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COMPARISON OF HEMODYNAMIC CHARACTERISTICS OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS (WITH PARTICULAR REFERENCE TO THE ADAPTABILITY OF THE HEART TO SUSTAINED HYPERTENSION)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

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BY

MARC A. PFEFFER

Oklahoma City, Oklahoma

1972

COMPARISON OF HEMODYNAMIC CHARACTERISTICS OF NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS (WITH PARTICULAR REFERENCE TO THE ADAPTABILITY OF THE HEART TO SUSTAINED HYPERTENSION)

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To My Wife, Jan, and Our Family

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CHAPTER I

INTRODUCTION

Historical Development of Arterial Pressure Measurement

The first quantitative measurement of arterial blood pressure was made by Stephen Hales in 1733. Hales determined the height at which blood rose above the level of the heart by directly connecting a series of vertically positioned tubes (i.e., brass pipes, glass tubing and sometimes for pliancy, the windpipe of a goose), into an artery (1, 2). He clearly understood that the heart provided the necessary force for the circulation of blood, and correctly used the heart as the appropriate reference point for measuring the height above which the column of blood was supported. The introduction of the mercury filled U-tube manometer for blood pressure measurements, in 1828, by Poiseuille eliminated the need for long vertical manometers and introduced practical blood pressure measurements into the laboratory (3). By placing a float attached to a pointer at one end of the column of mercury Ludwig, in 1847, produced graphic recordings of arterial pulses on a smoked-drum kymograph (4). Even at this time it was recognized that, because of friction and inertia, the oscillations

recorded on the kymograph from the movements of the column of mercury did not accurately represent the actual pressure changes that occurred within the vessels during systole and diastole. However, mean arterial pressure was obtained from this apparatus by narrowing the caliber of the connection between the vessel and the column of mercury. Thus, the intermittent pressure changes in the large arteries were not observed, and consequently, only the mean pressure which the blood exerted upon the vascular walls was measured (5, 6).

The rapid pressure changes that occur in the arterial system were more accurately displayed by newer pressure measuring apparatus. In 1861, Chauveau and Marey inserted small elastic bags into the aorta and cardiac chambers (7). These elastic bags communicated by an air filled tube to a tambour and lever system for recording. This system produced remarkable tracings of the sequence of events in the cardiac cycle. Fick developed a C-spring manometer, in 1864, which also measured blood pressure with improved fidelity (8). It is of interest that Foster's 1878 Textbook of Physiology describes the controversy between Marey and Fick over atrial pressure recordings (6). Marey reported a steep rise and fall in atrial pressure (atrial systole) just prior to ventricular contraction, whereas Fick's measurements of atrial pressure did not reveal these large changes in atrial pressure. This discrepancy was attributed to the different types of manometers used, and illustrates the state of the art of pressure measurements at the turn of the century. Many manometers of varying design were in use and, as a result, there were a variety of pressure curves produced.

Otto Frank converted the measurement of pressure from an art to

a science by formulating the theoretical principles for the construction of efficient pressure measuring manometers with a high "figure of merit"[1] (9). He discarded the mechanical lever system and substituted the reflection of a narrow beam of light by a tiny mirror attached to the membrane. This provided for greater magnification of the reflected beam on a moving photosensitive surface. With the beam of light serving as the lever all the other movable masses were eliminated (10).

Such great cardiovascular physiologists as Frank, Wiggers and Hamilton used the optical technique to provide accurate and reliable information of pressure pulses in the cardiovascular system (10, 11). The decline in the use of optical manometers from the 1930's to the present was not the result of the lack of high fidelity recordings by these devices, but because such recordings could be obtained with less difficulty with electrical manometers. Based on the principle that slight distortions of stiff membranes could be used to alter currents or voltages, the present day electrical transducer, when coupled with the proper amplifier and recorder, reproduces pressure wave changes with accuracy. Because of the great potential for amplifying electrical changes, the sensitivity of these systems can be increased without decreasing the volume elasticity coefficient. The amplification of these systems can be so great that the slight electrical changes produced in the pressure transducers can provide the power to drive direct writing recording

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[1] Figure of merit is equal to the product of the magnification of the lever by the sensitivity of the elastic membrane and the volume elasticity coefficient divided by the effective mass. A high figure of merit was obtained by eliminating all elastic structures other than the membrane and reducing effective mass.

instruments (12). After moving away from mechanical writing devices to the optical system, this return to mechanical writing has been made without a loss of frequency response. It is of further personal interest, when one reflects upon these historical traditions of pressure recording instruments, that the modern day cardiovascular laboratory depends upon the mercury manometer to calibrate its most recent electronic transducers.

Hemodynamic Concepts

With each ventricular contraction blood is ejected into the aorta at a rate which exceeds the runoff through the peripheral arterioles and into the capillaries. During early systole the volume and pressure of blood in the aorta and its major arterial branches increase. In contrast, during diastole blood leaves these vessels at a greater rate than it enters, and arterial blood volume and pressure decline. During systole only part of the energy imparted by each cardiac contraction is utilized in the production of forward capillary flow. Most of the energy is converted to potential energy within the distensible walls of the arterial system. The temporary storage of most of the stroke volume by the arterial system, termed Windkessel effect, transforms the intermittent input into the arterial system into a more even outflow by elastic recoil during diastole.

The pulse pressure, the difference between systolic and diastolic arterial pressures, is principally a function of the stroke volume and the arterial capacitance (dV/dP, change in volume with respect to pressure). When arterial capacitance is small, the vessels are rigid, and slight changes in arterial volume produce relatively large changes in pressure. Conversely, when arterial capacitance is high, the same volume

increment increases pressure only modestly. Thus, given the same cardiac output and total peripheral resistance and, therefore, mean arterial pressure, the oscillations of pressure about the mean will be greater when the arterial capacitance is lower (13). If the distensible arterial system were replaced by rigid tubes, the capacitance would be extremely small and, therefore, the pulse pressure would be unusually large. In addition, there would be no Windkessel effect of the arterial system; consequently, blood flow through the arterioles would be intermittent.

The mean pressure that exists in the aorta and its branches during an entire cardiac cycle can be obtained by measuring the area under the arterial pressure curve and dividing by the cardiac cycle duration. More practically, mean arterial pressure can be determined by damping the phasic fluctuations in arterial pressure. This may be accomplished electronically or hydraulically; both methods greatly reduce the frequency response of the recording system so that only the mean arterial pressure is displayed.

Mean arterial pressure is the average pressure during an entire cardiac cycle which is available to propel blood through peripheral vessels. Since the arterial system is an open system, its volume is dependent on the rate of inflow (cardiac output) and the rate of outflow (peripheral runoff). The peripheral runoff, or flow, is determined by the mean arterial to right atrial pressure gradient and the resistance to flow. Since right atrial pressure is close to zero, mean arterial pressure itself is often used in place of the actual pressure gradient.

Resistance to the flow of blood in a vessel cannot be directly measured. It is calculated by dividing the pressure difference across a

vascular bed by the blood flow of that vasculature. For example, the resistance of the whole systemic circulation (i.e., total peripheral resistance) is obtained by dividing the mean arterial pressure by the cardiac output.[2] Calculations of total peripheral resistance provide an index of the difficulty in driving flow through the vasculature. The mean arterial pressure is therefore the product of the total peripheral resistance and cardiac output.

Arteries supplying the heart, brain, kidney, splanchnic organs, muscle, skin, and bone all have the same mean pressure. However, the blood flow to each of these systemic beds is different. This disproportionate distribution of the cardiac output is the result of the local resistance offered by each vasculature. The total peripheral resistance is the sum of all these individual resistances in series. From the Poiseuille relationship[3], it is apparent that resistance changes usually indicate alterations in arteriolar geometry. When pressure, vascular length, and blood viscosity are relatively constant, flow is proportional to the fourth power of the vascular radius. Thus, small changes in

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[2]When pressure is expressed as mm Hg and flow in ml/sec, the arbitrary units of resistance are in mm Hg/ml/sec or peripheral resistance units (PRU). Peripheral resistance units can also be expressed as mm Hg/L/min. It may also be calculated in c.g.s. units, with pressure in dyne/cm^2 (1 mm Hg = 1,333 dyne/cm^2) and flow in cm^3/sec (ml/sec); in this case, resistance units are in dyne sec/cm^5.

[3]Hagen in 1839 and Poiseuille in 1842, independently published experiments describing the factors regulating the laminar flow of fluid through rigid cylindrical tubes (14). Flow was found to be equal to the pressure difference across the tube divided by the resistance to flow afforded by the tube. The resistance (R) is equal to $8L\eta/\pi r^4$, where L is the length of tube, η the viscosity of the fluid, and r the radius of the tube. Though the Hagen-Poiseuille Law was derived for steady flow through rigid tubes, it does demonstrate that the radius is the major determinant of resistance.

arteriolar diameter can alter the distribution of cardiac output and the mean arterial pressure. By maintaining mean arterial pressure, regional tissue perfusion can be controlled by regulating the diameter of the "stopcocks" of the vascular system.

Regulation of Arterial Pressure

Since mean arterial pressure is determined by the product of total peripheral resistance and cardiac output, changes in the latter two variables obviously will alter arterial pressure. However, it is well known that during a variety of physiological conditions major changes in total peripheral resistance and cardiac output can occur without affecting mean arterial pressure. For example, when a large arteriovenous fistula is opened and closed, the total peripheral resistance can change as much as 100% without altering arterial pressure (15). In addition, during moderate degrees of hemorrhage cardiac output is progressively reduced without parallel reductions in arterial pressure (16). Perhaps the most dramatic example of the maintenance of mean arterial pressure with drastic, though proportional, changes in cardiac output and total peripheral resistance is exhibited in animals which demonstrate the "diving reflex" (17, 18). These animals can remain under water for extended periods of time because their oxygen reserves are delivered almost entirely to the heart and brain. Upon submersion there is a prompt vasoconstriction and bradycardia. The cardiac output may be reduced to as much as one-twentieth of the resting level, although the mean arterial pressure is maintained by an intense peripheral vasoconstriction (19). Upon resurfacing these changes are reversed and the oxygen debt of the underperfused

tissues is repaid by a cardiac output which may increase to amounts fifty times greater than during submersion (20). Clearly, the maintenance of relatively stable mean arterial pressure during drastic changes in cardiac output and total peripheral resistance demonstrates the remarkable ability of the cardiovascular system to regulate arterial pressure.

Many different systems are known to play important roles in this regulation of arterial pressure. All of these arterial pressure controlling systems combine to maintain arterial pressure at a stable level from second to second and month to month. Each system has different characteristics in terms of pressure ranges in which they function, time course of action, and gain (ability to return altered pressure back to original level). However, it must be emphasized that effective pressure control is the result of a concerted action of many pressure controlling sytems. The autonomic nervous system responds in seconds to sudden alterations in arterial pressure, whereas the regulation of arterial pressure by hormonal, body fluid, and renal controlling mechanisms requires longer periods (minutes, hours to days) to correct disturbances of arterial pressure. Each of the important pressure controlling systems will be briefly discussed to present only the highlights of a wide variety of systems with different mechanisms for detecting arterial pressure alterations and returning pressure to its original level.

Neural Regulation of Arterial Pressure

Autonomic nervous control of cardiovascular function is accomplished through vasomotor center regulation of cardiac performance and

of the caliber of peripheral blood vessels. Both the rate and the force of cardiac contraction are under the influence of both divisions of the autonomic nervous system, the sympathetic and parasympathetic.

Pre- and postganglionic neurons of the sympathetic division synapse in the thoracolumbar chain of sympathetic ganglia along both sides of the spinal cord or in collateral ganglia near the viscera (21). The neurotransmitter at this synapse is acetylcholine. The postganglionic adrenergic fibers are distributed throughout the body, most of which exert their action by the release of norepinephrine. Sympathetic input to the heart is mainly supplied through the stellate ganglion. Stimulation of cardiac sympathetic nerves produces a release of norepinephrine from the nerve ending, which increases the concentration of norepinephrine that is free to couple with myocardial beta-adrenergic receptors. Beta adrenergic stimulation, by increasing both the rate and force of cardiac contractions, improves the pumping performance of the heart.

The cardiac actions of the sympathetic division are actively opposed by the parasympathetic division of the autonomic nervous system. The vagus (tenth cranial) nerve supplies the parasympathetic innervation to the heart. In the parasympathetic division, the pre- to postganglionic synapse occurs very close to the end organ; thus, the postganglionic fibers are extremely short (1-300 mm) (22). As in the sympathetic division, the preganglionic neurons of the parasympathetic division secrete acetylcholine; but unlike the adrenergic system, short postganglionic neurons are also cholinergic. Vagal fibers are predominantly distributed near the sinoatrial and atrioventricular nodes (23), and the acetylcholine released decreases the rate of sinoatrial node discharge. Right vagal

nerve stimulation produces sinus bradycardia and can completely stop sinoatrial node activity for several seconds. Although the predominant effect of vagal stimulation is chronotropic (slowing cardiac rate), it also reduces the inotropic force of cardiac contraction (24).

The opposing sympathetic and parasympathetic cardiac effects are not simply algebraically additive (25). Although acetylcholine has only slight negative inotropic activity, vagal stimulation has a much more pronounced negative inotropic and chronotropic effect when the background of sympathetic activity is raised (26). Thus, the adrenergic and cholinergic nerve endings interact in a complex fashion to alter intrinsic cardiac performance.

Neural regulation of the peripheral circulation permits rapid adjustment of blood flow distribution. By far the most important factor in neurogenic control of vascular resistance are the adrenergic vasoconstrictor fibers to the precapillary arterioles. These fibers release the neurohumoral substance, norepinephrine, which couples with alpha receptors on the vascular smooth muscle membrane to effect vasoconstriction. Vasomotor activity is tonic (i.e., impulses continuously travel along adrenergic fibers) permitting the change in vessel geometry by altered activity of these vasoconstrictor fibers. Thus, vasoconstriction is produced by a relative increase, and vasodilation by a relative decrease, in the frequency of sympathetic vasomotor nerve firing. Though vasoconstrictor innervation is ubiquitous, coronary and cerebral vessels are only sparsely supplied. Stimulation of sympathetic activity by hemorrhage increases the fraction of the cardiac output delivered to the heart and brain at the expense of other organs which are more densely supplied with

sympathetic innervation (28). In addition to adrenergic vasoconstrictor fibers, skeletal muscle is also innervated with sympathetic vasodilator (cholinergic) fibers which are believed to increase skeletal muscle blood flow through acetylcholine release in anticipation of their use in emergency (i.e., fight or flight situation) (29). Thus, although the neural control of cardiac function is accomplished by the interplay of both sympathetic and parasympathetic innervation, neural regulation of peripheral vasculature is almost exclusively performed by the sympathetic system. In order for the vasomotor center to initiate the proper efferent outflow to the heart and vessels it must constantly receive afferent information on the status of cardiovascular function.

The central nervous system receives sensory input of arterial pressure levels on a beat to beat basis. Mechanoreceptors located mainly in the carotid sinus and aortic arch initiate impulses when stretched by the arterial pressure pulse. These impulses travel through the sinus nerve to the vasomotor center of the medulla, where afferent information from all mechanoreceptors is received. When arterial pressure is suddenly elevated, increased distension of mechanoreceptors enhance their firing rate (30). This initiates an integrated reflexive response from the vasomotor center thereby increasing parasympathetic activity to the heart and decreasing sympathetic activity to peripheral arterioles (i.e., vasodilation). Thus, the heart rate and the cardiac output are reduced and the vasodilation lowers the peripheral resistance. The net result is the return of arterial pressure towards pre-stimulated levels. The efferent limb of this reflex was actually discovered by Cyon and Ludwig, in 1866, when they showed that stimulation of the central end of the

depressor nerve of a rabbit caused a marked fall in arterial pressure and bradycardia (31).

Mechanoreceptor reflexive control is also exerted when arterial pressure is lower. For example, when an individual rapidly stands upright from the horizontal position, venous return and hence arterial pressure are reduced because of the redistribution of blood into distendable veins below the heart. However, the reduced pressure decreases mechanoreceptor firing and initiates a reflexive response from the vasomotor center. This response buffers the pressure drop by increasing sympathetic tone and therefore, compensating for gravitational shifts in blood distribution during postural changes. It is of interest that animals like snakes and eels which do not assume the upright position and have extremely poor vasomotor responses can be killed when their heads are immobilized in the vertical position (32). In this extreme case, arterial pressure is not maintained and cerebral perfusion is fatally reduced.

