnomalities observed in the SHR as compared to the WKY rats. It appears that cardiac secretin-VIP-sensitive adenylate cyclase activity was simultaneous to the development of hypertension in the SHR (84). Also, a decrease in the inotropic response of hypertrophic left ventricles in renal hypertensive rats to both VIP and glucagon was observed, indicating an impairment of the adenylate cyclase pathway independent of β-receptor alterations (85). Reduced levels of several neuropeptides including VIP, were observed in the spinal cord of the SHR as compared to the WKY rats (86). Furthermore, the density of nerves containing VIP was higher in the veins and superior mesenteric artery of SHRSP, but

lower in the cerebral arteries, as compared to WKY rats, however, no difference was observed in muscular mesenteric arteries (18). It was suggested that these differences in distribution of nerves containing VIP may be associated with the development of hypertension in the SHR (18). Additionally, Kawamura et al (1989) also found no difference between the densities of cholinergic nerve fibers, VIP-containing and substance P-containing nerve fibers in the mesenteric arteries of the SHR and WKY rats (17). There has also been a study involving hypertensive human subjects which examined the circulating levels of calcitonin gene-related peptide, substance P- and VIP- like immunoreactivity before and after treatment (87). Levels of VIP-like immunoreactivity did not differ before and after treatment for high blood pressure.

J. Statement of objectives

The objective of the research described herein was to attempt to determine if hypertension in the SHR model is associated with alterations to the VIP and forskolin effector systems as indicated by relaxation properties of the thoracic aorta and superior mesenteric artery. Thus, the hypothesis tested was that experimental hypertension is associated with impairments in the VIP effector system, in blood vessels from the SHR.

II MATERIALS AND METHODS

A. MATERIALS

1. Chemicals

Norepinephrine hydrochloride and papaverine (6,7-dimethoxy-1-veratrylisoquinoline) were obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A. VIP (human porcine, rat) and prostaglandin $F_{2\alpha}$ were purchased from Peninsula Laboratories Inc., Belmont, California, U.S.A. Forskolin (Coleus forskohlii, lyophilized) was obtained from Calbiochem, La Jolla, California, U.S.A.

The following chemicals were obtained from Fisher Scientific Co. and were utilized for the bioassay procedures: calcium chloride, dextrose, ethanol, glacial acetic acid, hydrochloric acid, magnesium chloride, potassium chloride, potassium phosphate monobasic and sodium bicarbonate.

Carbogen (95% O_2, 5%CO_2) was obtained from Union Carbide Canada Ltd, Edmonton, Alberta.

2. INSTRUMENTS AND APPARATUS

a) Blood Pressure Monitoring System

Systolic and mean blood pressures were determined using a photoelectric type sphygmomanometer, pulse amplifier (Mod 59), and recorder (Mod 45-SE120) obtained from IITC Life Science, Woodland Hills, California, U.S.A. Animals were placed in transparent rat holders (IITC Life Science) and pre-heated with an overhead heat-lamp (Fisher Scientific Co.).

b) Bioassay

Isometric tension changes were recorded through a force-displacement transducer coupled to a pen recorder obtained from Gould, Cleveland, Ohio, U.S.A. The water heaters models F3 and D1 were purchased from Haake, Berlin, Germany.

C) Miscellaneous

Chemical masses were determined on either a Mettler PE 3600 or a Mettler AE 163 balance (Mettler Instruments AG, Zurich, Switzerland). Animal weights were determined using a Triple Beam Balance obtained from Ohaus, Florham Park, New Jersey, U.S.A. Pipettors used included Gilson models P200 and P1000 (Mandel Scientific Company Ltd., Calgary, Alberta.) and

a Labsystems Finnpipette 1-5 ul from Labsystems Oy, Helsinki, Finland. A Vortex-Genie mixer (Scientific Industries Inc., Bohemia, New York, U.S.A. or a Corning PC-353 stirrer (Corning Glass Works, Corning, New York, U.S.A.) was used to mix solutions. The dissecting microscope used was a Stereozoom 7 obtained from Bausch and Lomb Optics Canada Ltd.

3. ANIMALS

Male SHR, WKY rats, and Wistar rats were obtained from rat colonies at Charles River Canada Inc., St. Constant, Quebec. Rats utilized were 12-14 weeks of age following blood pressure monitoring.

B. METHODS

1. Monitoring Blood Pressure

Systolic and mean blood pressure were monitored over a period of 1-2 weeks. Animals were warmed with an overhead lamp, approximately 0.5 m from the animals, for 30 minutes to facilitate pulse detection. Others have found that preheating will slightly increase rectal temperature - resulting in sufficient vasodilation to permit detection of tail pulses with the photoelectric sensor (88). The animal walked forwards into the rat holder which had an adjustable head gate

with a magnetically held tail section. There is a small focused light and a photoresistive cell mounted in a 5 cm long inflatable rubber cuff placed over the tail of the animal. The cuff is inflated and deflated manually. Pulse signals from the photocell are fed into an amplifier for regulation of gain, offset, and intensity of light source. Signals from the photocell amplifier and cuff pressure transducer are then recorded continuously on separate channels of the recorder. The IITC tail-cuff method of determining mean and systolic blood pressure has been validated by simultaneously measuring blood pressure directly, using catheters inserted into femoral and carotid arteries (88).

From each animal, three measurements per trial (day) for at least three trials were obtained over the period of at least one week.

2. Vessel Preparation

Animals were sacrificed by means of a carbon dioxide chamber, prior to immediate dissection of the blood vessels of interest: thoracic aorta, superior mesenteric artery, carotid artery, and iliac artery. Vessels were carefully removed to avoid stretching or puncturing, and immediately placed in iced and aerated Krebs-Henseleit solution of the following composition: NaCl 115 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, and dextrose 10 mM.

Vessels were threaded onto a wire which was then fixed to the base of a gelatinous Petri dish containing Krebs-Henseleit solution. The vessels were trimmed of any fat or connective tissue with surgical scissors and forceps, to maximize drug effects. The arteries were then cut at an angle to produce a helical strip. Thoracic aortae yielded three segments while superior mesenteric, carotid, and iliac arteries yield one segment. Each strip was approximately 1 mm in width and 1 cm in length. Suture thread was then tied onto each end of the vessel segment in order to connect it to the transducer. The proximal portion of the strip was tied to a lever of the force-displacement transducer.

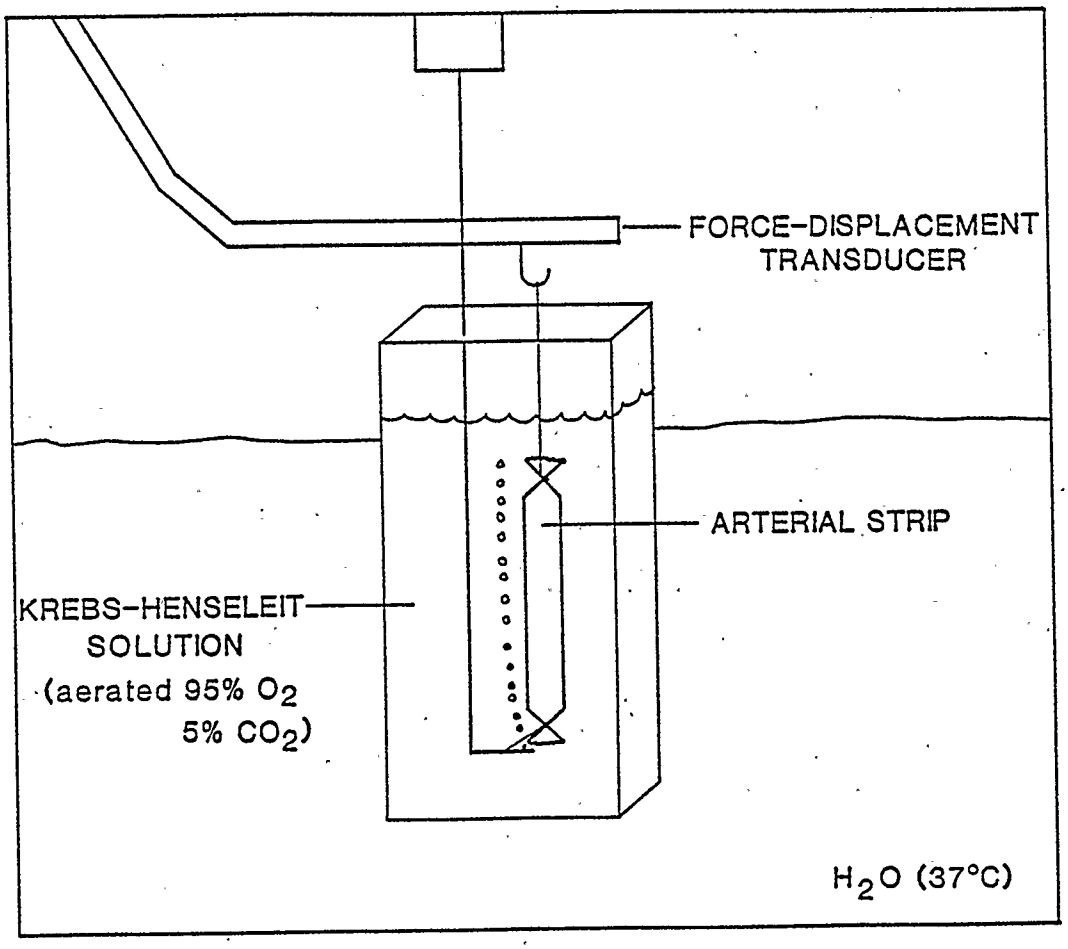
3. Bioassay

Figure 4 illustrates the tissue bath assay utilized to assess in vitro isometric tension changes. Thoracic strips were suspended under a resting tension of 1.0 gram, while superior mesenteric, carotid, and iliac arteries were suspended under a resting tension of 0.5 gram . These are the standards commonly utilized for these vessels (18,82). Arterial strips were equilibrated in 3 ml tissue baths containing Krebs-Henseleit solution at 37°C and aerated continuously with 95% O_2 and 5% CO_2 for one hour.

The drugs utilized in the experiments were added directly to the tissue bath. Preliminary studies were undertaken to

FIGURE 4. Tissue Bath Assay Utilized To Assess In Vitro Isometric Tension Changes.

Tension changes were recorded through a force-displacement transducer.


FORCE-DISPLACEMENT
TRANSDUCER
ARTERIAL STRIP
KREBS-HENSELEIT
SOLUTION
(aerated 95% O_2
5% CO_2)
H_2O (37°C)

identify the most effective agonist concentrations for observing the vasorelaxant effect of VIP in the normotensive Wistar thoracic aorta, superior mesenteric artery, iliac artery, and carotid artery. The agonists observed initially included norepinephrine, prostaglandin $F_2\alpha$, and KCl. KCl was studied at the concentration of 40 mM. Dose-response relationships were examined for norepinephrine and prostaglandin $F_2\alpha$ induced contractions as well as VIP and forskolin induced relaxations. When observing a dose-response relationship, very low concentrations of the hormone/chemical were injected into the tissue bath, initially achieving no effect. Concentrations of the hormone/chemical increased gradually to achieve either a small contractile effect for norepinephrine and prostaglandin $F_2\alpha$, or a small relaxant effect for VIP and forskolin. Hormone/chemical concentrations were increased by small increments until a maximal effect was observed.

In the VIP experiments, norepinephrine 0.1 μM in aortae and 1 μM in superior mesenteric artery, was utilized to contract vessels intially. The tissue baths were then washed with Krebs-Henseleit solution and allowed to equilibrate for one hour. Fresh buffer was added every 20 minutes. Norepinephrine was again added to produce a sustained contraction. Vessels in which a sustained contraction could not be achieved were not used for study. VIP was then added to examine the relaxant response - the concentration in the

tissue bath was 0.1 μM. Finally, papaverine was injected into the tissue bath, achieving a final concentration of 0.1 mM, which was taken as 100% relaxation. The relaxant response to papaverine was utilized to determine the percent relaxation of VIP (and forskolin).

In the forskolin studies, an initial application of norepinephrine was followed by forskolin 0.1 μM. Papaverine was then injected to achieve a concentration of 0.1 mM, with the relaxation defined as 100% relaxation.

4.Statistical Analysis

Statistical analysis of the data was conducted by one-way analysis of variance followed by the Duncan's Multiple Range Test at the 5% level of significance to examine differences between SHR, Wistar rat, and WKY rat groups. Two groups of data were considered to be significantly different when $p<0.05$. Before analysis, percentage data was subject to arcsin $\sqrt{\%}$ transformation according to Rohlf and Sokal, 1969 (89). Angular transformations were utilized in order to have data that more closely conformed to a normal distribution (90). The angular transformation is especially appropriate for percentages and proportions (91).

III RESULTS

A. Mean and Systolic Blood Pressure

The mean and systolic blood pressures were determined for the SHR, WKY rats, and Wistar rats. The averaged systolic blood pressure values were 196 ± 6 for SHR, 128 ± 4 for WKY rats, and 133 ± 6 for Wistar rats (mm Hg, mean ± SEM) . These values are consistent with those in the literature (92,93). The averaged mean blood pressure values for SHR, WKY rats, and Wistar rats were 156 ± 9, 107 ± 8, and 109 ± 4 (mm Hg, mean ± SEM). For both systolic blood pressure and mean blood pressure, there are significant differences between the SHR readings as compared to those of the Wistar rats and WKY rats ($p<0.05$).

B. Preliminary Results - Effects of Various Agonists and VIP

Initially, the blood vessels studied included thoracic aortae, superior mesenteric artery, iliac artery, and carotid artery. Firstly, a dose-response curve was constructed for norepinephrine and prostaglandin $F_{2\alpha}$ (Figures 5 and 6). It was found that the most effective concentration of norepinephrine for use in the contractility studies was 0.1 μM for thoracic aorta (n=13) and 1 μM for superior mesenteric artery (n=7). These concentrations maintain a sustained

FIGURE 5. Norepinephrine Induced Contractions for Thoracic Aorta, Carotid Artery, Iliac Artery, and Superior Mesenteric Artery in the Wistar Rat using the Tissue Bath Assay.

The symbols and number of experiments are as follows: ● thoracic aorta n=13, ■ iliac artery n=7, △ superior mesenteric artery n=7, and ▲ carotid artery n=1.

Symbols represent mean changes in tension (mg) and vertical bars represent SEM.

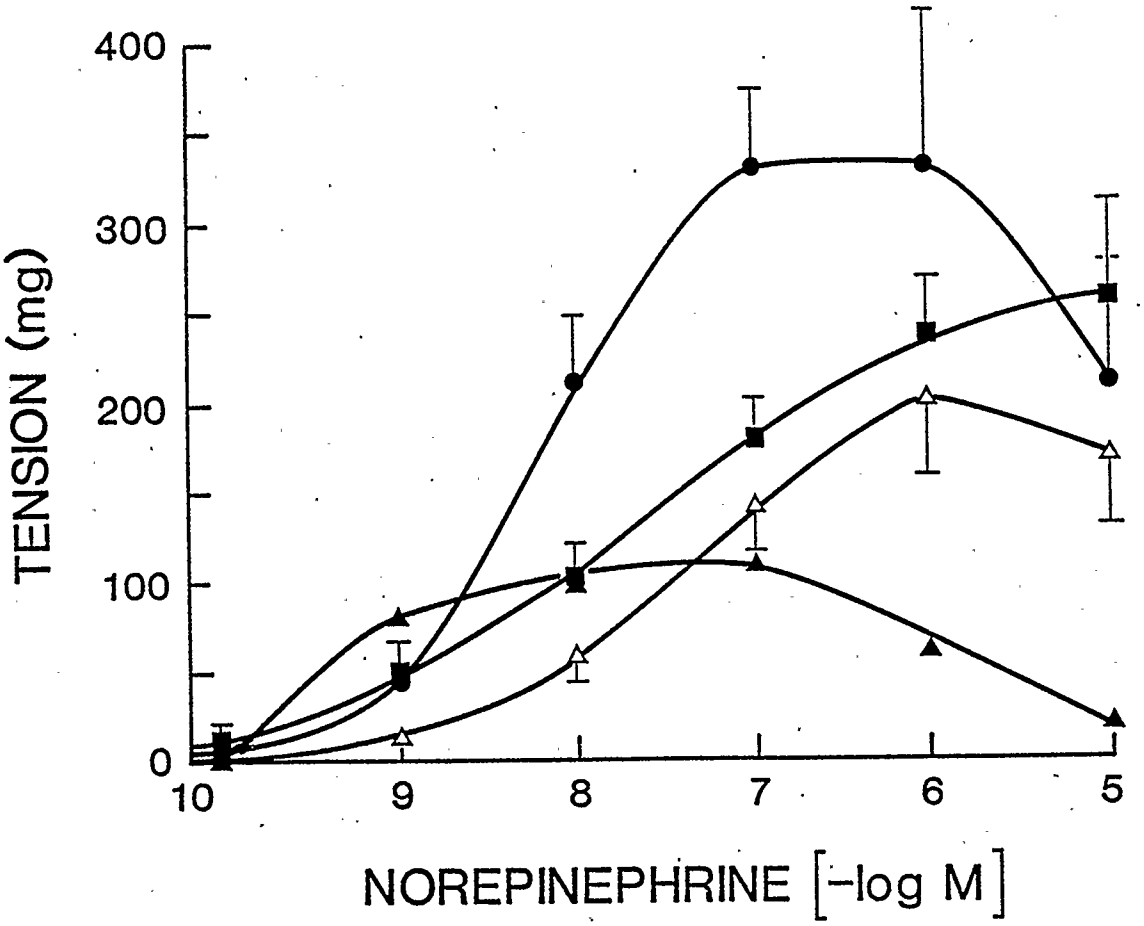
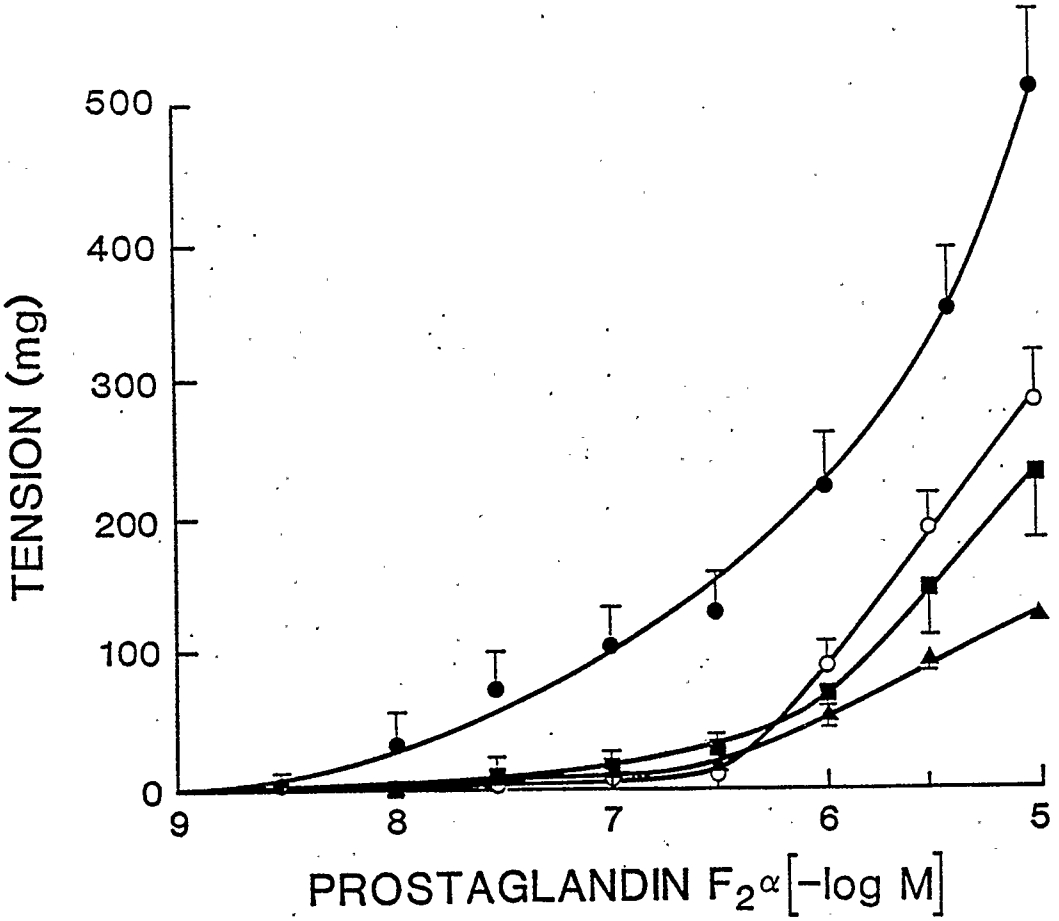