Although chemoreceptor function is mainly concerned with augmentation of ventilatory rate and depth, they can, like mechanoreceptors, increase sympathetic activity and hence arterial pressure. Corneille Heymans demonstrated that small vascular bodies arising from the internal carotid artery and aorta (glomus caroticum and glomus aorticum, respectively) contained chemoreceptors (33). These receptors could produce reflexive changes in respiration and cardiovascular function when stimulated with reductions in arterial blood oxygen tension or increases in carbon dioxide levels. Although stimulation of these receptors primarily affects respiration, the vasomotor center is also influenced. By

reflexively increasing peripheral resistance, arterial pressure is raised resulting in better perfusion of aortic and carotid bodies and consequently the brain.

A marked reduction in arterial pressure can result in ischemia to the vasomotor center itself. Although this pressure regulating system functions only at low levels of arterial pressure, vasomotor center ischemia can produce powerful sympathetic stimulation (34). This mechanism is, in effect, a last effort to raise arterial pressure by enhancing cardiac activity and increasing peripheral resistance. These neural mechanisms which were briefly discussed, can be termed acute regulators of arterial pressure tending immediately to buffer pressure changes.

Renal Body Fluid and Hormonal Regulation of Arterial Pressure

Fluid exchange between the plasma and interstitial fluid compartment plays a more intermediate time role in regulating arterial pressure. Normally, the hydrostatic and colloid osmotic pressure in the capillaries balance each other, and no net change in the volume of these fluid compartments occurs. However, this balance of the pressure forces can be disturbed by relative changes in the relationship between the pre- and post-capillary resistances and thus, capillary hydrostatic pressure. When arterial pressure is slightly decreased, as in moderate hemorrhage, reduced mechanoreceptor firing initiates a reflexive increase in sympathetic vasomotor outflow. Capillary pressure is reduced because of the relative increase in pre- to post-capillary resistances. From the Starling hypothesis (of 1896) describing fluid movements across capillary endothelium (35), it is known that when the capillary hydrostatic pressure is lowered with plasma oncotic, interstitial fluid oncotic and interstitial

fluid hydrostatic pressures remaining constant, the net movement of fluid is directed from the interstitial to plasma compartment. This absorption of interstitial fluids tends to return the reduced arterial pressure toward normal. Conversely, increased capillary hydrostatic pressure (for example, resulting increased blood volume, or increased venous pressure) may cause a net filtration of plasma into the interstitial space. Thus, the capillary shift mechanism of pressure regulation finely adjusts plasma volume in the opposite direction as the pressure change, thereby tending to maintain arterial pressure.

Regulation of extracellular fluid volume is probably one of the most important mechanisms in the long-term regulation of arterial pressure. Thus, with any disturbance of arterial pressure, the extracellular fluid volume changes until pressure is restored to normal levels. When pressure is elevated, extracellular fluid volume may decline over periods of days and weeks until pressure has returned to normal. Only at normal arterial pressure levels is the output of salt and water equal to the intake, and the rate of change of extracellular fluid volume then is zero (36). Thus, this slow onset, long-term system has an infinite gain (i.e., it will return arterial pressure to its original level), so that extracellular fluid volume will continue to change until that level of arterial pressure is achieved which causes an equilibrium between intake and output of water (37). Because of their role in the maintenance of arterial pressure, these factors involved in extracellular fluid volume regulation will be discussed in relation to pressure changes.

Reduction of arterial pressure increases the release of renin from the kidney (38). Renin is a proteolytic enzyme which produces

angiotensin I from the plasma protein angiotensinogen. In the plasma, probably in the lesser circulation, a converting enzyme cleaves a dipeptide from the decapeptide, angiotensin I, to form an octapeptide, angiotensin II. Angiotensin II has several effects which elevate arterial pressure. On a weight basis angiotensin II is the most potent vasopressor agent known, and as such directly constricts peripheral arterioles to increase total peripheral resistance (39). It also stimulates the release of aldosterone from the adrenal cortex, promoting sodium retention and extracellular fluid volume expansion (40). More recently, angiotensin also has been shown to elevate arterial pressure indirectly through an effect upon the central nervous system (41-43). This action appears to involve the area postrema, a region in the caudal medulla where the blood-brain barrier is permeable to this small octapeptide (44). The resulting rise in arterial pressure produced by this central action of angiotensin most likely is mediated through increased adrenergic vasomotor outflow to increased peripheral resistance (45), although a withdrawal of parasympathetic activity has also been proposed (46). Angiotensin, therefore, raises arterial pressure by several mechanisms each of which serves to remove the stimulus for the renin release--the reduced arterial pressure.

Renin release seems to be controlled by intrarenal and extrarenal mechanisms. The intrarenal baroreceptor and macula densa theories suggest that the stimulus for renin release occurs within the kidney. According to the baroreceptor theory, with reduced renal arterial pressure a decreased stretch on the afferent arteriolar wall is "sensed" by the juxtaglomerular cells surrounding these vessels, thereby releasing stored

renin from its cytoplasmic granules (47). The macula densa theory suggests that the renin release from the juxtaglomerular cells is controlled by changes in the sodium load presented to the distal tubule. The macula densa is an accumulation of epithelial cells at the distal tubule as it passes in close proximity to its own glomerulus (48). With a change in the load of filtered sodium, the macula densa cells through, an as yet unknown mechanism, relay this information to the juxtaglomerular cells which release renin from its granular store (48-50).

Neural and hormonal factors also modify renin release (51). Thus, renal sympathetic nerve stimulation and circulating catecholamines increase renin release (52). These adrenergic influences may affect renin release directly or indirectly by altering the filtered load of sodium. The importance of the renin-angiotensin system in arterial pressure homeostasis is emphasized by the multiplicity of factors involved in the control of renin release and by the variety of mechanisms which angiotensin elevates arterial pressure.

Antidiuretic hormone (ADH, vasopressin) is another hormone which regulates arterial pressure by controlling renal water retention and directly constricting vascular smooth muscle. This octapeptide is produced in the supraoptic and paraventricular nuclei of the hypothalamus and is stored in the posterior pituitary gland (53). Antidiuretic hormone controls distal tubular and collecting duct water permeability (54). In its presence water is reabsorbed by the distal tubule and collecting duct following the osmotic gradient generated by the countercurrent mechanism, thereby producing a concentrated urine (urine osmolality greater than plasma). Hypothalamic osmoreceptors (and possibly

others less central) respond to plasma osmolality. When osmolality increases (as in contraction of intravascular volume), the posterior pituitary releases antidiuretic hormone. Osmoreceptor stimulation also stimulates thirst,so that in addition to the greater water retention by the kidney, there is increased desire for fluid intake. Antidiuretic secretion is also affected by low-pressure volume receptors located at the venoatrial junctions (55). Thus, atrial distention initiates cardiovascular reflexes which influence centers responsible for inhibition of ADH (56). As a result of the reduced ADH levels, the urine becomes less concentrated and intravascular volume decreases. Antidiuretic hormone also produces peripheral vasoconstriction; however, the levels of circulating hormone required for this effect are much higher than the amounts normally secreted in the regulation of distal tubule and collecting duct water reabsorption (57). This effect of the hormone is especially significant during hemorrhagic hypotension where large amounts of antidiuretic hormone are released (58).

Several other hormones have direct effects on vascular caliber and, therefore, resistance. During sympathetic stimulation, whether tonically mediated or through increased vasomotor outflow, there is a release of norepinephrine from the adrenergic nerve endings. This release of the neurohumoral substance is enhanced by the secretion of a mixture of norepinephrine and epinephrine from the adrenal medulla. The actions of these and other catecholamines were recently popularized by Ahlquist when he proposed the alpha and beta receptor classification (59). Briefly, norepinephrine combines much more readily with alpha receptors in vascular smooth muscle to cause constriction. Epinephrine can stimulate alpha

receptors; however, it has a greater affinity for beta receptors, causing vasodilation. Both norepinephrine and epinephrine stimulate cardiac beta receptors, thereby increasing the rate and force of cardiac contractions. Nevertheless, the release of these catecholamines into the blood by the adrenal medulla is much less important in cardiovascular regulation than direct sympathetic discharge, which releases norepinephrine at the target effector site (60).

In the discussion of arterial pressure regulation neural and hormonal mechanisms,which can alter extracellular fluid volume, have been presented. A scheme by which extracellular fluid volume changes are believed to alter arterial pressure may therefore be envisioned as follows. An increased extracellular fluid volume would expand the blood volume; this, in turn, would increase the mean circulatory pressure- the pressure at all points of the circulatory system if the heart were stopped and blood were rapidly redistributed so that all vascular pressures were equal (61). This mean circulatory pressure is a measurement which is theoretically determined by the holding capacity of the circulatory system and the blood volume, and thus provides a measurement of the degree of filling of the circulatory system. Increasing mean circulatory pressure results in almost proportional changes in venous return (62). Thus, an increased extracellular fluid volume can raise the cardiac output and thereby elevate arterial pressure. If an elevated cardiac output is sustained, the excess flow through peripheral tissues could, by local autoregulatory mechanisms,produce general vasoconstriction (63). Local autoregulation tends to stabilize blood flow through tissues during variations in perfusion pressure (64). For example, when the tissue is

under-perfused, myogenic activity of vascular smooth muscle and the vasodilator effect of increasing concentrations of local metabolites combine to decrease vascular tone and permit flow to return towards normal (65). Excess flow would increase the myogenic activity and decrease the concentration of metabolites, thereby raising vascular tone and return flow towards its original level.

Guyton and several others have postulated a mechanism whereby an increase in extracellular fluid volume can produce sustained arterial pressure elevation (66-69). The initial alteration in extracellular fluid volume primarily causes an increased cardiac output. Later, with total body autoregulation (tendency to maintain constant flow), the output returns to normal as the resistance rises. This scheme emphasizes the basic hemodynamic concept discussed previously, namely that mechanisms which regulate arterial pressure manifest their actions by altering either cardiac output and/or total peripheral resistance.

Assessment of Cardiac Performance

In the past twenty years the traditional assessment of the heart's performance in terms of its ability to pump blood has been amplified to encompass further understanding of the heart as a muscle. The quantity of blood the heart pumps is determined primarily by the metabolic needs of the tissues (70). The heart, however, may play a dominant role in controlling its own output in extreme conditions, such as during heavy exercise or when there is a marked impairment of ventricular function (36). The huge reserve capacity of the heart as a pump serves to mask defects in the performance of the heart as a muscle. Primarily as a result of the pioneer work on skeletal muscle mechanics by A. V. Hill (71, 72) it is

now possible to describe cardiac muscle performance in terms of the ability of the myocardium to develop force of contraction and velocity of shortening. The force-velocity relationship[4] of 1938 has since become a basic tool for the understanding of muscle performance. Simply stated, the velocity of muscular shortening is inversely related to the load against which the muscle contracts (i.e., the greater the load the muscle must contract against, the slower will be its velocity of shortening). In the late 1950's and early 1960's the force-velocity curves for cardiac muscle were examined (73, 74).

The force-velocity relation describes the contractile state of muscle. At any given length skeletal muscle has a single force-velocity curve. Thus, if the initial fiber length (determined by the preload) is constant, then the velocity of shortening of the muscle at any given afterload (load against which the muscle must contract) will be the same each time the muscle contracts. Therefore, the velocity of shortening of a theoretically unloaded muscle (maximum velocity of shortening or Vmax) and the force developed when the velocity of shortening is zero (maximum force, isometric contraction) remain unchanged. However, if the initial muscle length were increased, the ensuing contraction would produce a greater maximum isometric force (Po) without changing the maximum velocity of shortening (Vmax). This fundamental relationship between initial muscle length and force of contraction was observed in

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[4]The Hill equation, $(P + a)(V + b) = (Po + a)b$, characterizes the hyperbolic curve of the force-velocity relation (71). P is the force produced; V is the velocity of shortening; Po is the maximum force at zero speed; and a and b are constants chosen to best fit the equation to the observed values. The constant b has the dimensions of velocity, while constant a has the dimensions of force.

skeletal muscle by Fick in 1882 (75) and Blix in 1895 (76). In 1895, Frank demonstrated that the relationship between initial muscle length and force of its contraction was also valid for heart muscle (77). And 20 years later, Starling quantitatively studied the effects of increasing diastolic volume (fiber length) on the stroke volume of mammalian hearts. Using a heart-lung preparation he demonstrated that by raising the height of the venous reservoir (and hence ventricular filling pressure and volume) the ventricular stroke output was increased (78). These studies led to the formulation of what is now called Starling's Law of the Heart. "The law of the heart is, therefore, the same as that of skeletal muscle, namely, that the mechanical energy set free on passage from the resting to the contracted state depends on the area of 'chemically active surfaces', i.e., on the length of the muscle fiber" (78).

The significance of the Frank-Starling relationship in the intact circulation was questioned seriously because of findings that the heart could contract at times more forcefully even though end diastolic pressure (and therefore muscle length) was reduced (78, 80). Sarnoff did much to reconcile what appeared to be conflicting views by introducing the ventricular function curve and demonstrating that cardiac performance could be considered best asfunctioning in terms of a family of Starling (or ventricular function) curves (81, 82). These ventricular fuction curves related stroke work (stroke volume times mean arterial pressure) to end diastolic pressure, and provided a graphic illustration of ventricular performance. They clearly demonstrated that as end diastolic pressure (and hence muscle fiber length) was increased, the stroke work concomitantly increased. This intrinsic Frank-Starling cardiac response

was termed heterometric autoregulation because it involved changes in fiber length.

The ventricular function curve was utilized to describe shifts from one Starling curve to another. Such changes were termed homeometric autoregulation, since the heart could alter its output at the same fiber length by changing the contractile state of the myocardium (83). The characteristics of homeometric autoregulation (or alterations of myocardial contractility) are described best by shifts in the force-velocity relation of isolated cardiac muscle. As mentioned previously, alterations in the preload change the maximum force the muscle develops (Po), but not the maximum velocity of shortening (Vmax). However, even if the preload of cardiac muscle remains constant, the force-velocity relation may be shifted in another characteristic manner by various inotropic interventions which alter the myocardial contractile state (73, 74). For example, if the positive inotropic agent,norepinephrine, were added to the fluid bath surrounding a contracting cardiac muscle, both the maximum force (Po) and velocity of shortening (Vmax) would increase. Conversely, agents which depress myocardial contractility (i.e., negative inotropic agents such as pentobarbital) decrease the maximum velocity of shortening (Vmax). Therefore, changes in Vmax define the inotropic or contractile state of the myocardium and are not affected by preload alterations (84). In summary, shifts in the ventricular function curve, where stroke work changes at the same end diastolic pressure (or homeometric autoregulation) are produced by alterations in the contractile state of the myocardium. The force-velocity relation characterizes the two fundamental ways in which cardiac muscle function may be altered: one, by changing the initial

muscle length (the Frank-Starling relationship) and two, by changing the myocardial contractile state (contractility) (85).

According to Huxley's sliding filament hypothesis of muscle contraction, changes in the initial muscle length (determined by preload) alter the overlap of actin and myosin myofilaments within the sarcomere (86). Stretching the sarcomere increases the overlap between the thick (myosin) and thin (actin) filaments, and provides a greater area for their interaction. When the muscle is stretched beyond its optimal length for contraction, the filaments become partially disengaged and the number of reactive sites between thick and thin filaments become reduced, thereby producing the well-known descending limb of the length-tension relationship (87). Sarcomere length of intact left ventricular myocardium has been shown to be a function of filling pressure (88). Such studies have provided an ultrastructural basis for the Frank-Starling relation; namely, that the normal left ventricle functions along the ascending portion of the length-tension curve, that is to say, increases in end-diastolic pressure are associated with sarcomere length changes.

Variations in contractility, characterized by alterations in the maximum velocity of shortening, are conceptually viewed as changes in the rate at which chemical energy is converted to mechanical energy at the force-generating sites (90). The contractility of skeletal muscle fibers cannot be altered; however, on any given contraction, more or less fibers can be recruited to contract. In contrast, with each cardiac contraction the number of cells activated remains constant; performance can be altered, however, by changing the contractile state of heart muscle--the rate at which force is generated by the contractile filaments

(91), a more or less forceful contraction can be produced.

In the intact heart the autonomic nervous system and circulating hormones influence its contractile state; and the degree of ventricular filling during diastole determines the initial fiber length. Together, these two modes of altering cardiac performance combine to adjust continually the pumping characteristics of the heart to the work load. The Frank-Starling mechanism provides for a delicate adjustment between the strength (or force) of contraction and the degree of ventricular filling, thereby balancing the variable input and output of the right and left ventricles on a beat to beat basis (92, 93). In addition, variations in the contractile state of the ventricle greatly alter its pumping performance; thus sympathetic stimulation, by increasing contractility, permits the heart to eject large quantities of blood without over-distending the ventricular cavity (or increasing the preload). In fact, high end-diastolic pressures are usually associated with a ventricle in overt failure, since in this case the diseased myocardium must resort to large preload increases just to eject its normal stroke volume. Hence, the normal myocardium uses the Frank-Starling relation for beat to beat adjustments; it superimposes on this mechanism changes in the contractile state to provide the tremendous reserve capacity it possesses.