400
300
200
100
0
TENSION (mg)
10
9
8
7
6
5
NOREPINEPHRINE [-log M]

FIGURE 6. Prostaglandin $F_{2\alpha}$ Induced Contractions for Thoracic Aorta, Carotid Artery, Iliac Artery, and Superior Mesenteric Artery in the Wistar Rat using the Tissue Bath Assay.

The symbols and number of experiments are as follows: ● thoracic aorta n=14, O superior mesenteric artery n=8, ■ iliac artery n=7, and ▲ carotid artery n=4.

Symbols represent mean changes in tension (mg) and vertical bars represent SEM.


500
400
300
200
100
0
TENSION (mg)
9
8
7
6
5
PROSTAGLANDIN $F_2\alpha$ [-log M]

contraction, as well as provide a contraction of sufficient magnitude to observe the vasorelaxation response. Sustained contractions were difficult to achieve in carotid (n=1) and iliac (n=7) arteries, and the response to VIP in iliac arteries was minimal (Figures 7 and 8). A contraction of sufficient magnitude necessary to examine the effects of VIP in the carotid artery could not be obtained . As a result of these findings, the iliac and carotid arteries were excluded from study.

Figure 6 shows that the prostaglandin $F_2\alpha$ dose-response curves initially indicated potential for use of prostaglandin $F_2\alpha$ as an agonist in high concentrations (1 μM to 10 μM) for thoracic aortae (n=14) and superior mesenteric arteries (n=8). As was the case with the norepinephrine studies, stable contractions of sufficient strength could not be achieved in iliac (n=7) and carotid arteries (n=4). When the effects of VIP were studied in prostaglandin $F_2\alpha$ pre-contracted vessels, no relaxation was observed (Figure 9).

VIP dose-response curves for the iliac artery, superior mesenteric artery, and thoracic aorta are illustrated in Figure 7. As was previously stated, VIP had very little effect on the iliac artery (n=9). The response to VIP of thoracic aortae pre-contracted with norepinephrine 1 μM was also minimal. The superior mesenteric artery (n=17) pre-contracted with norepinephrine 1 μM and the thoracic aortae pre-contracted with norepinephrine 0.1 μM (n=8), and

FIGURE 7. VIP Induced Relaxation in Thoracic Aorta, Iliac Artery, and Superior Mesenteric Artery in the Wistar Rat using the Tissue Bath Assay.

The symbols and number of experiments are as follows: ■ iliac artery precontracted with norepinephrine 1 μM n=9, O thoracic aorta precontracted with norepinephrine 1 μM n=32, ● superior mesenteric artery precontracted with norepinephrine 1 μM n=17, ▲ thoracic aorta precontracted with norepinephrine 0.1 μM n=8,and △ thoracic aorta precontracted with norepinephrine 0.01 μM n=8.

Symbols represent mean changes in relaxation (%) and vertical bars represent SEM.

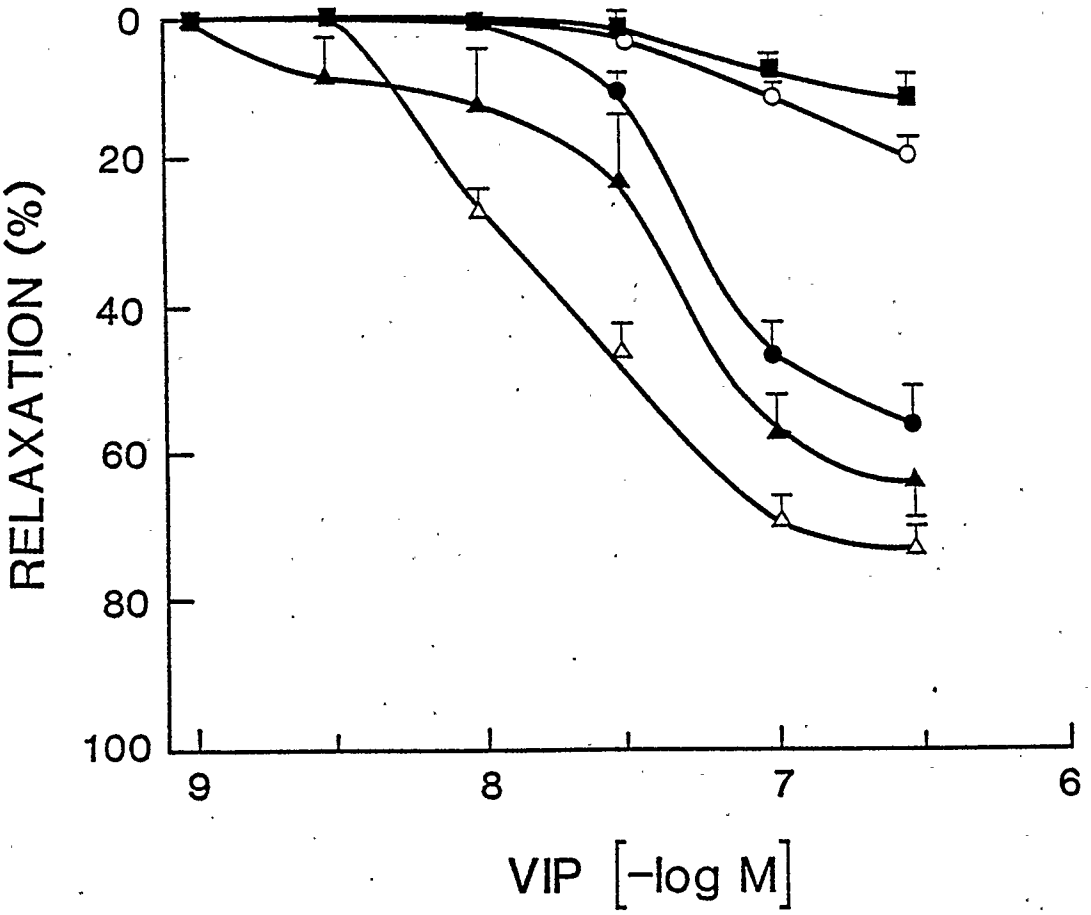

RELAXATION (%)
0
20
40
60
80
100
9
8
7
6
VIP [-log M]

FIGURE 8. Contraction Induced by Several Agents in Thoracic Aorta, Superior Mesenteric Artery, Iliac Artery, and Carotid Artery in the Wistar Rat using the Tissue Bath Assay.

The symbols are as follows:  thoracic aorta,  superior mesenteric artery,  iliac artery, and  carotid artery.

Abbreviations are as follows: NE, norepinephrine; KCl, potassium chloride; and $PGF_2\alpha$, prostaglandin $F_2\alpha$.

Bar length represents mean changes in contactility (mg) and horizontal bar lines represent SEM.

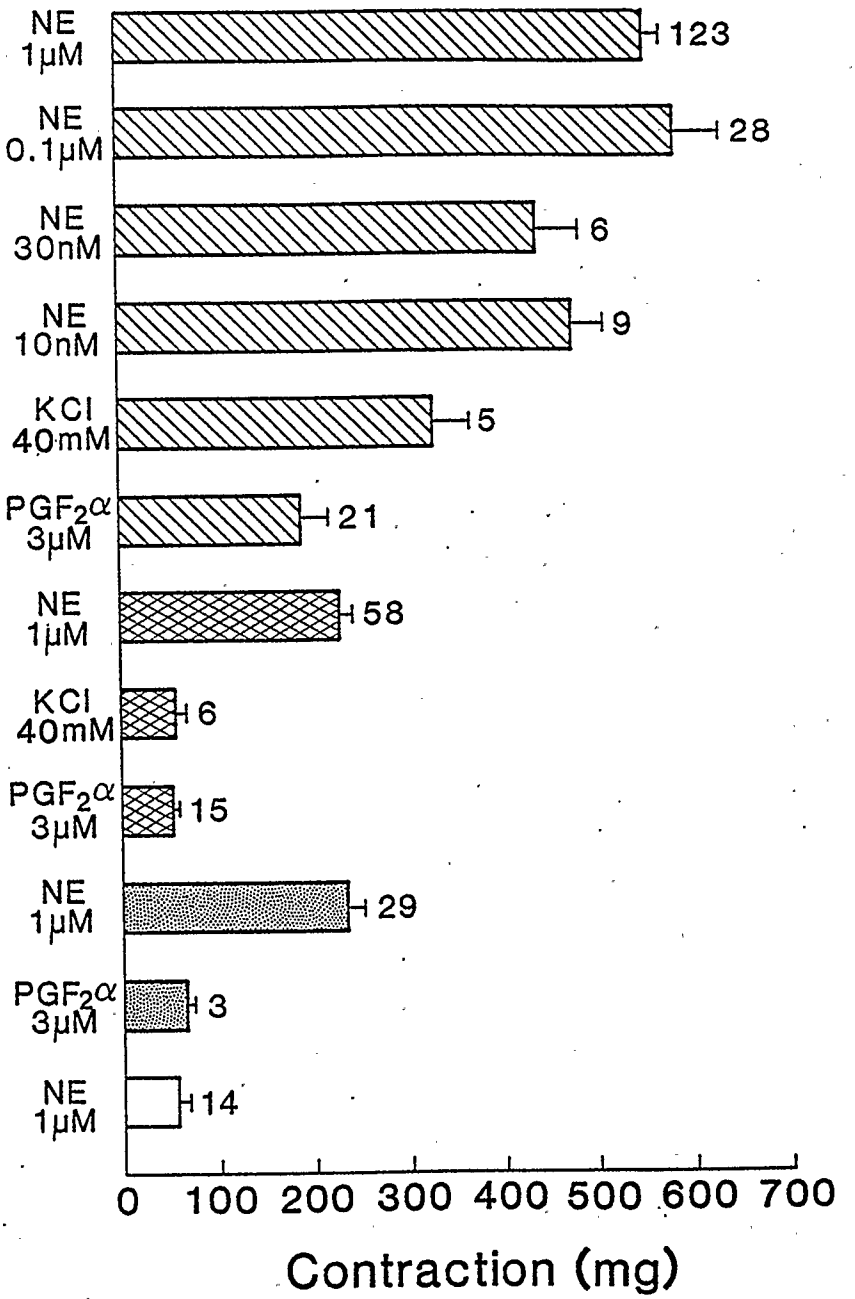
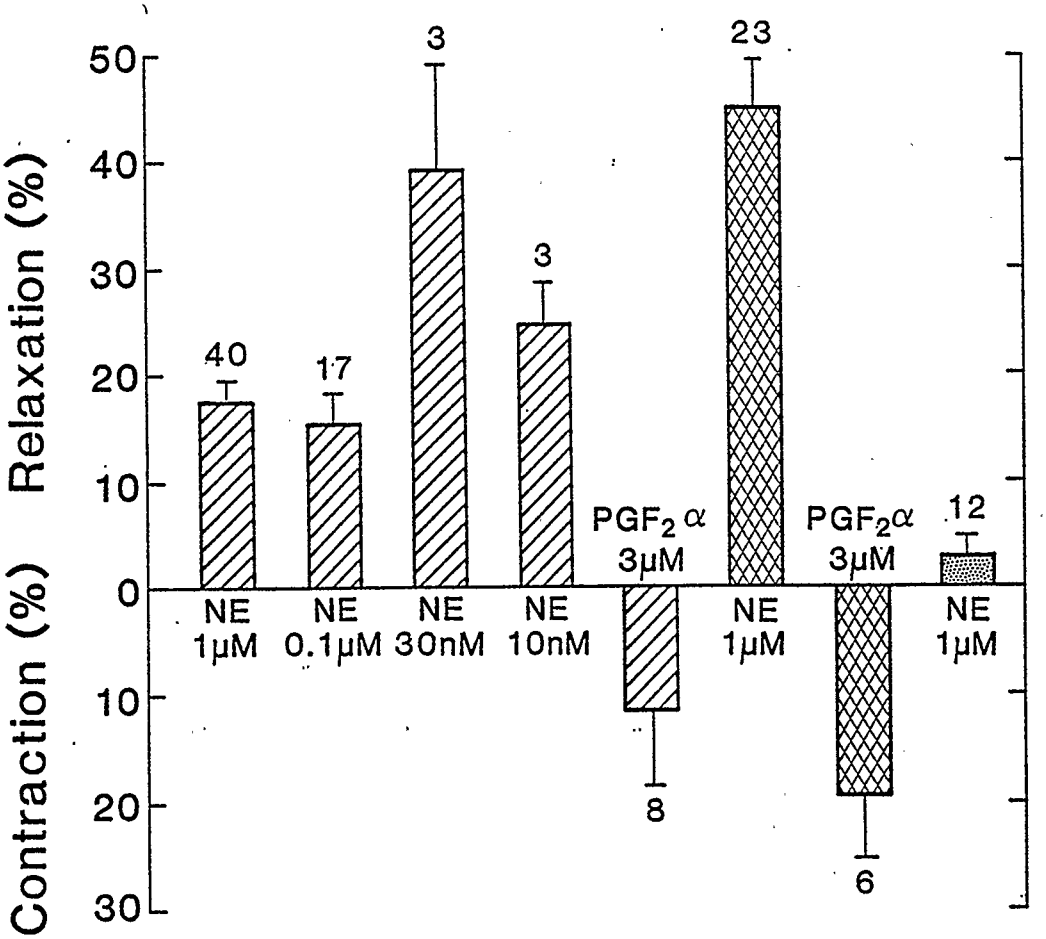

NE 1μM
123
NE 0.1μM
28
NE 30nM
6
NE 10nM
9
KCl 40mM
5
PGF2α 3μM
21
NE 1μM
58
KCl 40mM
6
PGF2α 3μM
15
NE 1μM
29
PGF2α 3μM
3
NE 1μM
14
0 100 200 300 400 500 600 700
Contraction (mg)

FIGURE 9. Effect of VIP (0.1 μM) After Addition of Various Agents in the Thoracic Aorta, Superior Mesenteric Artery, and Iliac Artery in the Wistar Rat using the Tissue Bath Assay.

The symbols are as follows: ▨ thoracic aorta, ▩ superior mesenteric artery, and ▦ iliac artery.

Bar height represents mean changes in relaxation (%) or contraction (%) and vertical lines represent SEM.


Contraction (%) Relaxation (%)
50
40
30
20
10
0
10
20
30
40
17
3
3
8
23
6
12
NE 1μM
NE 0.1μM
NE 30nM
NE 10nM
PGF2α 3μM
NE 1μM
PGF2α 3μM
NE 1μM

norepinephrine 0.01 μM (n=8) achieved the greatest relaxation to VIP. It was decided that it might be most appropriate to utilize a single dosage of VIP and inject it into the tissue bath of the pre-contracted vessels. A final concentration of VIP 0.1 μM was utilized as it produced a relaxation of sufficient size to be easily measured.

The contractile effects of various agonists are summarized in Figure 8. In the thoracic aorta, the strongest contraction was observed when norepinephrine 0.1 μM (n=28) was in the tissue bath. Other concentrations of norepinephrine, KCl 40 mM (n=5), and prostaglandin $F_2\alpha$ 3 μM (n=21) were not as effective in evoking a strong contractile response. In the superior mesenteric artery, the magnitude of contraction achieved with norepinephrine 1 μM (n=58) almost quadrupled those obtained with KCl 40 mM (n=6) and prostaglandin $F_2\alpha$ 3 μM (n=15). In studying the iliac artery, it was found that contractions of sufficient magnitude could not be obtained with prostaglandin $F_2\alpha$ 3 μM (n=3). Although satisfactory contractions were obtained with norepinephrine 1 μM (n=29) in the iliac artery, the effect of VIP on this vessel was very small; consequently, the iliac artery was no longer utilized for study. Lastly, in the carotid artery, norepinephrine 1 μM (n=14) could not produce a contraction of sufficient magnitude to examine the relaxant response of VIP.

Figure 9 illustrates the effects of VIP 0.1 μM after the addition of various agonists in the thoracic aorta, superior

mesenteric artery, and iliac artery. As previously mentioned, no relaxation was observed in vessels pre-contracted with prostaglandin $F_{2\alpha}$. Conversely, the vessels' contractility was augmented. It should be noted that small contractions produced by VIP in arterial segments (guinea-pig uterine artery) precontracted by 3 μM prostaglandin $F_{2\alpha}$ have been observed (49). Thoracic aortae (n=8) and superior mesenteric artery (n=6) pre-contracted with prostaglandin $F_{2\alpha}$ 3 μM increased the contraction size upon the addition of VIP 0.1 μM. Thoracic aortae were also pre-contracted with concentrations of norepinephrine of 1 μM (n=40), 0.1 μM (n=17), 0.03 μM (n=3), and 0.01 μM (n=3) . Although the greatest amount of relaxation was observed when thoracic aorta was pre-contracted with norepinephrine 0.03 μM, the magnitude of the relaxation was more difficult to measure because the size of the contraction was relatively smaller. Superior mesenteric artery was the most sensitive to VIP of the vessels studied in terms of its relaxant response, while the iliac artery (n=12) was the least sensitive.