Studies of isolated heart muscle (73, 74, 89, 95) stressed the importance of the initial velocity of shortening (i.e., Vmax) in determining its contractile state. Vmax theoretically expresses the rate at which chemical energy is converted to mechanical contraction (96). In recent years, the principles of isolated muscle mechanics have been applied to the intact heart. When myocardial contractile state is augmented, the

rate at which pressure is developed within the ventricle is also increased (97, 98). Thus, the maximum rate of rise of intraventricular pressure (max.dp/dt) has been used to evaluate its contractile properties (98-100). However, unlike the theoretical Vmax from isolated muscle strip studies, the max.dp/dt is influenced not only by the contractile state but also by the loading conditions of the ventricle (98,100, 101). Thus, elevations of either end diastolic pressure (preload) or aortic diastolic pressure (after load) will increase the max.dp/dt even though the contractile state of the ventricle may remain unchanged. To circumvent this inherent problem with the max. dp/dt several investigators have advocated the use of ratios of dp/dt to the simultaneously developed intraventricular pressure (the dp/dt/p) (101-103), and these indices of myocardial contractility have been shown to be relatively insensitive to pre and afterload alterations.

Another approach to evaluating the contractile state of the intact ventricle is based upon the observations of Spencer and Greiss (104) and of Rushmer (105) that the ventricle functions as an impulse generator during early systole. As such, the ventricle develops pressure rapidly and when the ventricular pressure exceeds aortic pressure, outflow of blood is rapidly set into motion. Once the blood is in motion, it will continue because of inertia until the resistance to flow stops its forward movement. This physical property-momentum, when applied to cardiac dynamics, places great import on the initial phase of ventricular ejection. Indeed, Noble has demonstrated that during late systolic ejection (when aortic pressure is higher than intraventricular) blood leaves the entricle under its own momentum (106). Rushmer has made the analogy "that

ejection of blood from both ventricles, particularly the left, is more like striking a piston with a mallet than like squeezing blood out of the chamber" (105). This mallet-like action of the heart is characterized by the peak flow velocity and the maximum acceleration of ascending aortic blood flow. These latter two variables have been shown to be sensitive indices of myocardial contractility, closely related to the maximum initial velocity of shortening of left ventricular muscle (Vmax) (107, 108), only shown in in vitro preparations.

Hypertension

A persistently elevated arterial pressure is a sign of a variety of hypertensive diseases. Hypertension obviously denotes the existance of an abnormal relationship of cardiac output and peripheral resistance to flow. As such, it may be construed as a disease of regulation, since normal arterial pressure levels are closely maintained by the proportional control of these two hemodynamic determinates: blood flow and resistance. Various etiological and mechanistic types of hypertension have been characterized by abnormalities in these hemodynamic variables (109). The most common category of high arterial pressure in man is essential hypertension (hypertension of unknown cause). As Pickering so strongly emphasizes, "blood pressure is a graded characteristic like height, and the differences between individuals are quantitative not qualitative" (110). Essential hypertensive individuals, thus, represent one section of the population having arterial pressure above an arbitrarily defined value, and having no known disease to which the elevated pressure can be attributed. Significantly related to the degree of arterial pressure elevation is a marked shortening of life expectancy (111).

Experimental work in hypertension received a great impetus in 1934 when Goldblatt successfully produced hypertension in experimental animals (112). He constricted both renal arteries of dogs with silver clamps to the degree which he believed reduced renal pressure and flow without materially decreasing renal excretory function.[5] Along with renal arterial constriction, experimental hypertension has been induced also by such diverse procedures as bilateral encapsulation of the kidneys in latex or cellophane (114), treatment with deoxycorticosterone and high salt diet (115), sino-aortic denervation (116), regeneration of adrenal cortex after its enucleation (117), and chronic stimulation of the hypothalamus defense area (118). It is well to note that all of these experimental models tend to focus attention to a distinct causative factor in the pathogenesis of hypertension. Although valuable physiological information of events secondary to the hypertension have been obtained from such experimental studies, none represent the counterpart for the most common type of hypertension in man-essential hypertension. Perhaps genetic strains of hypertensive animals could provide an experimental approach to the natural history of the pathophysiological changes associated with the development of progressive hypertensive cardiovascular disease. Several investigative groups set about to provide such a model.

By selective inbreeding Alexander, Hinshaw and Drury developed a strain of spontaneously hypertensive rabbits (119) and Smirk and Hall produced a strain of genetically hypertensive rats (120). Dahl was able to develop two strains of Sprague-Dawley rats, one resistant and one prone

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[5]Though Goldblatt refers to the clamped kidneys as ischemic, evidence was soon provided that flow to the kidney was not necessarily reduced by the clamp (113).

to develop hypertension when chronically fed high levels of sodium chloride (121). Okamoto and Aoki, using selective brother to sister inbreeding techniques, developed a strain of hypertensive Wistar rats which demonstrated a 100 percent incidence of hypertension in their offspring (122, 123). This latter strain of animals is referred to as Spontaneously Hypertensive Rats (SHR).

Spontaneously Hypertensive Rats

Okamoto and Aoki bred an otherwise normal male Wistar which showed persistently elevated arterial pressure (systolic pressure between 150 and 175 mm Hg) with a female Wistar rat whose pressure was slightly above average (130 to 140 mm Hg). From these parents, F_1 rats with elevated pressures were selected and mated brother to sister to produce the F_2 generation. In their original report Okamoto and Aoki continued this process through the F_6 generation (122), and subsequently they showed chronological changes in body weight and systolic pressure up through the F_{12} generation of these spontaneously hypertensive rats (SHR) (123). By five weeks of age systolic pressure of their SHR group was significantly higher (135 mm Hg) than their age-matched, control, normotensive Wistar (NR) rats (112 mm Hg). During the next 40 weeks pressure continually rose in the SHR group, but the pressures of the NR group stabilized by 10 weeks of age. Thus, SHR male rats of the F_6 generation had systolic pressures of 192 and 201 mm Hg at twenty and forty weeks, respectively, and NR rats of the same sex and age had systolic pressures of 138 and 130 mm Hg, respectively. Because of the strong genetic element, without a specific causative mechanism identified, this strain of rats seems particularly promising as a model of essential hypertensive man.

Since the introduction and distribution of these animals throughout the world-wide scientific community, many factors have been studied to determine their contribution to the pathogenesis and maintenance of the elevated pressure of the SHR rats. Thus far, the expectations that they seem to be an excellent experimental model for essential hypertensive man appear to have been confirmed (124). Analysis of the pressures of a hybrid progeny of SHR and NR rats revealed intermediate pressures ranging from NR to SHR group pressures (125, 126). These findings strongly favor the polygenic mode of inheritance and lend further support to Pickering's description (vida supra).

Environmental influences have also been examined in the SHR rats. Louis, et al. maintained four groups of SHR rats on either "salt free", 0.1%, 1.0% or 4.0% NaCl diets for ten weeks and found significant differences in blood pressure development only in the extreme groups ("salt free" and 4.0% NaCl) (127). However, chronic ingestion of even 1.0% NaCl solution has been shown to accelerate the severity of the hypertensive disease of these SHR rats and decrease the already shortened life expectancy of these animals (128, 129). It is important to note, however, that hypertension does develop in SHR rats maintained on a "salt-free" (<0.01%) diet (127). Yamori, et al., using rather extreme procedures of immobilization, combined visual, auditory and electrical stimuli, and cold exposure on NR and SHR rats, demonstrated that these environmental factors play a role in the development of hypertension in SHR rats (130). These forms of chronic stress augmented the pressure development of the SHR rats, while only slight increases in pressure were observed in similarly stressed NR rats. Thus, although the SHR rat develops severe and

sustained hypertension without excessive salt administration or chronic stress, it is clear that these environmental conditions can accelerate the progression of the disease.

Several investigators have reported that the renin-angiotensin system does not play a significant role in the pathogenesis of the hypertension in the SHR rat. Thus, Sokabe reported decreased renin activity from SHR kidney (131, 132), and the juxtaglomerular apparatus granularity was also reduced (133). Koletsky, Shook and Rivera-Velez, studying juxtaglomerular cell granulation, renal vein plasma renin activity and vasopressor activity of renal vein blood in 2 to 6 month and 7 to 12 month SHR rats, also found reduced renin activity in the older SHR rats; and bioassays of renal venous blood from the younger group demonstrated no renal pressor substance (134).

Other endocrine organs have been implicated in the development of the elevated arterial pressure of the SHR rats. Thus, Aoki has demonstrated that either hypophysectomy, bilateral adrenalectomy or thyroidectomy prevents the development of hypertension in these rats (135). Although these procedures greatly alter normal physiological control, it is of interest that oral administration of thyroid powder (300 mg/kg body weight) accelerated the development of the elevated arterial pressure of these rats (136). Thyroid activity measured by ^{131}I uptake by thyroid gland (3 and 24 hours), ^{131}I release by thyroid gland, 24-hour conversion ratio and thyroid secretion rate (minimal exogenous thyroxine required to inhibit release of ^{131}I from thyroid gland) indicate that the thyroid activity of the SHR rats is greater than NR (137). In addition, propylthiouracil (137), methylthiouracil (135) and radiothyroidectomy (136)

therapy directed at reducing thyroid function significantly lowered systolic arterial pressure of SHR rats.

Neural factors have also been suggested to participate in the pathogenesis of SHR hypertension. Okamoto, et al., demonstrated that, while mesencephalic transection (cerveau isolé) of the central nervous system did not produce any change in arterial pressure of either NR or SHR rats, encéphale isolé (transection at the base of the brain) did produce a sudden fall in both groups (138). Since this latter reduction in pressure was greater in the SHR group, the authors suggested that a midbrain center participates in the maintenance of SHR hypertension. In addition, Yamori and Okamoto, using a "micro-knife", selectively separated the hypothalamus from the mesencephalon, and in so doing, they reduced pressure and heart rate of both NR and SHR rats which was greater in the SHR (139). The nervous system was implicated further when spontaneous splanchnic (sympathetic) nerve activity was shown to be increased in SHR rats (138).

This possible autonomic nervous involvement in the SHR rat was beclouded by several reports concerning norepinephrine metabolism. Thus, Yamori, Lovenberg and Sjoerdsma reported that norepinephrine levels in the SHR hypothalamus and lower brainstem were reduced (140). However, Nakamura, Gerold and Thoenen failed to confirm that observation (141). Since an increase in tissue concentration of catecholamines in these brain centers has been said to have a hypotensive action (142), a diminished tissue level may be related to hypertension. There seems to be better agreement concerning norepinephrine studies in other tissues. Thus, while endogenous cardiac norepinephrine concentrations are no different

between NR and SHR rats (143), cardiac norepinephrine synthesis (^{14}C-tyrosine incorporation) (144) and turnover (^{3}H-norepinephrine disappearance) rates were reduced in SHR rats (141, 145). This suggested a compensatory mechanism resulting from the hypertension, rather than an increased norepinephrine turnover producing the hypertension. Depletion of other tissue norepinephrine with 6-hydroxydopamine (a synthetic catecholamine which selectively destroys sympathetic nerve terminals) (146) did not prevent the development of hypertension in the SHR rats (147). Therefore, it appears that only a fraction of normal tissue norepinephrine store is necessary for the maintenance of SHR hypertension.

Increased vascular resistance to the flow of blood in clinical essential hypertension may be produced by: increased adrenergic vascular tone, increased responsiveness of vascular smooth muscle to normal sympathetic activity, increased vascular tone as a result of imbalance between local vasoconstrictors and vasodilators, structural changes in the vasculature, or any combination of mechanisms (14). Although there is some evidence that these factors may participate in increasing vascular resistance in the SHR rat, no measurement of flow in the intact SHR rat has been reported. Reactivity of aortic strips to norepinephrine of NR and SHR were compared to determine whether vascular smooth muscle of the SHR rats is hypersensitive to norepinephrine. Spector, et al., reported that SHR aortic strips were less reactive than strips obtained from Carworth Farm Wistar NR (148). In contrast, Hallbäck, Lundgren and Weiss demonstrated similar responsiveness in aortic strips from NR and SHR rats (149). This latter finding was confirmed by Clineschmidt, et al., using a second control group of normotensive Wistar rats (NIH) (150).

In that same study Carworth Farm Wistar, NIH Wistar and SHR rats were compared. Just as Spector reported the Carworth Farm Wistar rat aortic strips developed almost twice the tension as the other two groups. However, the SHR rat vascular smooth muscle does not seem to be more reactive to norepinephrine than appropriate control rats. By way of comment, however, the aortic strip preparation permits evaluation of smooth muscle contraction and sensitivity, but the findings may not be analogus to the responses which might occur in arterioles.

Arterial pressure responses to norepinephrine have also been shown to be similar in pithed NR and SHR rats (151). These findings suggest that the elevated pressure in the SHR is not maintained by an increased sensitivity to norepinephrine. Nevertheless, in intact SHR rats a greater "response index" (magnitude of the pressure rise times duration of the response) to norepinephrine administered as a bolus has been reported (152). This response is difficult to interpret since much more than the vascular response to the constrictor agent is involved.

Ichijima performed extensive morphological studies on jejunal small arteries and arterioles (internal diameter 150 to 10μ) of NR and SHR rats, and reported that the mean luminal diameter of these vessels were smaller in SHR rats (153).

"In situ" flow resistance of these two groups was also compared by Folkow et al. by constructing pressure-flow curves of a perfused, maximally dilated systemic vasculature (154). In this latter study the hypertensives always had lower flow rate and, therefore, higher systemic flow resistance at equal perfusion pressures. Since this increased resistance was found during maximal vasodilation, it was believed to be the result of morphological vascular changes in which the wall/lumen

ratio of the resistance vessels was increased by a generalized thickening of arterioles. Such thickening of non-contractile elements of arteriolar walls in hypertension has been hypothesized as a "water logging" effect (155). Indeed the thoracic aorta of SHR rats is heavier and contains more water, sodium and potassium than NR (156, 157). However, hypertropic changes in the contractile portion of the arteriole must also be considered since the resistance of perfused hindquarters of SHR rats when maximally stimulated with norepinephrine can be about 40 per cent greater than NR rat resistance (158). An increased wall/lumen ratio could explain these increased resistances since the constriction of the thickened arteriolar wall with norepinephrine would produce a greater luminal reduction. Indeed, structural adaptations of the resistance vessels to alterations in pressure loads have been demonstrated (159). Inherent in each of these above reported findings on vascular smooth muscle reactivity is the preconception that, just as in essential hypertensive man, the total peripheral resistance in the SHR rats is increased. However, to date, there is no report of vascular resistance calculation from "in vivo" hemodynamic studies.

Although sympathetic stimulation may raise arterial pressure by increasing cardiac performance, as well as by vasoconstriction, the former effect has received very little alteration in SHR rats. Thus far, only Fujiwara, Kuchii and Shibata have shown differences in the responses of spontaneously beating in vitro atria of NR and SHR rats to beta-adrenergic stimulation (160). The responses of the intact SHR heart to adrenergic stimulation has yet to be reported.

The studies reported herein were designed to provide new information

about the hemodynamics of the SHR rats. Specifically, they were performed to answer the following questions:

1) What are the hemodynamic characteristics of the SHR rat? Is the elevated pressure associated with either an abnormal cardiac output or resistance or both?

2) Do the left ventricles of the SHR rat eject blood with the same vigor as NR rats? Does the left ventricular performance remain unchanged as it hypertrophies? Is the effect of aging on cardiac function the same in NR and SHR rats?

3) What are the relative roles of sympathetic and parasympathetic activity on cardiac function of NR and SHR rats? Are the cardiac responses to beta-adrenergic stimulation similar in NR and SHR rats?

4) What are the hemodynamic responses of both NR and SHR rats to further increases in arterial pressure? Is the baroreceptor reflex present in the SHR group?

CHAPTER II

METHODS AND MATERIALS

The studies which are the subject of this thesis were performed on 156 male Wistar rats. All rats were housed in plastic cages (16" x 20" x 8 1/2") with a maximum of four animals per cage. Food (Purina rat chow) and tap water were provided for the animals ad libitum. Of the total number of successful studies (156 animals), 93 were performed on normotensive Wistar control rats (NR). These rats were purchased from West Jersey Biological Supplies (Wenonah, New Jersey) and were the lineal descendants of the original Wistar colony. The remaining 63 animals studied were of the Spontaneously Hypertensive strain of Wistar rats (SHR) developed by Okamoto and Aoki (122, 123, 161). These Japanese investigators provided the National Heart Institute of the National Institutes of Health (Bethesda, Maryland) with breeders of the F_{13} generation. Through the generosity of Dr. Albert Sjoerdsma of the National Heart Institute, 4 female and 2 male SHR rats of the F_{19} generation were given to Dr. Edward D. Frohlich at the University of Oklahoma Health Sciences Center. From these breeders a large colony of SHR rats was established by brother to sister inbreeding. All the SHR rats used in the present studies were of either the F_{20}, F_{21}, F_{22} or F_{23} generations and are all from the University of Oklahoma Health Sciences Center Hypertensive Rat Colony. Both NR and SHR rats were housed at the Animal Facilities of the University

of Oklahoma Health Sciences Center.