Dose-response curves were also constructed for thoracic aorta (n=7) and superior mesenteric artery (n=6) using forskolin. In order to test the hypothesis that VIP-sensitive adenylate cyclase activity may have been abnormal in the superior mesenteric artery of hypertensive animals, forskolin, a direct activator of adenylate cyclase, was studied.

For thoracic aorta, the data presented describes tension

changes in the mid-thoracic region. Studies were undertaken to compare proximal, middle, and distal regions of the thoracic aorta; however, the findings did not influence the outcome of the results (data not shown). Also, the middle region appeared to be less prone to damage during dissection, and thus, more reliable than the proximal and distal regions.

C.VIP Vasorelaxation Studies

The relaxant responses to VIP were examined in norepinephrine pre-contracted strips of superior mesenteric artery and thoracic aorta from SHR, Wistar rats, and WKY rats. Table 1 summarizes the tension changes which were observed when norepinephrine, papaverine and VIP were utilized in the VIP vasorelaxation studies. As can be seen in Figure 10 , the contractile response of Wistar rat vessels to norepinephrine was significantly greater than in SHR and WKY rat vessels ($p<0.05$). On the other hand, no significant differences were observed between WKY rats and SHR.

Similarly, the relaxant responses to papaverine in both superior mesenteric artery and thoracic aortae were significantly greater in Wistar rats than in SHR and WKY rats ($p<0.05$), but no significant differences were detected between WKY rats and SHR ($p>0.05$). Relaxation induced by 0.1 mM papaverine was taken as 100% (as in 41,65,66,94,95).

TABLE 1

Tension Changes Observed in the VIP Studies

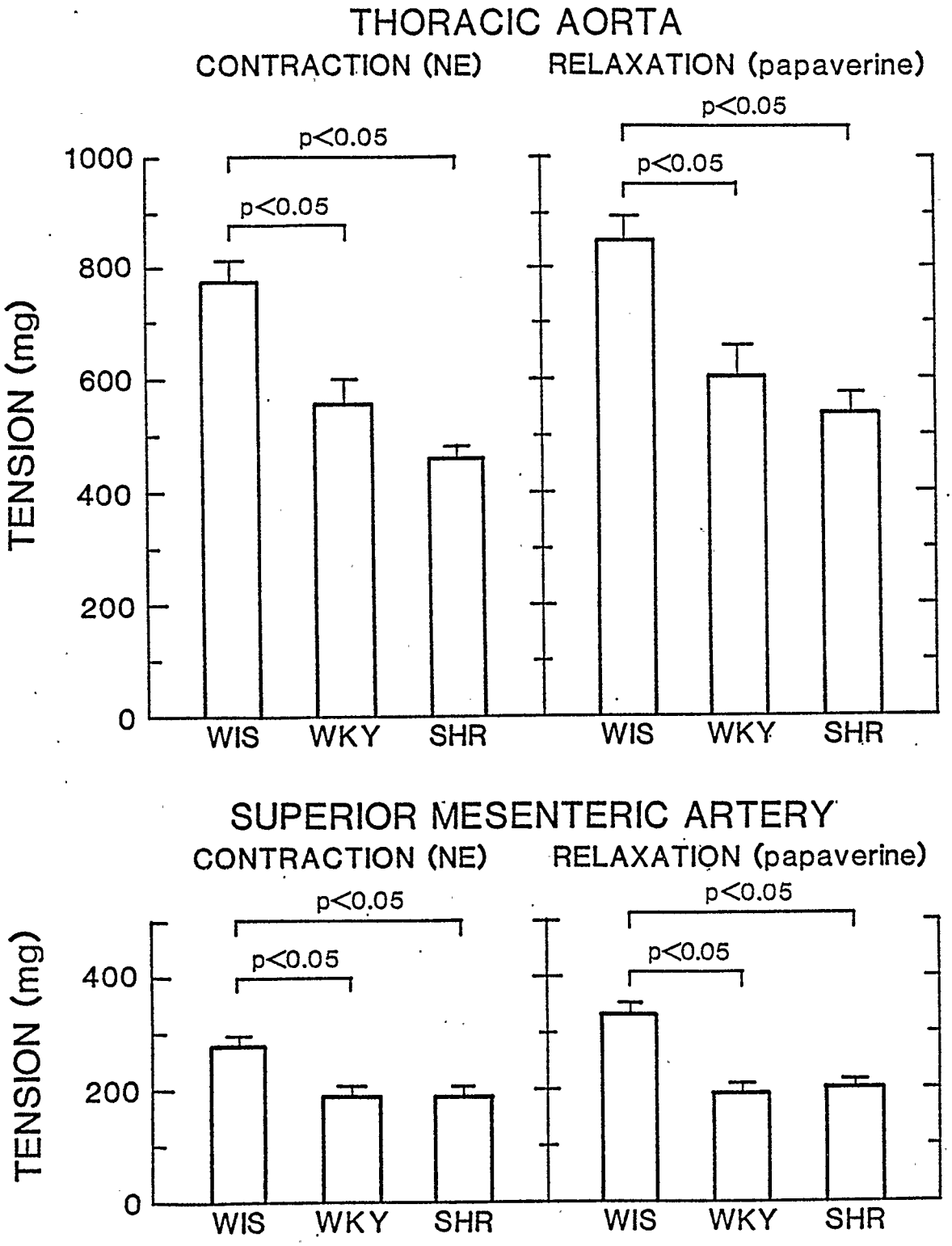
AGENT	CHANGE IN TENSION (mean ± SEM)		
	WISTAR	WKY	SHR
A.Superior Mesenteric Artery			
	n=16	n=12	n=16
Norepinephrine (mg)	278 ± 16	192 ± 13	188 ± 13
Papaverine (mg)	313 ± 19	193 ± 14	202 ± 12
VIP (mg)	143 ± 20	105 ± 12	48 ± 8
VIP (%)	46 ± 6	54 ± 4	24 ± 4
B.Thoracic Aorta			
	n=15	n=10	n=14
Norepinephrine (mg)	772 ± 43	564 ± 45	461 ± 21
Papaverine (mg)	849 ± 39	604 ± 54	531 ± 34
VIP (mg)	292 ± 59	140 ± 20	193 ± 33
VIP (%)	34 ± 6	28 ± 7	38 ± 7

FIGURE 10. Tension Changes Induced by Norepinephrine and Papaverine in Thoracic Aorta and Superior Mesenteric Artery in the Tissue Bath Assay.

Thoracic aorta was precontracted with norepinephrine 0.1 μM and superior mesenteric artery with norepinephrine 1 μM. Papaverine 0.1 mM was used for both thoracic aorta and superior mesenteric artery. The number of experiments are as follows: Wistar rat n=16, WKY rat n=12, and SHR n=16 in the thoracic aorta; and Wistar rat n=15, WKY rat n=10, and SHR n=14 in the superior mesenteric artery.

Vertical bars represent changes in tension (mg) and vertical lines represent SEM.

Refer to text for explanation of significant differences ($p<0.05$).


THORACIC AORTA
CONTRACTION (NE)
RELAXATION (papaverine)
p<0.05
p<0.05
p<0.05
p<0.05
TENSION (mg)
1000
800
600
400
200
0
WIS
WKY
SHR
WIS
WKY
SHR
SUPERIOR MESENTERIC ARTERY
CONTRACTION (NE)
RELAXATION (papaverine)
p<0.05
p<0.05
p<0.05
p<0.05
TENSION (mg)
400
200
0
WIS
WKY
SHR
WIS
WKY
SHR

The relaxant response to VIP was significantly diminished in the SHR superior mesenteric artery (n=16) as compared to Wistar rats (n=16) and WKY rats (n=12) ($p<0.05$), but this was not the case in the thoracic aorta. Figure 11 illustrates this reduction of relaxation in the superior mesenteric artery when measured not only as a percent of papaverine relaxation, but also as an absolute measure of tension. Accordingly, tension changes were greater in thoracic aortae than in the superior mesenteric artery. There was no significant difference in VIP relaxation of thoracic aortae between SHR (n=14), Wistar rats (n=15), and WKY rats (n=10) as measured by percent relaxation ($p>0.05$), however, when observed as an absolute measure of tension, the relaxation of WKY was significantly lower than that of Wistar rats ($p<0.05$).

D. Forskolin Vasorelaxation Studies

Table 2 summarizes the responses of norepinephrine, papaverine and forskolin in the forskolin relaxation studies. It should be noted that values obtained for relaxation of papaverine in the forskolin studies were significantly lower in the Wistar rat and WKY rat, but not in the SHR, as compared to values obtained for the VIP studies. Figure 12 illustrates forskolin dose-response curves for thoracic aorta (n=7) and superior mesenteric artery (n=6). These values are consistent with other studies reported in the literature (66,94). As

FIGURE 11. VIP Vasorelaxation on Thoracic Aorta and Superior Mesenteric Artery in the Tissue Bath Assay.

The symbols are as follows: A = Relaxation (mg) induced by VIP 0.1 μM, B = Relaxation (normalized to 0.1 mM papaverine relaxation) induced by VIP 0.1 μM.

The experimental numbers in the thoracic aorta were as follows: n=16 for Wistar rat, n=12 for WKY rat, and n=16 for SHR. In the superior mesenteric artery experimental numbers are Wistar rat n=15, WKY rat n=10, and SHR n=14.

Thoracic aorta were precontracted with norepinephrine 0.1 μM and superior mesenteric artery were precontracted with norepinephrine 1 μM.