Surgical Preparation

Each rat's body weight was determined on a top loading balance (Triple Beam) just prior to each study. After the induction of anesthesia (vida infra) the rat was placed upon the operating table in a supine position with extremities gently taped to the operating board. An incision was made through the skin along the ventral midline from the neck to the mid-thoracic level. By blunt dissection the left carotid artery was exposed and carefully separated from the vagus nerve. A cannula was then inserted into this vessel and advanced centrally. This arterial cannula was constructed by connecting a 2.5 cm segment of PE-50 (id .023 x od .038 inch) tubing into a 5.0 cm segment of silastic tubing (id .040 x od .085 inch) by interposing a short (5 mm) segment of PE-90 (id .034 x od .050 inch) tubing between the PE-50 and silastic tubing. Silastic tubing was utilized to render the catheter more flexible.

The right external jugular vein was cleared of surrounding tissue, a venous catheter was inserted into it and advanced towards the right atrium. The venous catheter consisted of 4 cm PE-90 inserted into 4 cm of silastic tubing. Both arterial and venous catheters, pre-filled with heparinized saline (10 units/ml), were connected by 18 gauge needles to Statham P23 Db and P23Bb strain gauges, respectively. Zero references for both transducers, which were fixed at midchest level, were checked frequently during the course of the experiment. Pressure signals were continuously displayed on a Hewlett Packard multichannel recorder (Model 7784A). Arterial pressure was considered technically satisfactory only if the dicrotic notch was readily apparent (162).

Once both arterial and venous catheters were in place mean pressure values were electronically determined and high speed recordings (100 mm/sec) of phasic fluctuations in these pressures were also obtained. These measurements of systolic and diastolic arterial pressure, mean arterial and venous pressures and heart rate were defined as the prethoracotomy control values.

Following recording of the prethoracotomy control values a tracheostomy was performed. The tracheal cannula (6 cm PE-240 id .066 x od .095 inch) was inserted and then connected to a constant volume respirator, Harvard Apparatus, Model 680. The rats were ventilated with room air at a minute volume which was preselected according to the body weight of the animal from the Harvard ventilation graph (163). Sometimes tidal volume and rate were readjusted so that arterial and venous pressures were only minimally altered by the changes in artificial respiratory pressures.

The chest was entered through an incision extending from the sternoclavicular junction to the fourth or fifth costal level. A microretractor was used to provide exposure of the superior aspect of the heart and the great vessels. Following thoracotomy, pressure and heart rate measurements were again recorded; these measurements are referred to as open-chest control values. Using forceps, the pericardium was incised and a suture was carefully placed around the ascending aorta. This suture permitted the gentle lifting of the aorta so that a 2.0 mm electromagnetic flow probe (Micron Instruments) could be placed around it. The flow probe position was adjusted and taped in that position which permitted the clearest flow tracing without altering arterial or

venous pressures or constricting the aortic luminal diameter.

Flow Probe Calibration

The electromagnetic flow probes were calibrated by *in vivo* and *in vitro* techniques. *In vitro* calibration was accomplished by perfusing an excised portion of rat aorta with whole blood at various rates by either an infusion pump (Harvard Apparatus) or a perfusion pump (Sigmamotor). The flow probe was also calibrated *in vivo* by placing it around a branch of a femoral artery of an anesthetized dog. The small artery was cannulated just distal to the probe and the entire outflow from the small vessel was collected in a graduated cylinder. Flow rates were adjusted by clamping the femoral artery. In addition, several simultaneous measurements of electromagnetically recorded ascending aortic flow (using these calibration lines) and cardiac output, determined by conventional indicator dilution techniques (indocyanine green), were found to be in close agreement.

Control Hemodynamic Measurements and Calculations

At the completion of surgery, phasic arterial and venous pressures and ascending aortic blood flow (and their respective electronically integrated mean values) were recorded continuously. During an initial twenty minute control period the value of each of the various hemodynamic indices was recorded four times at five minute intervals: 0, 5, 10, and 15 minutes post surgery. These four measurements for each hemodynamic variable were then averaged to provide the control values for each rat.

Cardiac output, as measured electromagnetically from the ascending aortic flow probe, was defined as mean aortic flow less coronary arterial

flow. The flat diastolic portion of the aortic flow tracing, recorded at a paper speed of 100 mm/sec and a frequency response which was flat to 30 Hz, was used as the zero reference for the cardiac output measurement. The assumption that the response of the system was adequate to return to zero flow between beats at heart rates approximating 400 beats/min was supported by observations in animals with atrioventricular block (Figure 1). Similarly, when the heart was arrested with intravenous acetylcholine, it was apparent that the zero-flow level determined was accurate (Figure 2).

Other hemodynamic measurements were obtained by analysis of high speed tracings (100 mm/sec) of the phasic changes in pressure and flow. Thus, heart rate was determined from beat to beat intervals; and systolic ejection time was measured from the arterial pressure tracing from the beginning of the systolic upstroke to the dicrotic notch. The highest deflection of the aortic flow velocity tracing above zero reference was interpreted as the peak aortic flow velocity. The maximum acceleration of aortic flow (df/dt, rate of change of flow) was determined in earlier studies by measuring the maximal slope of the aortic flow velocity tracing. In latter studies an electronic derivative computer was used and since no difference was found between the maximum acceleration of aortic flow obtained by both mechanical and electronic procedures, the latter method was used thereafter. This measurement and all other phasic indices reported represent the average of at least five consecutive pulsations.

These measurements permitted the calculation of many other important hemodynamic variables. Thus, cardiac index is expressed as the cardiac output divided by the body weight in kilograms, and represents

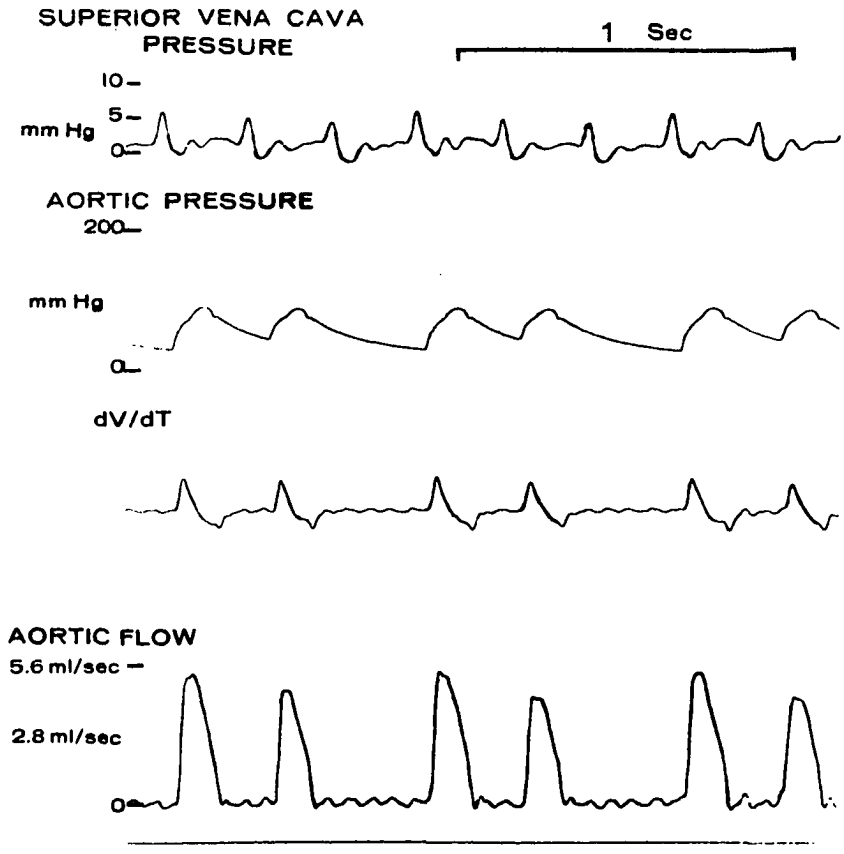

Figure 1. Recording from a rat with atrioventricular block. Note regular atrial systole (upper channel). Zero flow reference of aortic flow velocity is same during short and long diastolic intervals (lowest channel). Also note increased ventricular ejection that is recorded after prolonged filling times.

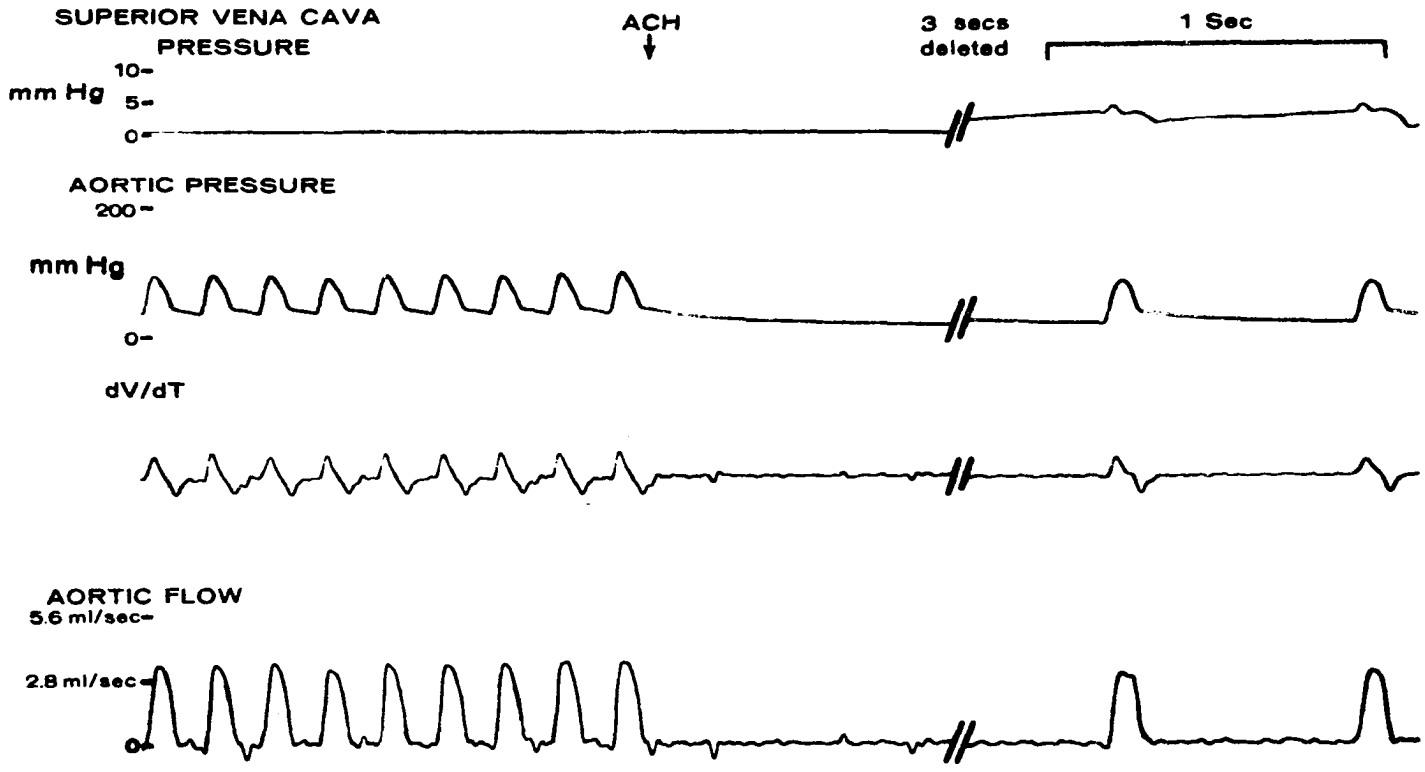

Figure 2. Recording of cardiac arrest induced by acetylcholine with subsequent resumption of cardiac rhythmicity. Superior vena cava pressures are not recorded during acetylcholine administration since this catheter was used for drug infusion. Note zero-flow level determined at a wide range of heart rates is the same.

blood flow per kilogram of noncardiac tissue (since coronary flow is not measured). Stroke volume was determined by dividing the cardiac output by heart rate; and left ventricular ejection rate was calculated by dividing stroke volume by systolic ejection time. The total peripheral resistance was obtained by dividing mean arterial pressure by cardiac output (expressed as mm Hg/ml/min); and, to equilibrate for weight differences among rats, peripheral resistance index was also calculated as the quotient of mean arterial pressure and cardiac index (expressed as mm Hg/ml/min/kg). The direct measurements of aortic flow and pressure also permitted calculations of left ventricular stroke and minute work. Left ventricular stroke work in gram-meters/beat was obtained by multiplying stroke volume by the difference of mean aortic pressure and right atrial pressure, and then multiplying the product by .0136 to convert mm Hg to meters water. Left ventricular minute work was then obtained by multiplying stroke work by heart rate.

Anesthesia Comparison

Thirty male NR rats ranging from 225-500 grams in weight (average 379 grams), and 16 to 21 weeks of age (average 19 weeks) were divided into three groups of ten. One group was anesthetized with urethan (1.4 gram/kilogram, subcutaneously), the second with pentobarbital (40 milligram/kilogram, intraperitoneally, Nembutal, Abbott Laboratories) and the third group with ether (small animal anesthesia chamber induction). After the initial induction of anesthesia, approximately 60, 20 and 5 minutes with urethan, pentobarbital and ether, respectively, the surgical procedures described above for obtaining the physiological measurements were performed.

Once all hemodynamic variables were technically satisfactory following surgical preparation, high speed tracings were recorded every five minutes for a one hour period. During this one hour observation period, rectal temperature was monitored continuously using an electronic thermistor probe; when necessary body temperature was maintained above 35°C with an infrared lamp. In no instance did rectal temperature exceed 38°C. All rats anesthetized with urethan required no supplemental dose of the anesthetic agent. In contrast, the pentobarbital-anesthetized animals did require one additional dose of 10 mg/kg during surgery; and, of course, the ether-anesthetized rats were ventilated continually with low levels of ether, controlled by an "etherizing apparatus" (Phipps and Bird).

Control Observation Period

The remaining 126 male rats used in these investigations were anesthetized with ether. After instrumentation of the rat (with arterial and venous catheters and ascending aortic blood flow probe) control hemodynamic measurements were obtained at five minute intervals for a twenty minute period. Each hemodynamic variable was therefore measured or calculated four times, and averaged in order to provide one single control value for each subsequent test hemodynamic variable for the individual rat. Control hemodynamic measurements were obtained thusly in 20 young (9-12 week) rats (10 NR and 10 SHR), 80 mature (18-33 week) rats (40 NR and 40 SHR), and 26 old (62-97 week) rats (13 NR and 13 SHR). At the completion of each study the heart was removed, and the right and left ventricle plus septum wet weights were determined on an analytical balance (Mettler).

Pharmacological Assessment of Autonomic Activity

Parasympathetic Inhibition

After control measurements were recorded, atropine sulfate (Burns Pharmaceuticals) was administered intravenously to 20 young (10 NR and 10 SHR) and 20 mature (10 NR and 10 SHR) male ether-anesthetized rats. First, 0.5 mg/kg atropine was given; and hemodynamic variables were recorded 3 minutes later from high speed tracings. Then, a second dose (of 0.5 mg/kg) was administered and hemodynamic values again recorded after another 3 minutes. Alterations from the control hemodynamic measurements induced by atropine were interpreted to indicate the degree of vagal tone prior to atropine. Thus, those animals with the greatest amount of "control" vagal tone were interpreted as having the greatest parasympathetic inhibitory action by atropine. Conversely, those rats with the least vagal tone were those demonstrating the smallest changes following atropine.

Combined Parasympathetic and Sympathetic Inhibition

Following parasympathetic inhibition with atropine, a beta-adrenergic blocking drug, sotalol hydrochloride (Mead Johnson), was also administered to the same 40 rats. This beta-adrenergic inhibiting drug was selected because of its low degree of nonspecific and negative inotropic activity in comparison to other beta-blocking drugs (i.e., propranolol) (164). Administration of atropine had to precede sotalol, for if the drugs were given in the reverse order, vagal tone would have been diminished with the greatly reduced cardiac function in sympathetically blocked rats. Sotalol was dissolved in saline in a concentration of

40 mg/ml, and two intravenous doses of 10 mg/kg sotalol were given at three minute intervals. Hemodynamic measurements were made prior to and three minutes after each of these dosages. A final dose of 20 mg/kg (cumulative dose 40 mg/kg) was then administered, and hemodynamic measurements were then obtained at five minute intervals for 15 minutes. Cardiac performance at this point was considered to be "intrinsic" since most of the extrinsic neurohumoral influences were inhibited.

Cardiac Stimulation

Cardiac responses of NR and SHR rats to beta-adrenergic stimulation were compared using a wide dose range of isoproterenol or norepinephrine.

Isoproterenol. After the usual twenty minute control period, isoproterenol hydrochloride (Winthrop Laboratories) was infused into a femoral vein in progressively increasing doses of 0.16, 0.32, 0.64, 1.28, 2.56, 5.12 and 10.24 μg/kg/min. The administration of these doses was accomplished by adjusting the infusion rate of a 2 μg/ml solution of isoproterenol by a variable speed infusion pump (Harvard Apparatus, Model 904). The infusion of each dose of isoproterenol was maintained for two minutes, and during the terminal ten seconds of each infusion, high speed recordings of all hemodynamic measurements were obtained. Such tracings permitted comparison of each dose level with control measurements. In this manner, isoproterenol was administered to five male and five female (4 to 6 month) NR (average weight 417 and 278 grams, respectively) and an equal number of male and female aged matched SHR rats (average weights 370 and 224 grams, respectively). In this and all

subsequent studies body temperature was maintained between 35° and 38°C with an electric heat pad placed under the animal prior to surgery.

Norepinephrine. A second group of 20 male rats (10 NR and 10 SHR), 4 to 6 months of age, was given intravenous infusions of norepinephrine (Levophed, Winthrop Laboratories) following the 20 minute control period. By adjusting the infusion rate of a 10 µg/ml solution of norepinephrine (by base), each animal received a 40 second infusion at the following doses: 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 µg/kg/min. During the terminal 10 seconds of each infusion level, high speed recordings were obtained to allow comparison with the same hemodynamic information recorded during the control period. The hemodynamic responses of the NR and SHR groups to norepinephrine and isoproterenol were analyzed by comparing each group's last control measurement (at 15 minutes of control observation) of mean arterial pressure, heart rate, cardiac index, maximum acceleration of aortic flow and total peripheral resistance with its respective measurement obtained at each dose level. A paired t-test was used for this comparison of control and post-infusion hemodynamics (165).

Elevation of Arterial Pressure - Methoxamine

Cardiovascular adaptations to sustained arterial hypertension were examined by further increasing arterial pressure with the alpha-adrenergic agonist, methoxamine hydrochloride (Vasoxyl, Burroughs and Wellcome and Company). After control measurements were obtained, another group of 20 male rats (10 NR and 10 SHR) 4 to 6 months of age were given intravenous infusions of 0.02, 0.04, 0.08, 0.20, 0.40, 0.80, 1.60, and

3.20 mg/kg/min by adjusting the infusion rate of a 0.4 mg/ml solution of methoxamine. Each infusion lasted 40 seconds and high speed recordings of all hemodynamic measurements were obtained during the last 10 seconds of each infusion period. The observed changes in cardiac function during methoxamine infusion were interpreted as representing reflexive adjustments to the increased arterial pressure, since methoxamine is an alpha-adrenergic agonist with little direct or intrinsic cardiac effects.

In order to evaluate and compare the hemodynamic reflexive responses of NR and SHR rats to methoxamine infusions, an arbitrary reflexive index was established: the mean arterial pressure level at which heart rate, cardiac index, and maximum acceleration of aortic had consistently decreased. This level was defined as that pressure at which the variable decreased by more than one standard deviation from that animal's control value. This arterial pressure level then, represents the last measured mean arterial pressure (as pressure is being elevated with methoxamine) before significant reflexive reduction in that hemodynamic variable was measured. This arbitrary index of reflexive inhibition was only utilized to provide a basis for comparative analysis of NR and SHR group reflexive responses. These mean arterial pressures, above which reflexive activity was first observed in NR and SHR groups, were compared by the Mann-Whitney-U test, since this test is nonparametric and does not assume normal distribution (165).

CHAPTER III

RESULTS

Anesthesia Comparison

Control Values

Hemodynamic recordings taken immediately (0) and at 5, 10 and 15 minutes after completion of surgery, using three anesthetic agents (ether, pentobarbital or urethan), were averaged for each rat to obtain control hemodynamic data. The group means (and standard errors of the means) for each of these three anesthesia groups are presented in Table 1. The urethan-anesthetized rats had the widest pulse pressure and the lowest mean aortic pressure and heart rate. This reduced mean aortic pressure reflected a lower diastolic pressure (urethan 48, pentobarbital 63 and ether 54 mm Hg), since the systolic pressure of the urethan group was similar to the other groups (urethan 109, pentobarbital 103 and ether 110 mm Hg).

The mean aortic pressure of rats anesthetized with pentobarbital and ether was the same (pentobarbital 78 and ether 79 mm Hg); however, the pentobarbital-treated group of animals had the narrowest pulse pressure (urethan 61, pentobarbital 40 and ether 56 mm Hg), as well as the lowest peak aortic flow velocity, maximum flow acceleration, and left ventricular ejection rate. Although the rats of the urethan and

TABLE 1

COMPARISON OF HEMODYNAMIC INDICES IN RATS ANESTHETIZED WITH THREE DIFFERENT AGENTS

	Urethan	Pentobarbital	Ether
Arterial Pressure (mm Hg)			
Systolic	109 ± 6	103 ± 5	110 ± 7
Diastolic	48 ± 3+	63 ± 5	54 ± 5
Mean	70 ± 3	79 ± 4	78 ± 5
Heart Rate (beats/min)	303 ± 16+	352 ± 8	326 ± 14
Cardiac Index (ml/min/kg)	145 ± 7++	139 ± 7*	214 ± 20
Peak Flow Velocity (ml/sec)	4.4 ± 0.3	3.8 ± 0.2	4.7 ± 0.3
Maximum Flow Acceleration (ml/sec/sec)	201 ± 15	176 ± 10*	229 ± 14
Peripheral Resistance Index (mm Hg/ml/min/kg)	.490 ± .022	.581 ± .042*	.392 ± .046

Each value represents the average of recordings obtained after completion of the surgical procedures and then after 5, 10, 15, and 20 minutes for 10 rats (± 1 standard error of the mean).

+Difference between Urethan and Pentobarbital groups significant at the $p<.05$ level.

++Difference between Urethan and Ether groups significant at the $p<.05$ level.

*Difference between Pentobarbital and Ether at the significant $p<.05$ level.

pentobarbital groups both had similar cardiac indexes (urethan 145 and pentobarbital 139 ml/min per kg), the characteristics of left ventricular ejection were different. Thus, the urethan-anesthetized rats ejected blood from the ventricle more vigorously, as evidenced by a greater peak flow velocity (4.4 ml/sec) and maximum acceleration (201 ml/sec^2) than the pentobarbital-anesthetized rats (peak flow velocity 3.8 ml/sec; maximum flow acceleration 176 ml/sec^2). Finally, rats anesthetized with ether consistently had the highest cardiac index (214 ml/min per kg) and most rapid left ventricular ejection (peak flow velocity, 4.7 ml/sec; maximum flow acceleration, 229 ml/sec^2).

Stability. Averages for each of the hemodynamic indices were calculated for the three 20-minute segments of the one hour observation period for each rat. The urethan-anesthetized rats showed considerable stability with respect to mean aortic pressure, cardiac index, peak flow velocity, and left ventricular ejection rate. However, there was a significant tendency of heart rate ($p<.001$) and maximum flow acceleration ($p<.025$) to increase with time (Figure 3). Cardiac output remained stable despite the increasing heart rate as a result of a concomitant reduction in stroke volume. Although the pentobarbital-treated animals demonstrated no significant hemodynamic changes during the hour observation period, mean aortic pressure, cardiac index, maximum flow acceleration, and left ventricular ejection rate declined gradually (Figure 4). Those rats anesthetized with ether also showed no significant hemodynamic changes during the hour observation period, although mean aortic pressure and cardiac index declined slightly (Figure 5). Nevertheless, all indices of cardiac function remained higher in these rats than those initial values

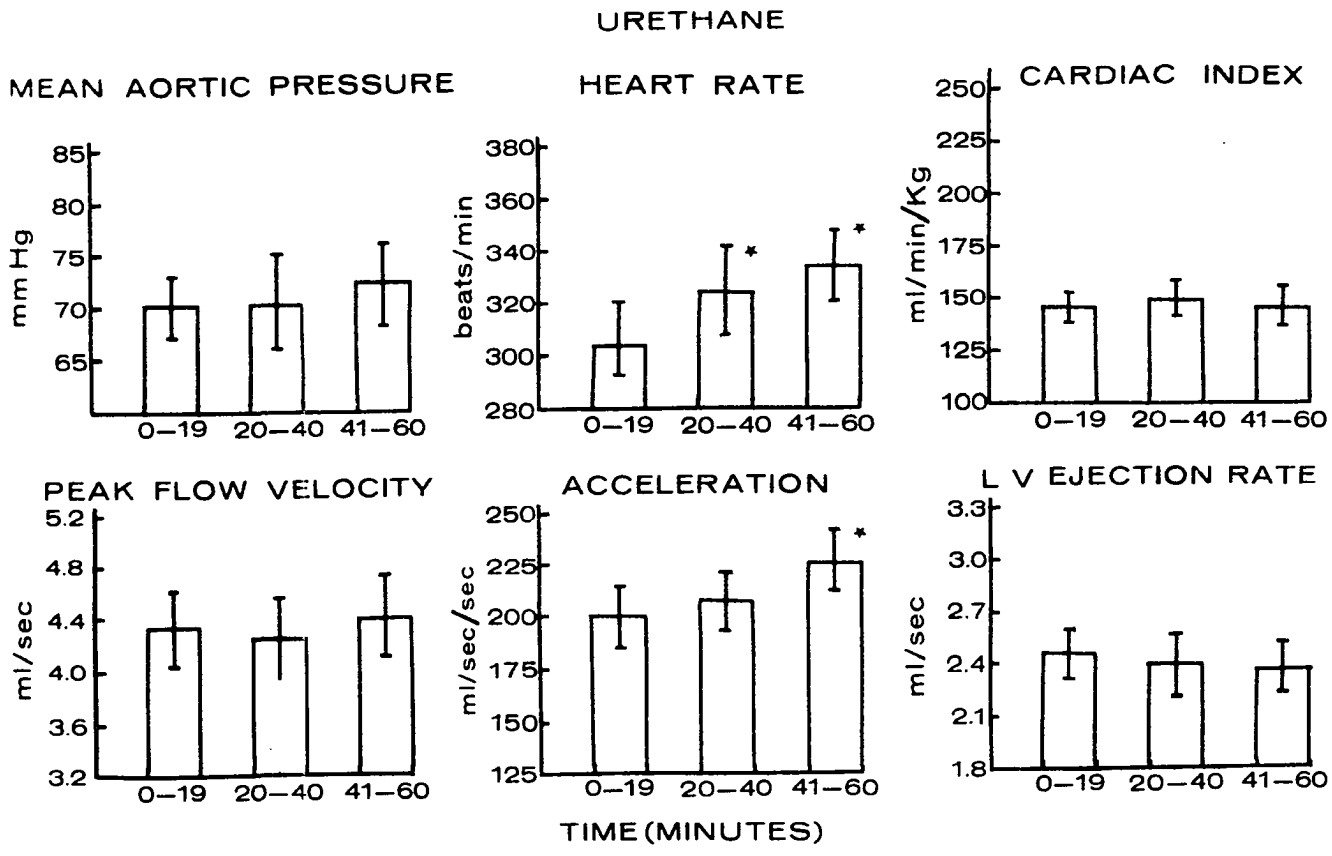

Figure 3. Hemodynamic stability of urethan-anesthetized rats. Shown are the means $\pm$ 1 standard error of mean of 20 minute averages for 10 rats. * $p<0.025$.

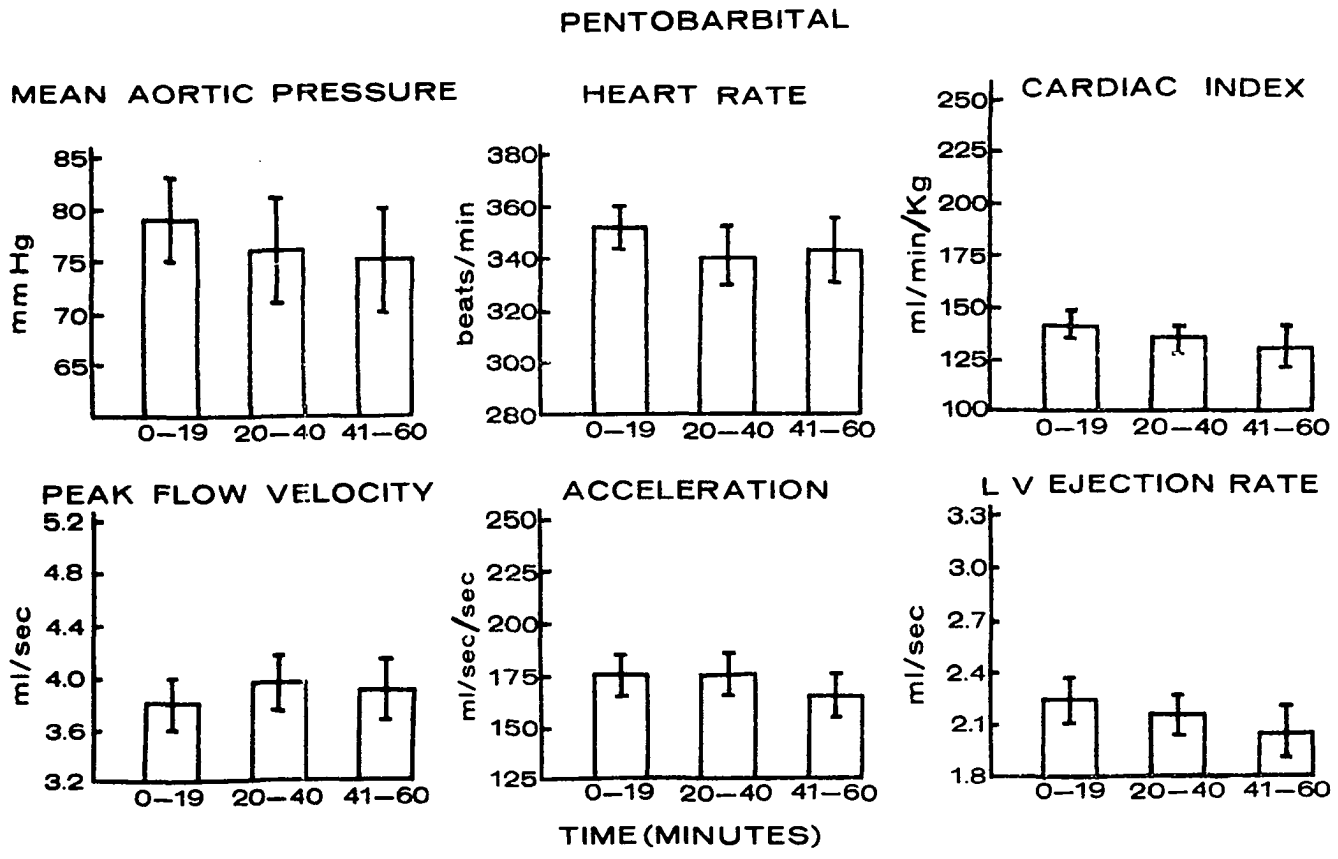

Figure 4. Hemodynamic stability of pentobarbital-anesthetized rats. Shown are the means $\pm$ 1 standard error of the mean of 20 minute averages for 10 rats.