Vertical bars represent mean changes in relaxation (mg or %) and vertical lines represent SEM.

Refer to text for explanation of significant differences ($p<0.05$).

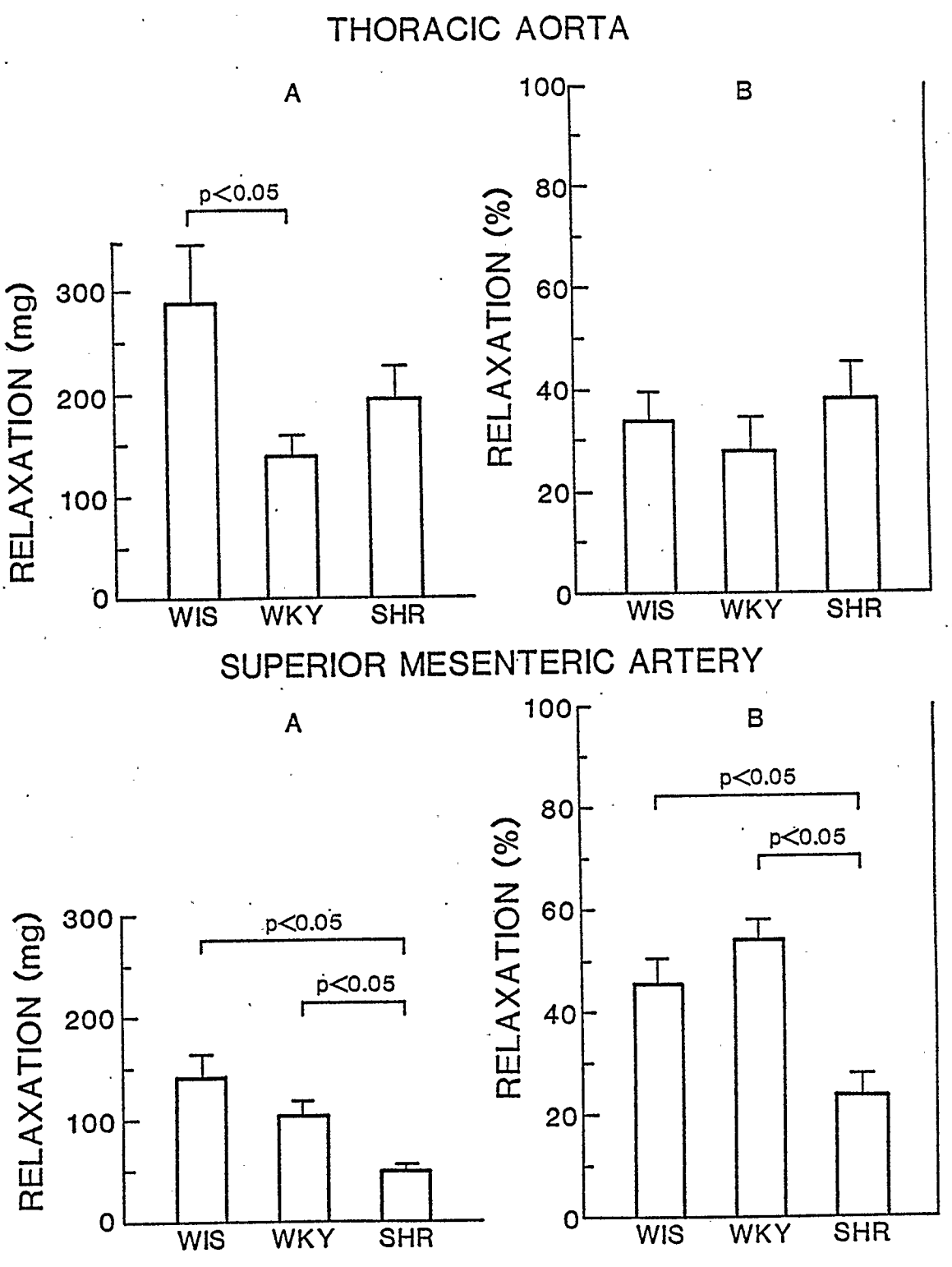

THORACIC AORTA
A
B
p<0.05
RELAXATION (mg)
RELAXATION (%)
0
100
200
300
20
40
60
80
WIS
WKY
SHR
SUPERIOR MESENTERIC ARTERY
A
B
p<0.05
p<0.05
p<0.05
RELAXATION (mg)
RELAXATION (%)
0
100
200
300
20
40
60
80
WIS
WKY
SHR

TABLE 2

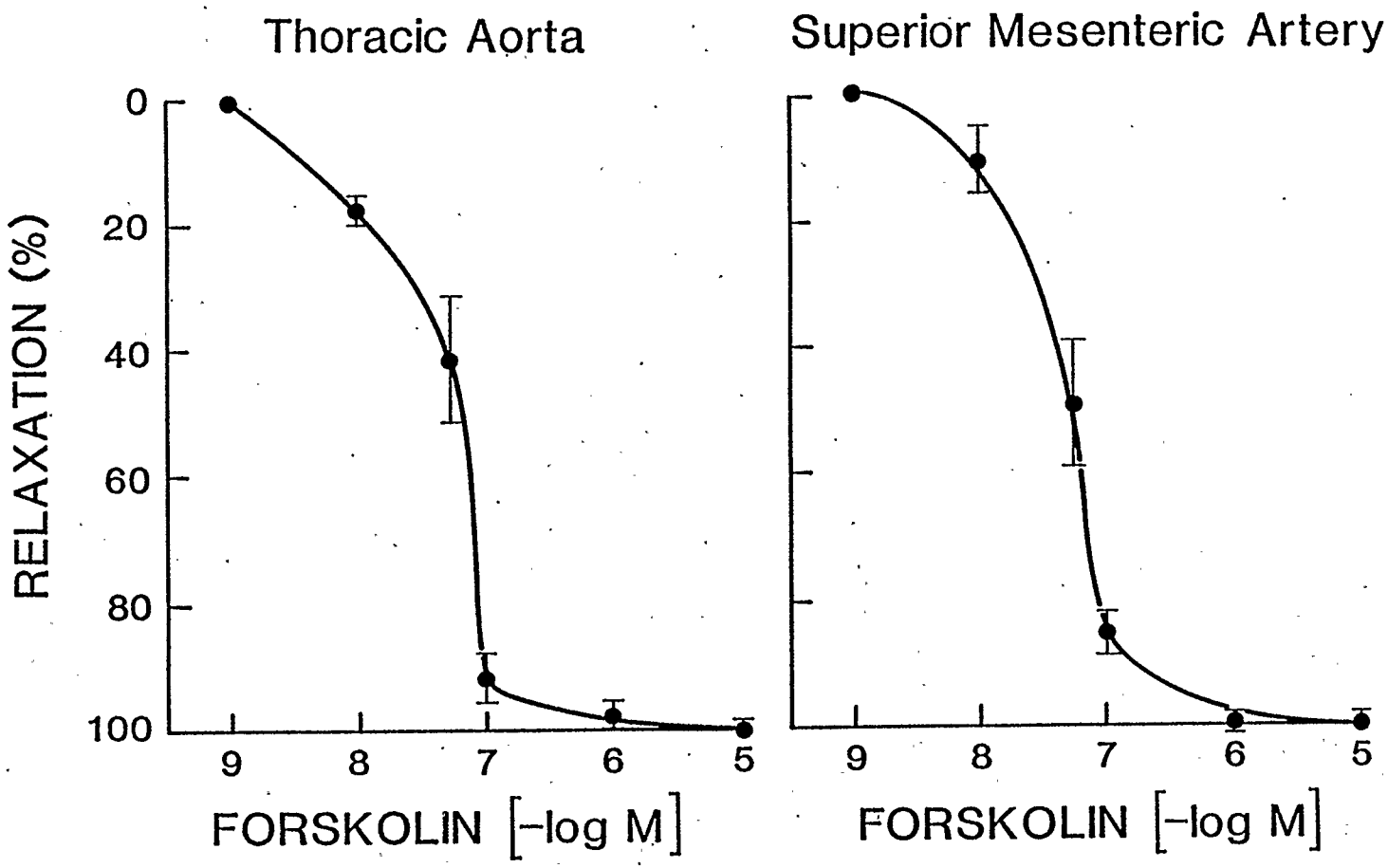
Tension Changes Observed in the Forskolin Studies

AGENT	CHANGE IN TENSION (mean ± SEM)		
	WISTAR	WKY	SHR
A.Superior Mesenteric Artery			
	n=12	n=11	n=9
Norepinephrine (mg)	206 ± 21	149 ± 10	189 ± 15
Papaverine (mg)	212 ± 21	156 ± 10	196 ± 14
Forskolin (mg)	134 ± 17	120 ± 8	90 ± 14
Forskolin (%)	64 ± 4	77 ± 3	45 ± 7
B.Thoracic Aorta			
	n=11	n=11	n=11
Norepinephrine (mg)	704 ± 38	389 ± 25	307 ± 28
Papaverine (mg)	773 ± 34	438 ± 18	400 ± 19
Forskolin (mg)	367 ± 45	342 ± 23	252 ± 20
Forskolin (%)	52 ± 7	77 ± 3	64 ± 5

FIGURE 12. Forskolin Induced Relaxation in Thoracic Aorta and Superior Mesenteric Artery of the Wistar Rat in the Tissue Bath Assay.

Thoracic Aortae were precontracted with norepinephrine 0.1 μM and superior mesenteric artery with 1 μM. Experimental numbers were n=7 for thoracic aorta and n=6 for superior mesenteric artery.