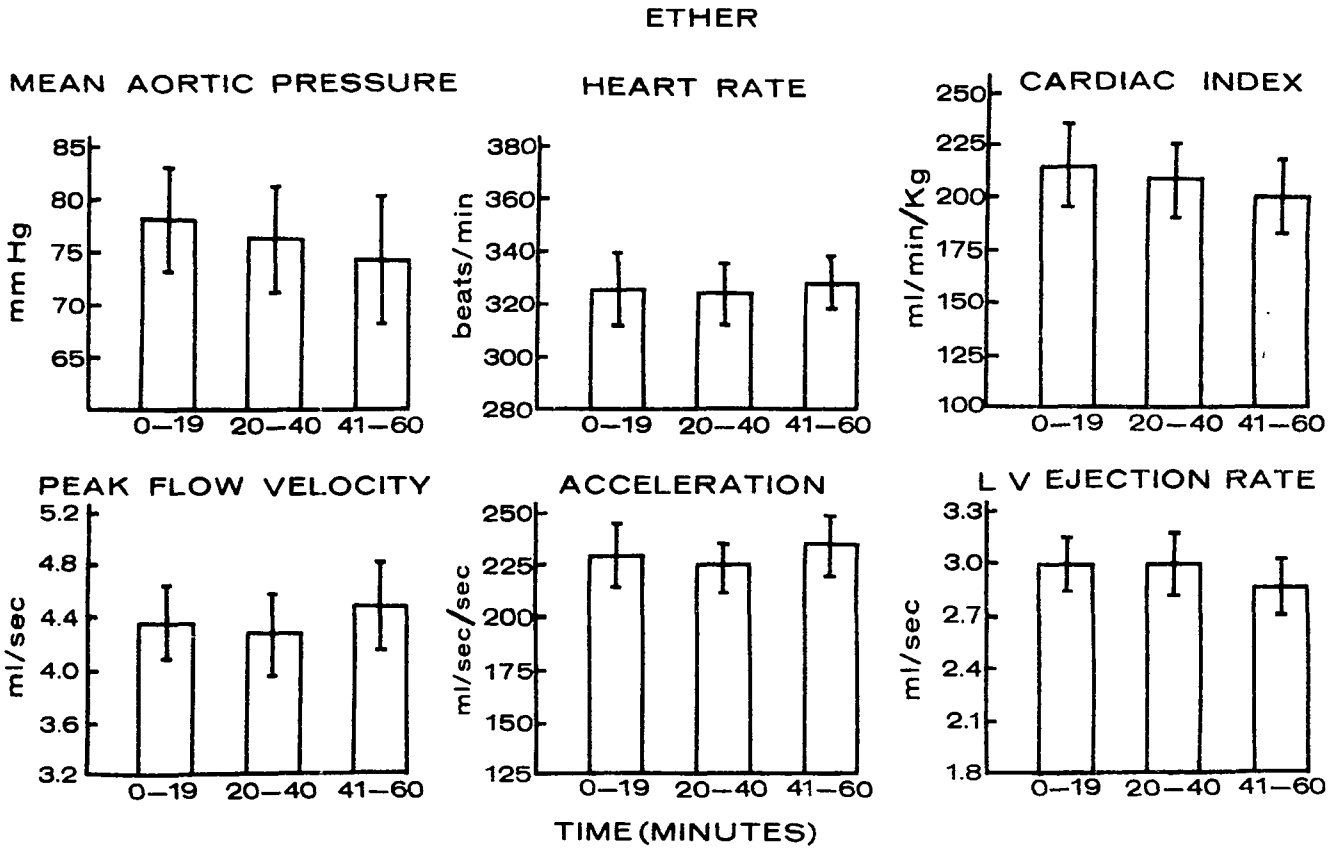

Figure 5. Hemodynamic stability of ether anesthetized rats. Shown are means ± 1 standard error of mean of 20 minute average for 10 rats.

obtained in those rats anesthetized with urethan or pentobarbital.

Comparison of Normotensive and Spontaneously Hypertensive Rats

Weight

The body weights of NR and SHR male rats of 9 to 12 weeks of age (Group I) were similar (247 $\pm$ 10 and 253 $\pm$ 5 grams, respectively). However, the left ventricular weights of the hypertensives were strikingly greater than the NR; this increased left ventricular mass was also found in the two older groups despite the reduced body weight as compared with their age-matched controls (Figure 6). The increasing left ventricular mass of aging NR rats was related to growth since a fairly stable ratio of left ventricular to body weight was observed in all age groups. In contrast, left ventricular mass of SHR rats increased more than would be expected by normal growth. Thus, left ventricular weight of the Group III SHR rats was 164 milligrams greater than those of Group II in whom no increase in body weight occurred (Figure 6). No difference in right ventricular weight was found between NR and SHR rats in each of the three age-matched groups.

Hemodynamics

Prethoracotomy systolic, diastolic, and mean arterial pressures and heart rate were greater in SHR rats than in their age-matched normotensive controls (Figure 7). Furthermore, the arterial pressure of the NR rats remained relatively stable as they grew, whereas the already elevated pressures of the young SHR rats increased progressively with age. Both groups (NR and SHR) exhibited decreasing heart rate with age, though the heart rates of the SHR rats were always faster. This reduction of

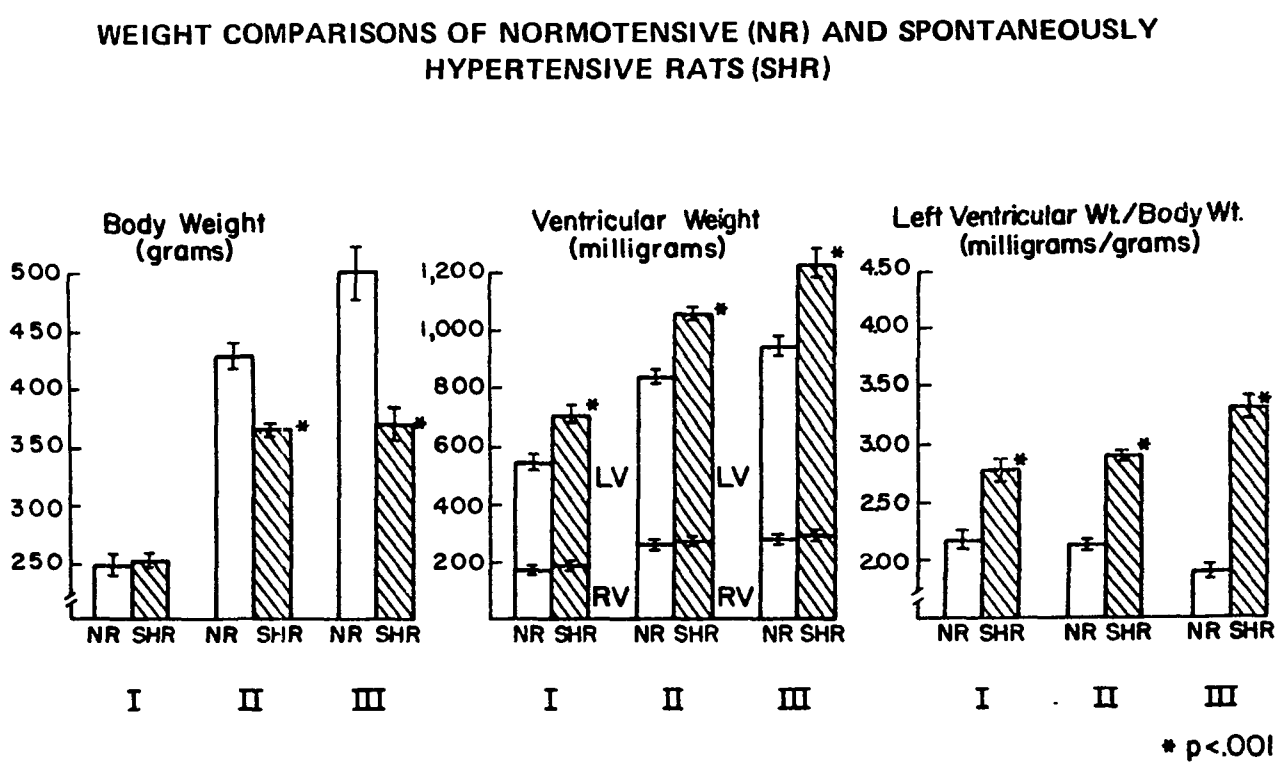

Figure 6. Comparison of body and ventricular weights of Group I (9-12 weeks), Group II (18 to 33 weeks), and Group III (62 to 97 week) normotensive (NR) and spontaneously hypertensive rats. Presented are the means $\pm$ 1 standard error of mean of 20 Group I, 80 Group II and 26 Group III rats (equal number of NR and SHR). LV-left ventricle, RV-right ventricle.

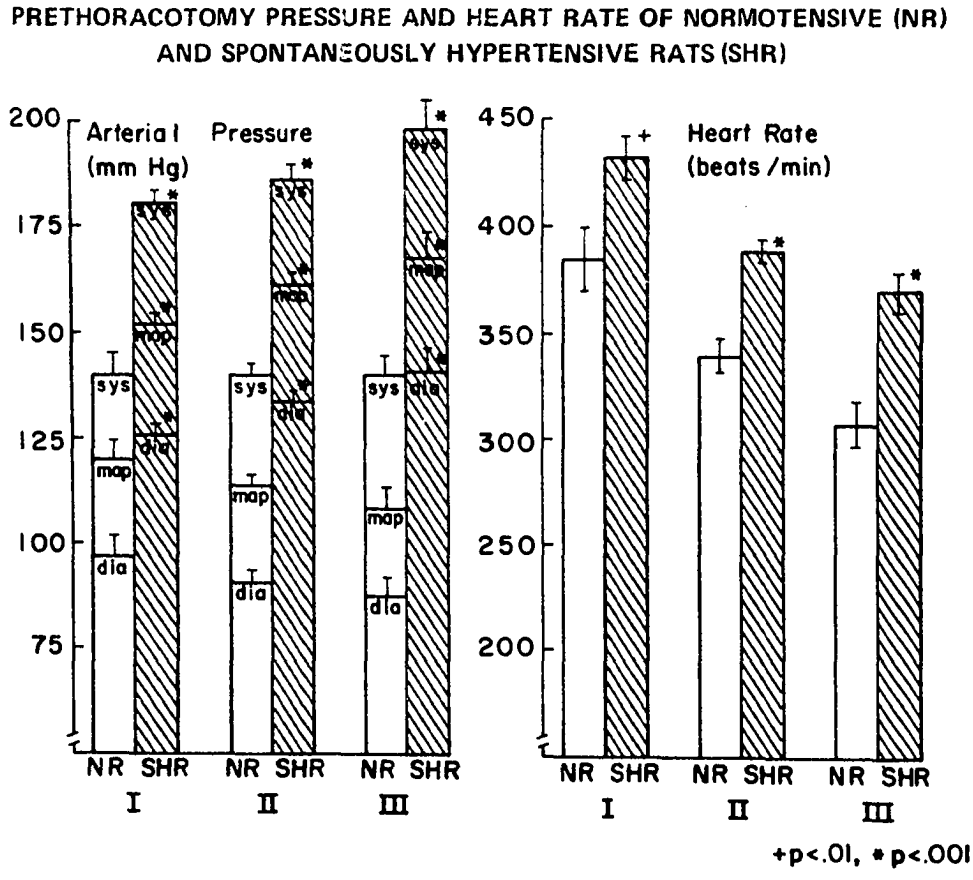

Figure 7. Comparison of arterial pressure and heart rates obtained prior to thoracotomy in ether-anesthetized matched normotensive (NR) and spontaneously hypertensive (SHR) rats. Presented are the means $\pm$ 1 standard error of mean of systolic (sys), mean (map), and diastolic (dia) arterial pressure and heart rate for 20 Group I (9 to 12 week), 80 Group II (8 to 33 week) and 26 Group III (62 to 97 week) NR and SHR rats.

heart rate with aging observed before thoracotomy was also observed after opening of the chest and placement of the ascending aortic flow probe. Even though the arterial pressures declined with thoracotomy, the SHR rats of all three age groups had greater systolic, diastolic, and mean arterial pressures ($p<.001$, Figure 8). Associated with this elevated arterial pressure was a normal cardiac output (Figure 8) and increased total peripheral resistance (Figure 9) in Groups II and III SHR rats. However, in the youngest SHR rats, output was significantly higher (72 $\pm$ 3 vs 58 $\pm$ 2 ml/min, respectively; $p<.001$); total peripheral resistance was normal.

Despite the faster heart rate of the Group I SHR rats, their stroke volume was the same as the age-matched NR (Figure 9). However, in both older SHR groups (II and III), stroke volume was reduced and because of their increased heart rate, cardiac output was normal. Of particular interest were the peak aortic blood flow velocity and maximum acceleration indices, which characterize the initial phase of ventricular ejection. In the young hypertensive rats both of these measurements were significantly greater ($p<.001$) than in age-matched controls (peak flow velocity, 4.8 $\pm$ 0.2 vs 3.7 $\pm$ 0.1 ml/sec; maximum acceleration, 275 $\pm$ 12 vs 202 $\pm$ 7 ml/sec^2, SHR vs NR, respectively). With aging, this pattern became reversed (Figure 10). Thus, in the Group II NR and SHR rats peak flow velocity and maximum acceleration were essentially the same (peak flow, 5.2 $\pm$ .1 and 5.2 $\pm$ .1 ml/sec; maximum acceleration, 249 $\pm$ 10 and 258 $\pm$ 8 ml/sec^2, SHR vs. NR, respectively). However, the oldest NR (Group III) maintained these levels of ventricular flow ejection (peak flow 5.1 $\pm$ 2 ml/sec and maximum acceleration 259 $\pm$ 13 ml/sec^2),

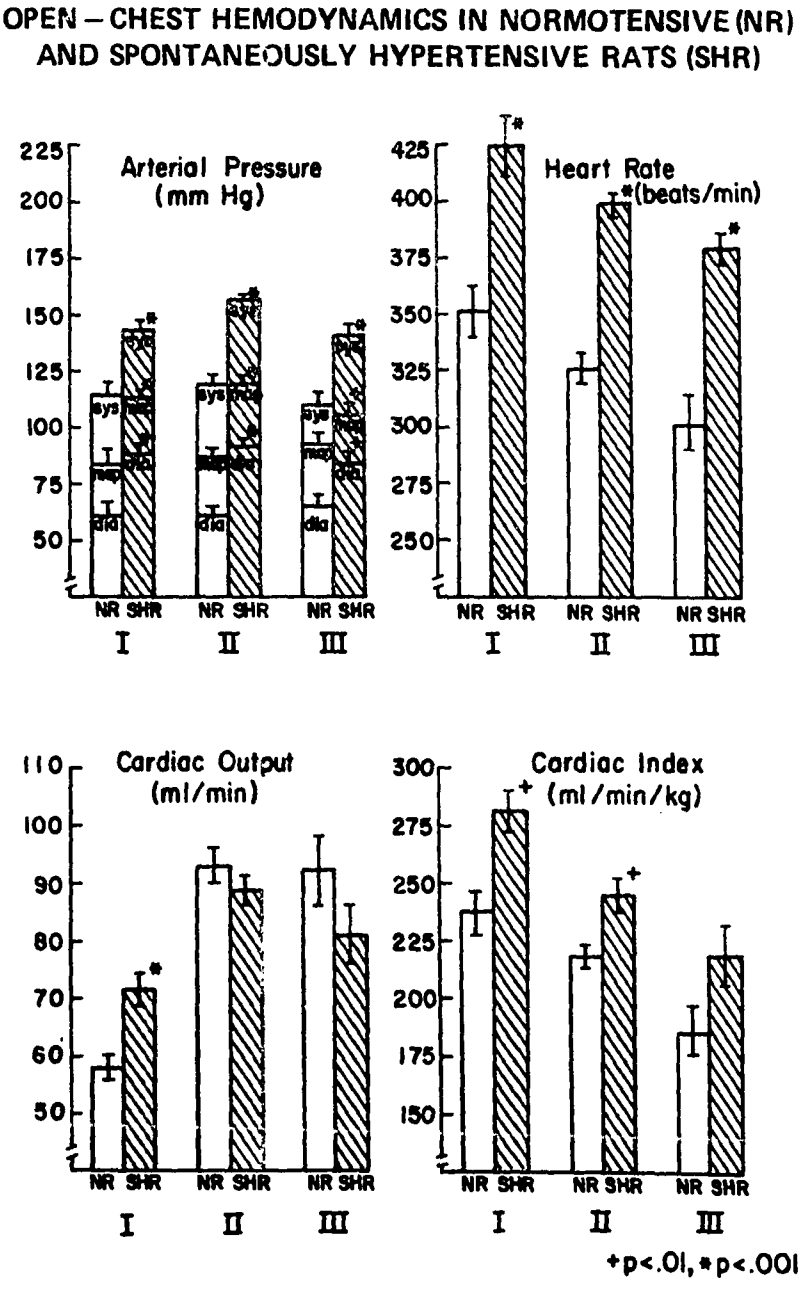

Figure 8. Hemodynamic comparison of open-chest ether-anesthetized matched normotensive (NR) and spontaneously hypertensive (SHR) rats. Presented are the means ± 1 standard error of mean for 20 Group I (9 to 12 week), 80 Group II (18 to 33 week) and 26 Group III (62 to 97 week) NR and SHR rats.

HEMODYNAMIC COMPARISON OF NORMOTENSIVE (NR) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

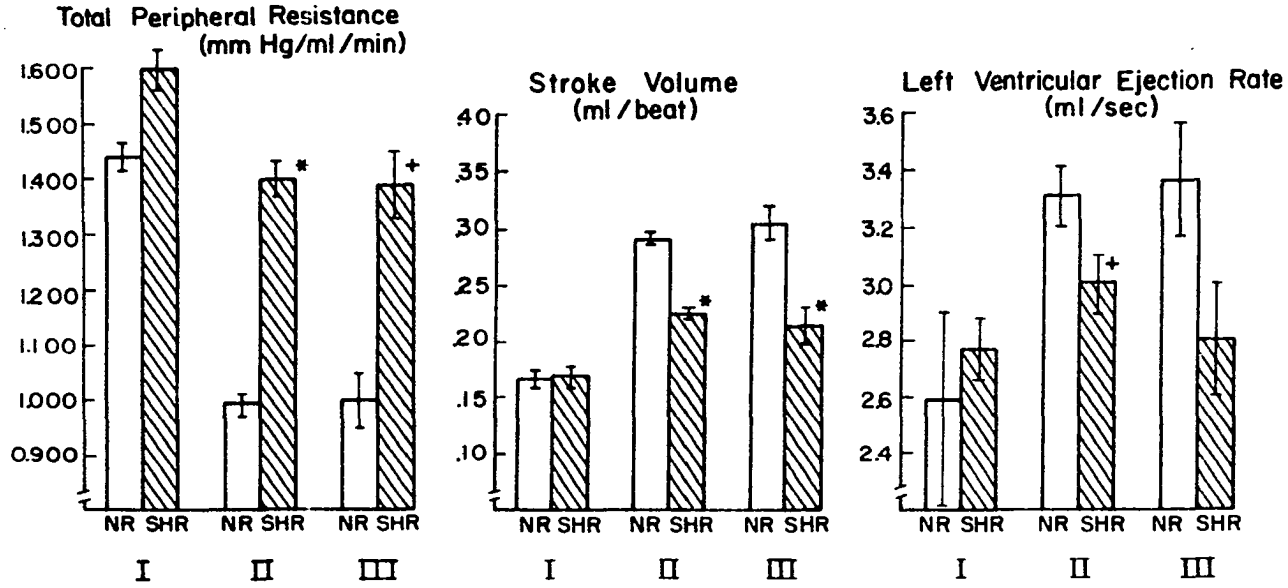

Figure 9. Calculations from hemodynamic measurements of open-chest ether-anesthetized matched normotensive (NR) and spontaneously hypertensive (SHR) rats. Presented are the means ± 1 standard error of the mean for 20 Group I (9 to 12 week), 80 Group II (18 to 33 week) and 26 Group III (62 to 97 week) NR and SHR rats.

despite measurable deterioration of cardiac function in the SHR (peak flow 4.0 $\pm$ .3 ml/sec and maximum acceleration 200 $\pm$ 18 ml/sec^2).

Another aspect of the failing nature of the old (Group III) hypertensive myocardium was underscored by the calculated left ventricular minute work (Figure 10). As previously discussed, the SHR left ventricular mass was greater than their age-matched NR. In the younger SHR rats (Groups I and II), this increased myocardial mass produced more external work, but even with the still further increased ventricular mass, the Group III SHR myocardium was no longer able to maintain the increased work load imposed by the disease. Therefore, the stroke work of only the youngest SHR rats (Group I) was greater than their respective normal group ($p<.001$) and the normal stroke work of the two older groups reflected their reduced stroke volume.

Hemodynamics: Aging. Arterial pressure levels of all age groups of normal rats studied remained relatively constant (Figure 7). However, cardiac output increased with age as the NR rats' body weight increased from Group I to Group II and III. However, this increased flow rate was not in proportion to the rats' growth, since the expression of flow with respect to body weight (cardiac index, ml/min/kg) revealed a progressive decline in this variable from Group I to III. Similarly, the NR heart rate decreased progressively from Group I to III; interestingly enough, however, ventricular ejection was poorest in the youngest rats with the fastest heart rate and highest cardiac index. Thus, stroke volume, left ventricular ejection rate (Figure 9), maximum acceleration of aortic flow, peak flow velocity, and stroke work (Figure 10) were lowest in the Group I NR, increasing significantly in Group II and stabilizing at that

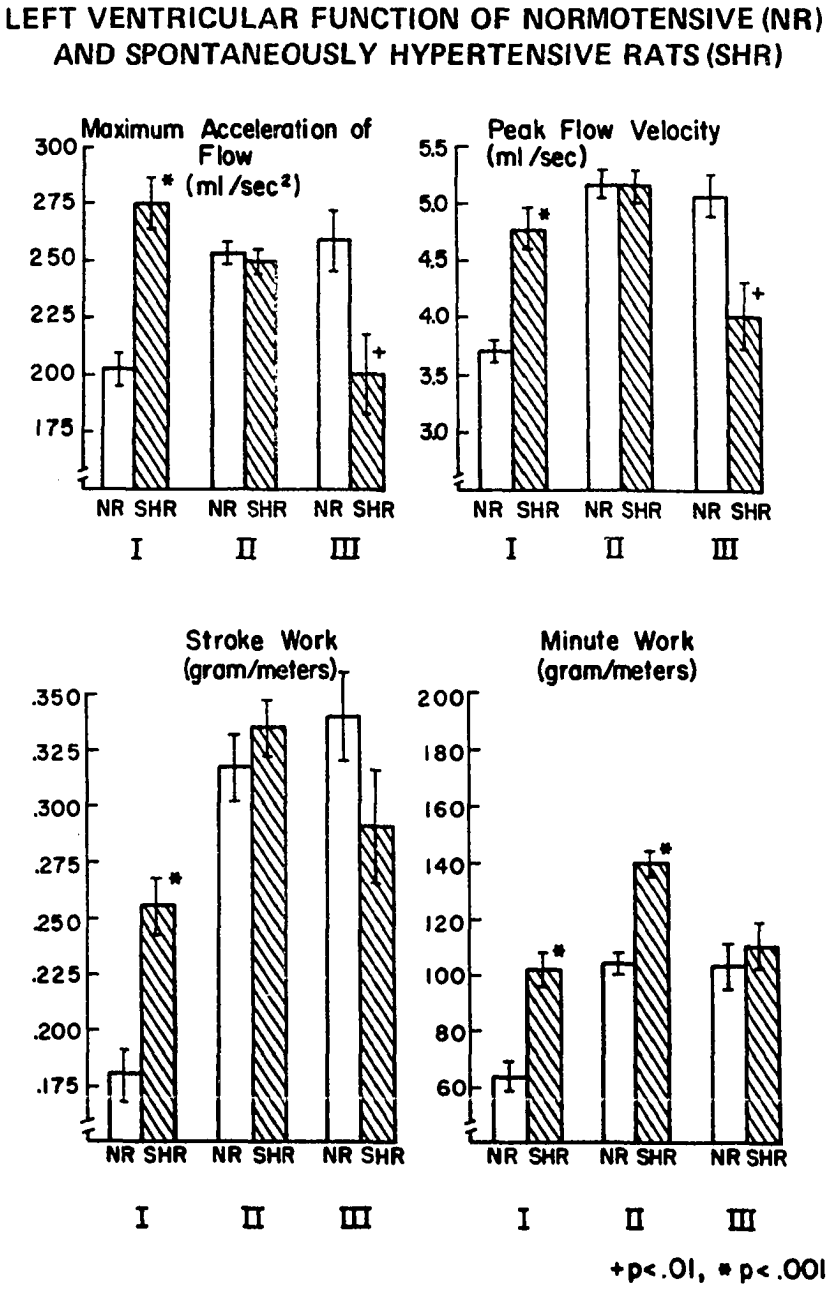

Figure 10. Left ventricular function of matched normotensive (NR) and spontaneously hypertensive (SHR) rats. Presented are the means ± 1 standard error of mean for 20 Group I (9 to 12 week), 80 Group II (18 to 33 week) and 26 Group III (62 to 97 week) NR and SHR rats.

level in Group III.

Unlike the NR, the elevated arterial pressure of the SHR rats increased further from Groups I to III (Figure 7). The decline in heart rate and cardiac index with aging was similar to those changes observed in the NR. However, several indices of ventricular function, which increased in the normals, were reduced in the oldest (Group III) SHR rats. Thus, the maximum acceleration of aortic flow was actually highest in the young Group I and lowest in Group III. In addition, the peak flow velocity was reduced in the Group III SHR rats with respect to Group II (Figure 10).

Pharmacological Assessment of Autonomic Activity

Parasympathetic Inhibition

Vagal influences on myocardial performance of NR and SHR rats were evaluated by studying alterations in cardiac performance after the administration of the parasympathetic inhibitor, atropine (Figure 11). Cumulative doses (0.5 and 1.0 mg/kg) of atropine markedly accelerated heart rate of normal rats. The young (9-12 week; Group I) NR rats increased heart rate from 350 $\pm$ 11 to 374 $\pm$ 10 beats/min with 0.5 mg/kg of atropine ($p<.05$); this failed to increase further (371 $\pm$ 10 beats/min) after the second 0.5 mg/kg dose. Similarly, the older (18-33 week; Group II) NR, having a slower pretreatment heart rate (306 $\pm$ 12 beats/min), increased to 380 $\pm$ 11 beats/min with 0.5 mg/kg of atropine ($p<.001$) and without increasing further with the second dose (383 $\pm$ 11 beats/min). In contrast, atropine had little effect on the heart rate of either the Group I or Group II SHR rats (Figure 11). Thus, the control heart rate of Group I

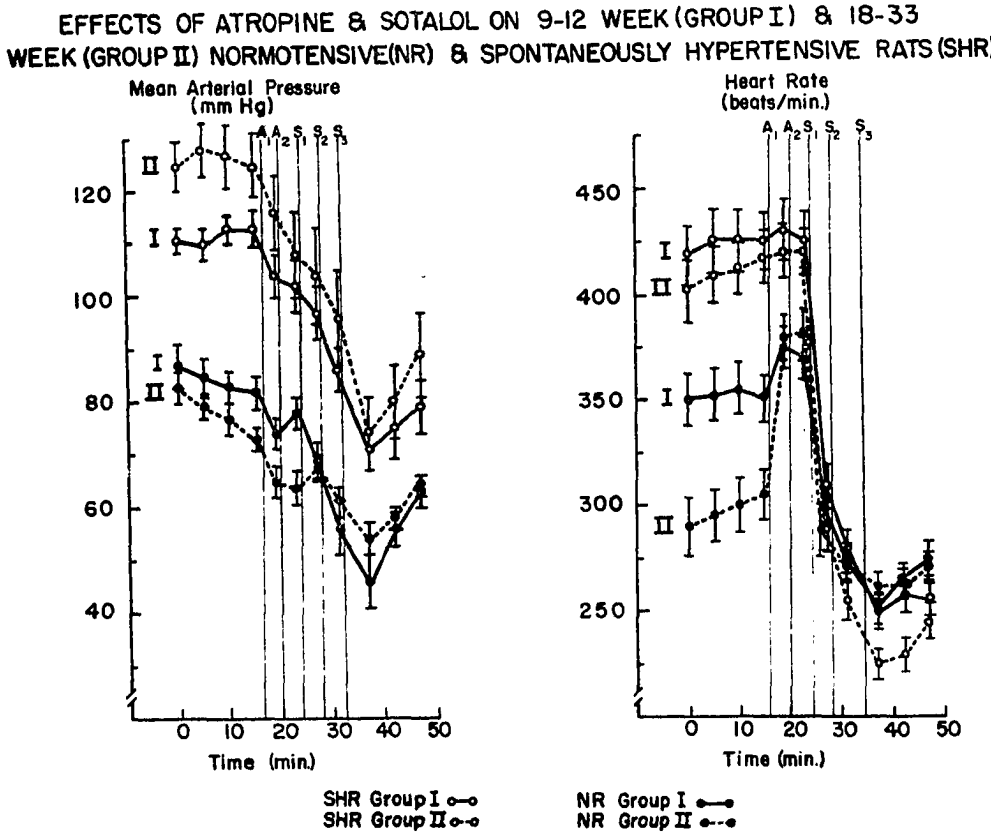

Figure 11. Effects of atropine and sotalol on mean arterial pressure and heart rate of 9 to 12 week (Group I) and 18 to 33 week (Group II) normotensive (NR) and spontaneously hypertensive (SHR) rats. Each point represents the mean value of 10 rats. Atropine (0.5 mg/kg) was given at each A_1 and A_2 period and sotalol (10 mg/kg) at S_1 and S_2 and 20 mg/kg at S_3. Therefore, the effects of atropine alone can be observed after A_1 and A_2.

SHR rats (424 ± 13 beats/min) failed to change after the two doses of atropine (430 ± 14 and 425 ± 13 beats/min, respectively); the control heart rate (417 ± 12 beats/min) also was not altered by atropine (420 ± 12 and 421 ± 11 beats/min, 0.5 and 1.0 mg/kg doses, respectively) in the Group II SHR rats.

Mean arterial pressure of both NR and SHR rats was reduced following atropine. Thus, pressures of the Group I NR fell from 82 ± 3 to 78 ± 3 mm Hg ($p<.05$) following the highest dose of atropine; in Group II NR, pressures were reduced from 73 ± 2 to 64 ± 3 mm Hg ($p<.05$). Response of arterial pressure was similar in the SHR rats; those of Group I fell from 113 ± 3 mm Hg to 102 ± 5 mm Hg ($p<.01$), and from 125 ± 6 to 108 ± 8 mm Hg ($p<.01$) in the Group II SHR rats. However, at no stage were the SHR and NR rats' mean arterial pressures at the same levels (Figure 11).

Atropine did not significantly reduce the cardiac index of the Group I NR (239 ± 11 ml/min/kg to 231 and 234 ml/min/kg, after 0.5 and 1.0 mg/kg atropine, respectively). The lower cardiac index of the Group II NR also failed to change following atropine (197 ± 9 vs 200 ± 15 ml/min/kg, control vs 1.0 mg/kg atropine, respectively). In contrast, the Group I SHR rats' cardiac index fell significantly from levels of 278 ± 8 ml/min/kg to 258 ± 9 and 253 ± 14 ml/min/kg after the 0.5 and 1.0 mg/kg doses of atropine ($p<.01$), respectively. The Group II SHR rats also had lower cardiac indexes than their younger hypertensive counterparts and, as with the Group II NR, flow failed to change after 1.0 mg/kg atropine (252 ± 15 and 246 ± 15 ml/min/kg, respectively).

The maximum acceleration of aortic flow of all groups was reduced following atropine. Thus, Group I NR fell from 205 ± 9 to 192 ± 4 ml/sec^2 after 1.0 mg/kg atropine ($p<.05$). This same dosage produced a measurable,

though insignificant, reduction in this variable from 307 ± 14 to 277 ± 20 ml/sec^2 in Group II NR. Similarly, maximum aortic flow acceleration of Group I SHR rats fell from 271 ± 12 to 241 ± 12 ml/sec^2 ($p<.05$) and in Group II it fell from 278 ± 12 to 258 ± 13 ml/sec^2 after 1.0 mg/kg atropine ($p<.05$).

Combined Parasympathetic and Beta-Adrenergic Inhibition

Sotalol, a beta-adrenergic blocking drug, produced major alterations in the hemodynamics of atropinized NR and SHR rats (Figures 11 and 12). Sotalol was given three times at 3 minute intervals to provide each animal with a cumulative dose of 40 mg/kg. Each hemodynamic variable measured fell sharply after sotalol and tended to recover slightly with time, stabilizing within 15 to 20 minutes. Although the full range of responses with time are presented in Figures 11 and 12, only those made 15 minutes after the last dose of sotalol will be discussed. At this time cardiac function is interpreted as being intrinsic, since Jose (166) described the simultaneous use of adequate doses of parasympathetic and beta-adrenergic inhibiting drugs as sufficient to counteract both of these neural types of extrinsic myocardial influences.

Although control and postatropine heart rates of NR and SHR rats differed following sotalol, the intrinsic rates became remarkably similar. Thus, the young and old NR had intrinsic heart rates of 274 ± 9 and 272 ± 7 beats/min, respectively; those of the young and old SHR were 257 ± 8 and 244 ± 8 beats/min, respectively.