Each symbol represents mean relaxation (%-normalized to 0.1 mM papverine relaxation) and vertical lines represent SEM.


Thoracic Aorta
Superior Mesenteric Artery
RELAXATION (%)
0
20
40
60
80
100
9
8
7
6
5
FORSKOLIN [-log M]

with VIP, the chosen dosage for single injection studies was 0.1 uM, because of its ability to produce sizeable relaxations which were easily measured. However, the usage of this dosage may have also contributed to difficulties comparing the groups, as 0.1 μM forskolin approaches the end of the dose-response curve. As a result, this dosage of forskolin may not have been as sensitive to any alterations in responsiveness as a lower concentration around the middle of the curve.

Figure 13 illustrates the relaxant response to forskolin 0.1 uM. Forskolin relaxation was significantly lower in the SHR superior mesenteric artery (n=9) as compared to Wistar rats (n=12) and WKY rats (n=11) , when measured as a percent of papaverine relaxation ($p<0.05$). However, when absolute measures of tension were examined, there was no significant difference between each of the three groups. In thoracic aortae, on the other hand, when forskolin relaxation is expressed as a percent of papaverine relaxation, there is significantly less relaxation in Wistar rats (n=11) as compared to WKY rats (n=11) ($p<0.05$). Furthermore, when forskolin relaxation is expressed as an absolute measure of tension, SHR values (n=11) are significantly lower than Wistar rat values ($p<0.05$), but not WKY values.

FIGURE 13. Forskolin Relaxation in Thoracic Aorta and Superior Mesenteric Artery in the Tissue Bath Assay.

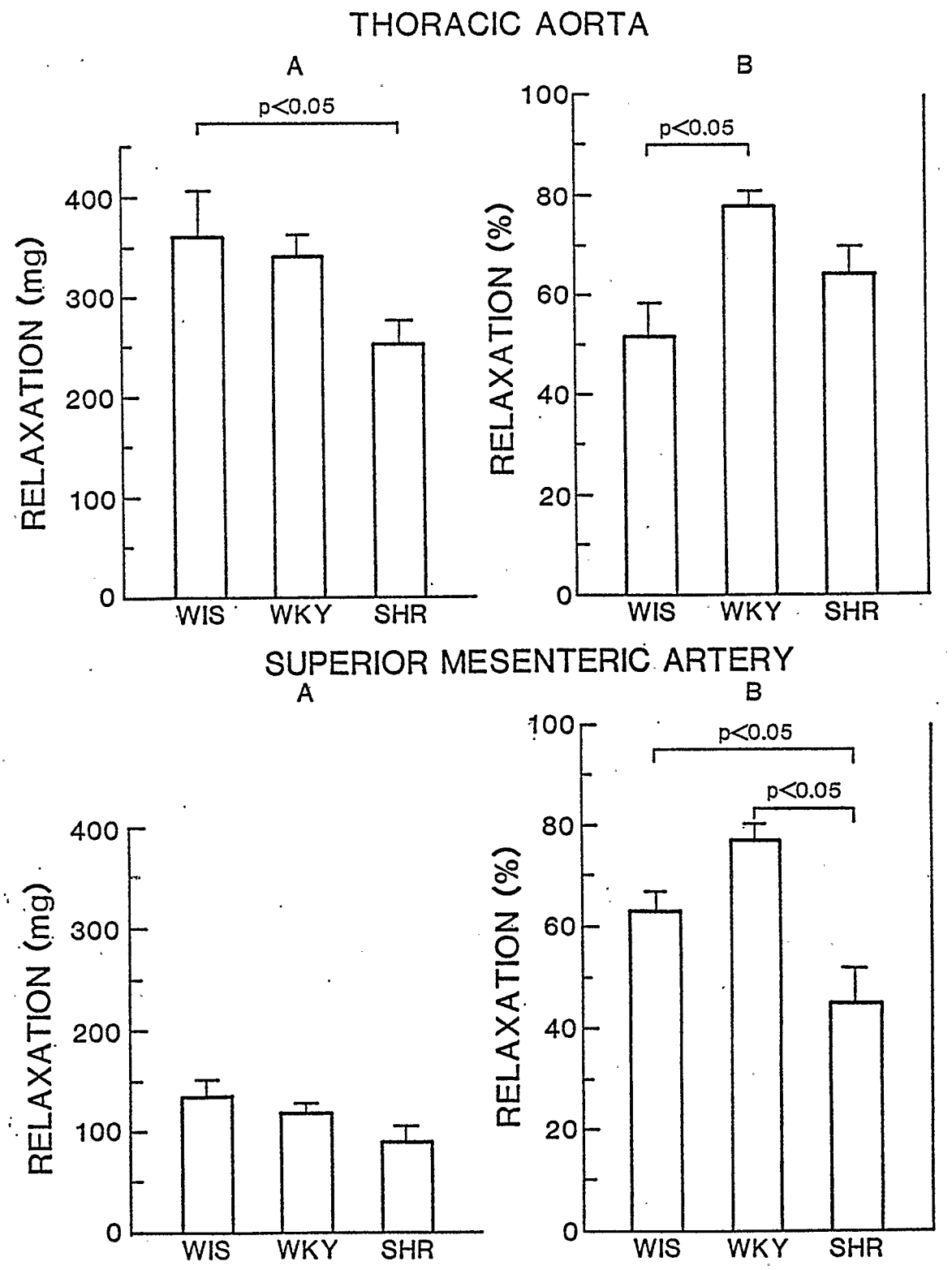
The symbols are as follows: A = Relaxation (mg) induced by forskolin 0.1 μM, and B = Relaxation (normalized to 0.1 mM papaverine relaxation) induced by forskolin 0.1 μM.

Thoracic aorta were precontracted with norepinephrine 0.1 μM and superior mesenteric artery with norepinephrine 1 μM.

The experimental numbers in the thoracic aorta are as follows: Wistar rat n=12, WKY rat n=11, and SHR n=9. In the superior mesenteric artery experimental numbers are n=11 for the Wistar rat, WKY rat and SHR.

Vertical bars represent mean changes in relaxation (% or mg) and vertical lines represent SEM.

Refer to text for explanation of significant differences ($p<0.05$).


THORACIC AORTA
A
p<0.05
RELAXATION (mg)
400
300
200
100
0
WIS
WKY
SHR
B
p<0.05
RELAXATION (%)
100
80
60
40
20
0
WIS
WKY
SHR
SUPERIOR MESENTERIC ARTERY
A
RELAXATION (mg)
400
300
200
100
0
WIS
WKY
SHR
B
p<0.05
p<0.05
RELAXATION (%)
100
80
60
40
20
0
WIS
WKY
SHR

IV. DISCUSSION

The experiments were performed to determine the effects of VIP and forskolin in norepinephrine pre-contracted strips of SHR, Wistar rat, and WKY rat thoracic aortae and superior mesenteric artery. Results show that there is a reduction in the relaxant response to VIP in the superior mesenteric artery of hypertensive animals. Studies utilizing forskolin do not provide a definitive conclusion regarding forskolin activity in the SHR. The findings are inconclusive, because results obtained when forskolin relaxation is expressed as an absolute measure of relaxation (mg), differ from results obtained when forskolin relaxation is normalized to papaverine relaxation, indicating that forskolin may be interfering with papaverine's ability to relax the vessels. Additionally, the relaxant response of papaverine was significantly lower in the Wistar rat and WKY rat in the forskolin studies as compared to the VIP studies. Because inconsistency in forskolin studies with the hypertensive rat has arisen in the literature (62-66), it appears that the effects of forskolin in the SHR have yet to be elucidated.

The mechanism of action of papaverine is proposed to involve the inhibition of cyclic nucleotide phosphodiesterase (69). Forskolin at 10 μM caused minimal inhibition of cyclic nucleotide phophodiesterase (61). It may be that forskolin is able to slightly interfere with papaverine's inhibition of

phosphodiesterase, resulting in a small diminishment of relaxation.

A. Methodology

Generally speaking, thoracic aorta was the vessel most sensitive to norepinephrine. In thoracic aorta, norepinephrine began to elicit contractions at 1 pM, while maximal contractions were observed at 0.1 μM to 1 μM. There was a reduction in the contractile activity at 10 μM, possibly due to desensitization or internalization of receptors.

In all the contractility studies, the thoracic aorta was the most reactive to each of the agonists. It is likely that the increased reactivity in the thoracic aorta is a function of the amount of smooth muscle mass available to respond to each agonist. Because the thoracic aorta is thicker than the other blood vessels, it may have possessed more tissue mass, resulting in greater contractions when measured in terms of isometric tension changes recorded through a force-displacement transducer.

The iliac artery followed the thoracic aorta in terms of the magnitudes obtained with norepinephrine. Again, slight contractions were observed first at 1 pM, while maximal contractions were observed first at 10 μM. It is possible that greater contractions may have been observed with higher concentrations of norepinephrine, however, because this vessel

was not useful for VIP relaxation studies, this parameter was not further explored.

In both the superior mesenteric and carotid arteries, contractions were initially observed at norepinephrine 0.1 nM. The peak contractions were observed at norepinephrine 1 μM and 0.1 μM respectively. Desensitization or internalization likely caused the diminishment in magnitude of the contractions beyond these concentrations. The carotid artery was the least responsive to norepinephrine of the vessels studied. This may be due to a reduction of adrenergic receptors in this vessel, or more likely, the difficulty in dissecting and preparing the vessel for study.

The effects of prostaglandin $F_{2\alpha}$ closely resembled the contractile effects of norepinephrine in the various vessels. Thoracic aorta was the most responsive, followed by superior mesenteric, iliac, and carotid arteries. In all vessels, contractility effects were initially observed at 3 nM, with the exception of the thoracic aorta which first exhibited contractile effects at 1 nM. Maximal contractions of all vessels was observed at 10 μM, so it is likely that greater contractions may have been achieved by higher concentrations of the hormone. However, not only was this not feasible for the numerous experiments, the relaxant response to VIP was not achieved in prostaglandin $F_{2\alpha}$ precontracted strips. Again, the greater contractile response of the thoracic aorta over the other vessels most likely can be explained by the greater

thickness of each segment, and the poor response of the carotid artery is likely due to damage incurred during preparation.

The relaxant response to VIP in norepinephrine precontracted vessels generally corresponds to the relative densities of VIP nerves illustrated in Figure 2. The exception is thoracic aortae precontracted with lower concentrations of norepinephrine, which appeared to achieve the greatest relaxation to VIP. However, because it was extremely difficult to obtain stable and consistent contractions which maintained a plateau long enough to examine VIP's relaxant effect, it was believed that this data was not completely reliable. As a result, higher concentrations were used for study.

Potassium chloride acts primarily by promoting entry of calcium from extracellular fluid, resulting in vasoconstriction. It is possible that the potassium effect was minimal as compared to norepinephrine, because vasoconstriction produced by potassium requires more calcium in the medium (96). Norepinephrine's effects, on the other hand, can still be substantial (80%) in a calcium-free medium as it's action is by the release of calcium from intracellular stores (96).

The dose-response curves of forskolin in norepinephrine precontracted strips were similar between the thoracic aorta and superior mesenteric artery. These responses were

anticipated as forskolin's relaxation is not induced by a receptor dependent mechanism.

B.VIP Relaxation

Norepinephrine, the agonist utilized, evoked significantly stronger contractions in Wistar rats than in WKY rats and SHR. This may be due to the fact that the Wistar rat is much larger than either the WKY rat or the SHR at the same age. It is conceivable that the thickness of each vessel segment was greater in the Wistar rat than those of the WKY rat or SHR, thus, producing a greater contractile effect as a greater smooth muscle mass would be available to respond to the norepinephrine. When measured in terms of isometric tension changes recorded through force-displacement, the end result was greater contractions for the Wistar rats. Blood pressure differences did not play a role in norepinephrine's contractile ability, as the normotensive WKY rat experienced contractions of the same magnitude as the SHR.

The relaxant response of forskolin and VIP was quantitated by comparison to maximal relaxation of the same arterial strip by 0.1 mM papaverine, which was taken as a 100% response. Because norepinephrine-induced contractions were greater in the Wistar rat than in the WKY rat or SHR, it was clear that papaverine-induced relaxations would be greater in the Wistar rat than in the WKY rat or SHR. Such was the case.

Additionally, the contractile effects of norepinephrine and the relaxant effects of papaverine are greater in the thoracic aorta than in the superior mesenteric artery. Like the difference previously mentioned between Wistar rat and both WKY rat and SHR, the magnitudes of norepinephrine contractions and papaverine relaxations in the different vessels can be attributed to a greater mass of the thoracic aorta segments than of the superior mesenteric artery segments.

It was found that there was no significant difference between SHR, WKY rat, and Wistar rat in the thoracic aorta, in terms of VIP induced relaxation when expressed as a percent of papaverine relaxation. Additionally, when VIP relaxation is expressed as an absolute measure of tension, a significant difference existed only between the two normotensive animals. These results indicate that VIP induced relaxation in the thoracic aorta is unaffected by a hypertensive state.

On the other hand, it was found that there was a significant reduction in the relaxant response to VIP in the SHR superior mesenteric artery as compared to WKY rats and Wistar rats. This was the case when VIP relaxation was expressed as both an absolute measure of tension and also as a percent of papaverine relaxation. There are a few possible explanations for the reduced VIP induced relaxation in the superior mesenteric artery in the hypertensive state. Firstly, it may be that morphological changes in the superior

mesenteric artery of the SHR occurred, versus that of normotensive animals, lending itself towards a diminishment of vasodilatory activity. Other studies suggesting a reduction of relaxation in the SHR include agents such as isoproterenol, a β- adrenoceptor agonist (62-64); norepinephrine, a β-adrenoceptor agonist (64-66); and epinephrine, a β-adrenoceptor agonist; dopamine, a β_1-adrenoceptor agonist; and glucagon, whose mechanism of action is similar to VIP (62). However no reduction of the dilators fenoterol, a β_2-adrenoceptor agonist (64); nifedipine, a calcium channel blocker; and nitroprusside, which decomposes to release nitric oxide and activates guanylate cyclase in vascular smooth muscle (65,66) was observed, suggesting this phenomenom is not generalized to all vasodilators.
Furthermore, significant changes in the wall stucture occurs in the aorta of the SHR too (74-81), however, no difference was observed in the VIP relaxation of either normotensive's or hypertensive's thoracic aortae.

Another factor which may contribute to the reduction of VIP induced relaxation in the superior mesenteric artery but not in the thoracic aorta, may be that the superior mesenteric artery is a much smaller and less resilient vessel than the thoracic aorta, and although one might expect to see changes in only the resistance vessels of the hypertensive animals, clearly the superior mesenteric artery more closely resembles this situation than the larger, thicker, more elastic aorta.

With the procedure utilized, it was not possible to examine vessels whose diameter was significantly smaller than the superior mesenteric artery, therefore, the results obtained from the superior mesenteric artery most closely approximate what one may find in the smaller vessels.

Lastly, there may be an abnormality in the VIP-effector system in the superior mesenteric artery of hypertensive animals. Because others have found a reduction in VIP-sensitive adenylate cyclase activity in the hearts of the SHR (84), and impairments of the adenylate cyclase pathway as indicated by a decrease in the inotropic response of left ventricle hypertrophy in hypertensive rats (85), the adenylate cyclase pathway was studied utilizing the in vitro bioassay.

C. Forskolin Relaxation

To further explore the possibility that an alteration in adenylate cyclase activity may be causing the observed reduction of relaxation in the SHR superior mesenteric artery, forskolin induced relaxation was observed. While VIP activates adenylate cyclase after interacting with a specific membrane bound receptor, forskolin directly acts upon the catalytic subunit of adenylate cyclase.

Results indicate that there is a reduction in forskolin induced relaxation in the superior mesenteric artery of the SHR, when expressed as percent relaxation. However, when

relaxation was expressed as an absolute measure of tension, there was no significant difference between each of the three groups. Furthermore, in the thoracic aorta there was a significant difference between the two normotensive animals when forskolin relaxation was expressed as percent relaxation, and a significant difference between Wistar rats and SHR when expressed as mg tension change.

Because no difference in forskolin relaxation was observed between the WKY rat and SHR in the thoacic aorta, it is conceivable that hypertension again, may not play a significant role in the relaxation properties of the thoracic aorta. Differences observed in the aortas of Wistar rats may be due to the heterogeneity of the outbred animal.

There was no significant difference in forskolin relaxation between Wistar rat, WKY rat, and SHR in the superior mesenteric artery, when expressed as an absolute measure of relaxation. However, when values were normalized to papaverine relaxation, there was a significant difference between the hypertensive and normotensive rats. This seems to suggest that papaverine relaxation is affected by prior addition of forskolin.

In the superior mesenteric artery, there was no significant difference ($p>0.05$) between papaverine relaxation in the SHR, for the VIP and forskolin studies, however, significant differences ($p<0.05$) did exist in the Wistar rats and WKY rats between the VIP and forskolin studies. It

appears that papaverine relaxation was not as great in the forskolin studies for the normotensive rats, suggesting that forskolin may interfere with papaverine's ability to relax vessels. These findings emphasize the importance of presenting relaxation data as absolute measures of relaxation in addition to normalization to papaverine. The present evidence does not permit a definitive conclusion regarding adenylate cyclase activity in the SHR.

The data available on forskolin relaxation in the literature indicates both a diminishment of relaxation as well as no reduction of relaxation in the SHR (63-66). These studies utilize various agonists prior to the addition of forskolin, which may contribute to some discrepancies. In addition, most express forskolin relaxation only as a percent of the maximum relaxation induced by 0.1 mM papaverine, and do not present the absolute measure of relaxation (mg) data. From the observations in the present study regarding means of expressing forskolin relaxation, this practice could be misleading. In short, not only the use of different agonists but also the normalization of forskolin data to papaverine only, may explain inconsistencies in the literature. In order to elucidate forskolin relaxation in the SHR, it may be necessary to conduct a study utilizing various agonists and constructing full dose-response curves.

It may be that the guanine nucleotide regulatory subunit of adenylate cyclase is altered in the hypertensive condition.

Published work indicates that a reduction of β-adrenoceptor responsiveness is due to a diminishment of the stimulatory GTP - binding protein (G_s) in the SHR (66,97). It is also conceivable that a reduced function of a GTP binding protein is responsible for a diminishment of VIP relaxation in the SHR superior mesenteric artery.

In conclusion, it is evident that there is a diminishment of VIP relaxation in the superior mesenteric artery of the SHR. Conversely, the experiments utilizing forskolin to probe adenylate cyclase in the superior mesenteric artery are inconclusive for reasons stated above. It remains to be determined whether the mechanism of the reduction in VIP relaxation occurs at the receptor or adenylate cyclase level. However, it is possible that factors contributing to the reduction of VIP relaxation in the superior mesenteric artery also contribute to a state of increased peripheral resistance, and thus, hypertension, in the SHR.

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