Control cardiac index of the young SHR was greater than that of their age-matched NR; however, their cardiac indexes after combined vagal

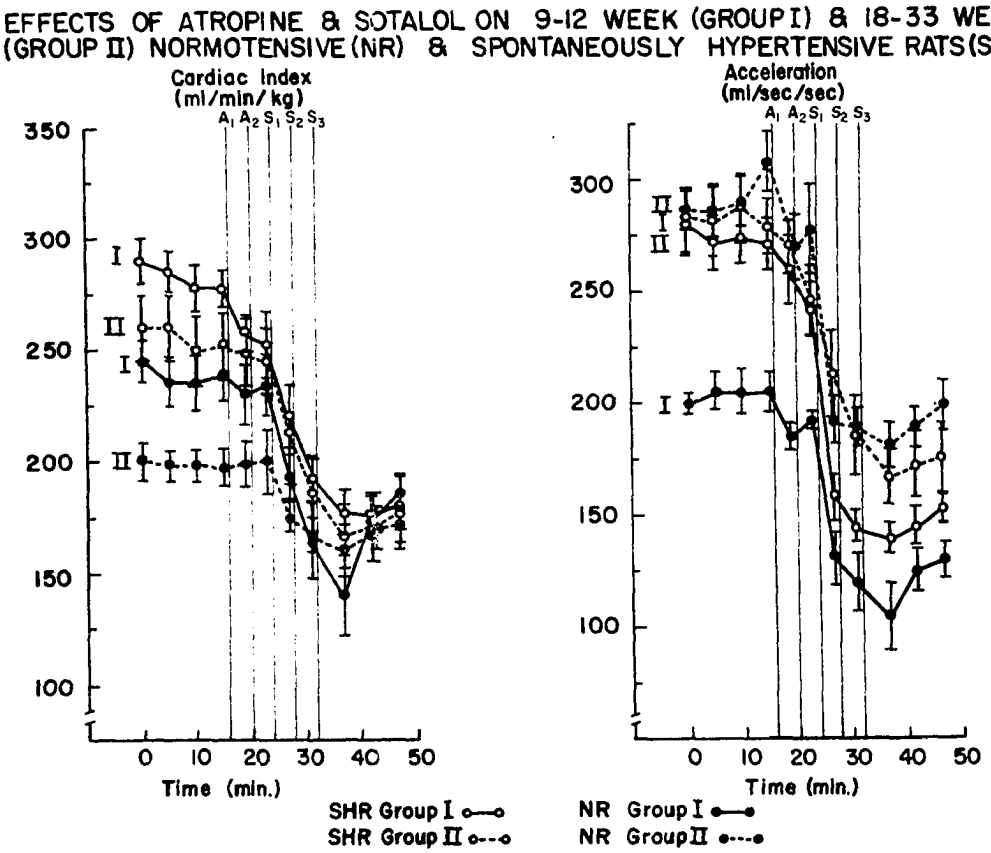

Figure 12. Effects of atropine and sotalol on cardiac index and maximum acceleration of aortic flow of 9 to 12 week (Group I) and 18 to 33 week (Group II) normotensive (NR) and spontaneously hypertensive (SHR) rats. Each point represents the mean value of 10 rats. Atropine (0.5 mg/kg) was given at each A_1 and A_2 period and sotalol (10 mg/kg) at S_1 and S_2 and 20 mg/kg at S_3.

and beta-adrenergic inhibition were the same (180 ± 11 and 185 ± 7 ml/min/kg, SHR and NR, respectively). Furthermore, the cardiac indexes of the older NR and SHR rats were reduced to similar levels after dual autonomic inhibition (SHR, 176 ± 18 and NR, 174 ± 10 ml/min/kg). Maximum acceleration of aortic flow of the young SHR rats was also greater than that of NR and, following pharmacological denervation, the intrinsic values did not differ significantly (SHR, 153 ± 7 ml/sec^2 and NR, 131 ± 8 ml/sec^2). The older rats had similar control levels of maximum flow acceleration; their intrinsic levels remained the same (SHR, 202 ± 13 and NR, 198 ± 11). However, the maximum flow acceleration of the older NR and SHR rats with pharmacologically denervated hearts was greater than that of the younger rats under the same conditions (vida infra).

Mean arterial pressure of both young and mature NR and SHR rats responded similarly to vagal and beta-adrenergic blockade (Figure 11). However, despite the fact that the intrinsic heart rate, cardiac index, and maximum acceleration of the respective NR and SHR groups were similar, arterial pressure in the SHR groups was always elevated with respect to their normotensive counterparts ($p<.01$). Thus, while mean arterial pressure of the young and mature NR fell to 63 ± 3 and 64 ± 2 mm Hg, respectively, after atropine and sotalol, pressures in the young and mature SHR were reduced to 79 ± 5 and 89 ± 8 mm Hg, respectively.

Intrinsic Cardiac Performance: Aging. Production of pharmacological cardiac denervation with sufficient doses of atropine and sotalol demonstrated that the slower heart rate in Group II NR resulted from extrinsic neurohumoral influences since the difference in heart rate between Group I and II no longer existed with total denervation. Moreover,

the difference in pretreatment heart rate between these two groups appears to be a result of increased parasympathetic participation with age, since atropine raised heart rates of these groups to the same level. Intrinsic cardiac index and arterial pressure of these NR were the same as shown by similar group levels after autonomic inhibition (Figure 12). However, the maximum acceleration of aortic flow, which was less in pretreated Group I NR, remained lower than Group II even after vagal and beta-adrenergic inhibition. Therefore, in contrast to the heart rate differences, the pretreatment differences in maximum flow acceleration appear to be intrinsic to the myocardium and not due to different levels of cardiac neurohumoral stimulation. Thus, although the intrinsic pressure and flow characteristics of the Group I and II NR were identical, the aortic flow acceleration of Group I NR suggests a reduced myocardial contractility at this age.

Following this same chemical cardiac denervation, no significant differences were observed in cardiac index, mean arterial pressure, heart rate, or maximum acceleration of aortic flow between Group I and II SHR rats. However, the mean arterial pressure of the Group II rats was slightly higher than Group I, although the cardiac indexes of the two groups were identical (Figures 11 and 12), confirming the progression of hypertensive vascular disease.

Cardiac Stimulation

Isoproterenol

Intravenous infusion of low doses of isoproterenol failed to stimulate chronotropically or inotropically the hypertrophied myocardium of

the SHR although these same doses significantly increased both of these cardiac responses in the normal rats (Figures 13-16). In the NR group, the lowest isoproterenol dose (0.16 μg/kg/min) increased heart rate and cardiac output significantly ($p<0.05$); this same dose produced no change in any hemodynamic variable measured in the SHR group. With the 0.32 μg/kg/min dose, cardiac function was further increased in NR; and by the 0.64 μg/kg/min level, the maximum response had been achieved. In contrast, in the SHR rats, only heart rate was increased by the 0.64 μg/kg/min infusion rate ($p<0.05$), and the peak cardiac responses to beta-adrenergic stimulation occurred only at the extremely high dose of 5.12 μg/kg/min. Thus, at the 0.64 μg/kg/min dose, NR heart rate had increased from a control value of 306 ± 15 to 367 ± 10 beats/min, but in the SHR, it only increased from 378 ± 15 to 402 ± 10 beats/min (Figure 13). At this same isoproterenol dosage cardiac output of the NR increased from 76 ± 6 to 88 ± 7 ml/min, whereas in the SHR rats it remained unchanged from control values 72 ± 6 to 72 ± 5 ml/min (Figure 14). Similarly, the peak flow velocity and maximum acceleration of aortic flow of the NR increased from 4.6 ± 0.2 to 5.1 ± 0.1 ml/sec, and 246 ± 14 to 293 ± 15 ml/sec^2, respectively from control to 0.64 μg/kg/min isoproterenol ($p<.005$). No significant change in these inotropic indexes was observed in the SHR at this infusion rate (4.8 ± 0.3 to 4.9 ± 0.2 ml/sec peak flow velocity and 254 ± 22 to 263 ± 15 ml/sec^2 maximum acceleration, control to 0.64 μg/kg/min, respectively) (Figure 15 and 16).

This marked shift to the right in the myocardial responses of SHR rats to increasing doses of isoproterenol was not observed with the arterial pressure response to this agent (Figure 17). Hence, at the

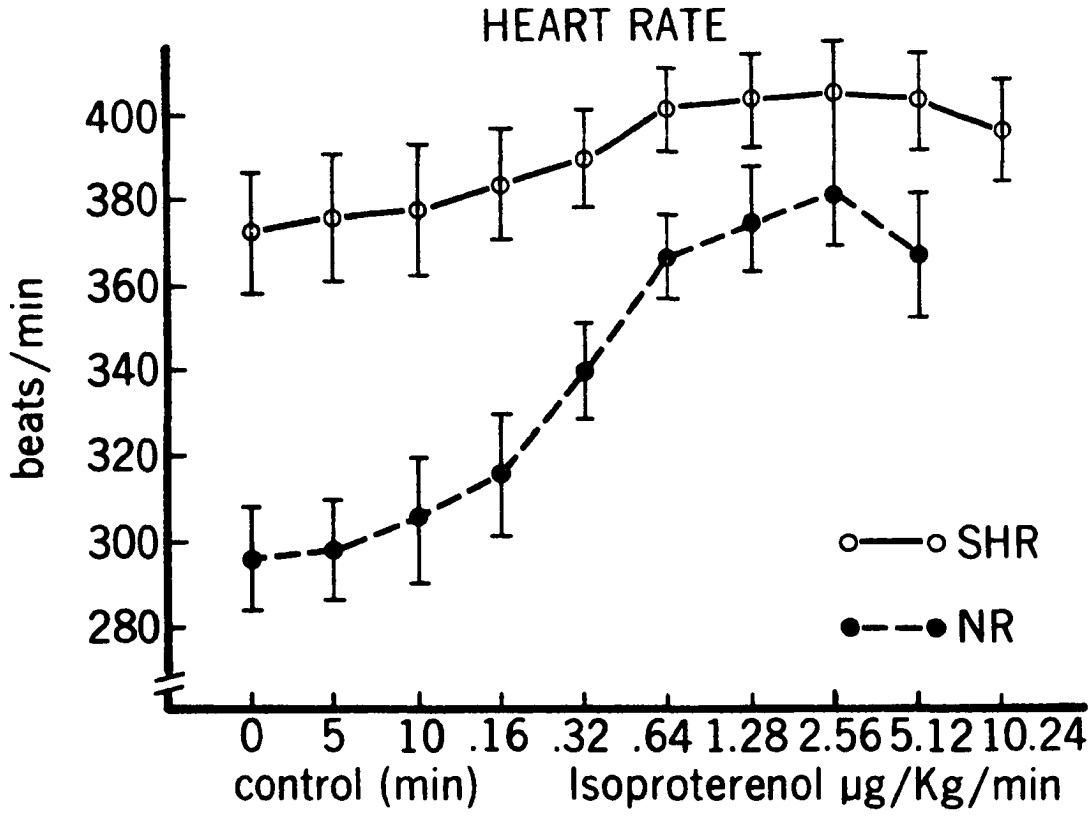

Figure 13. Heart rate responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean $\pm$ 1 standard error of mean values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2 minute infusions of increasing doses of isoproterenol. Time represented on abscissa applies only to the control period.

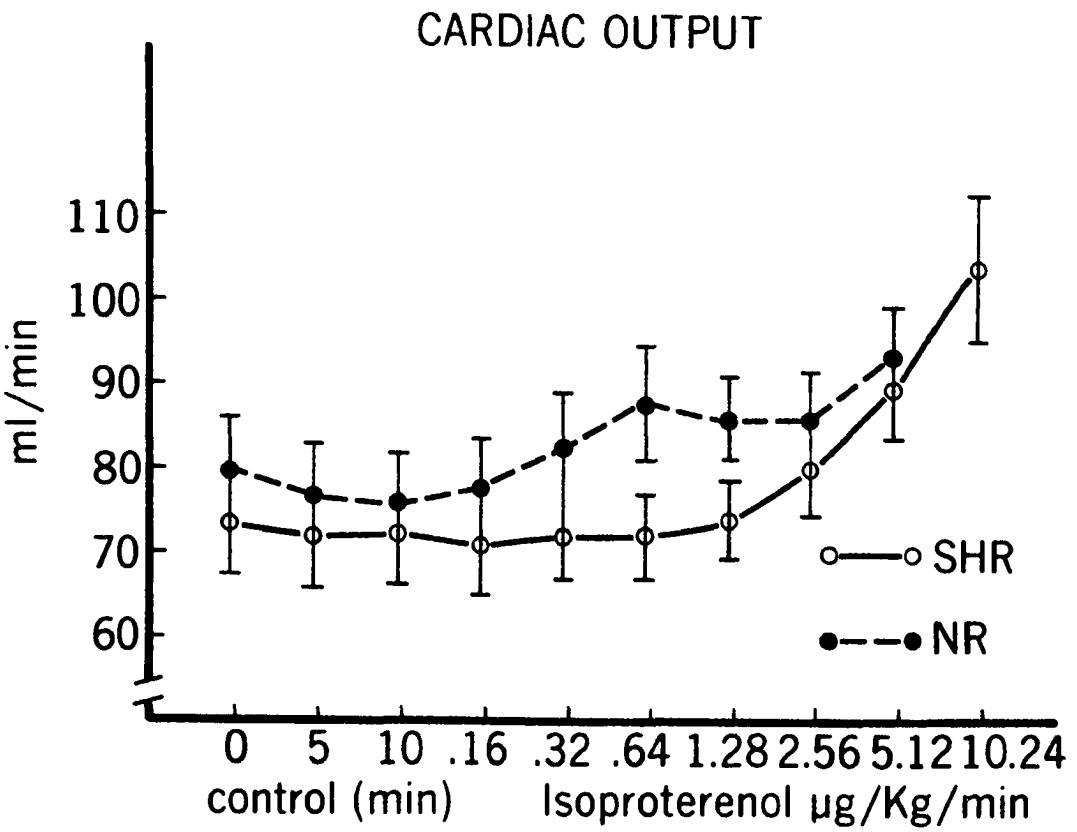

Figure 14. Cardiac output responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean $\pm$ 1 standard error of mean values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2 minute infusions of increasing doses of isoproterenol. Time represented on the abscissa applies only to the control period.

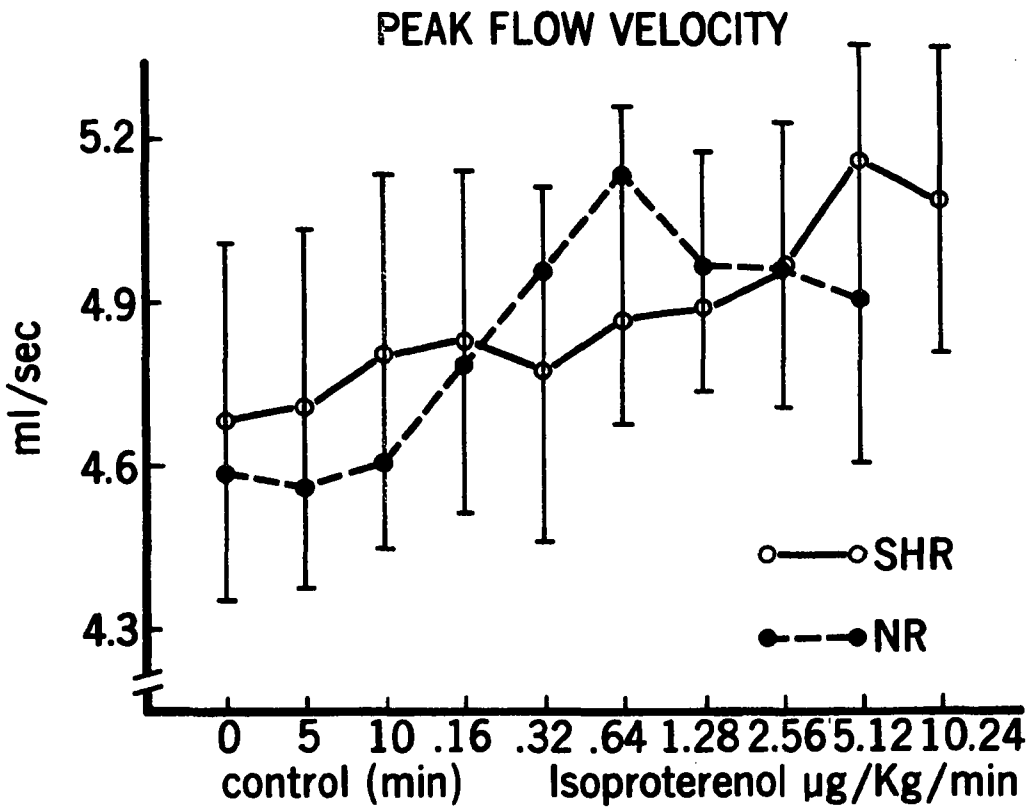

Figure 15. Peak ascending aortic flow velocity responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean $\pm$ 1 standard error of mean values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2 minute infusions of increasing doses of isoproterenol. Time represented on the abscissa applies only to the control period.

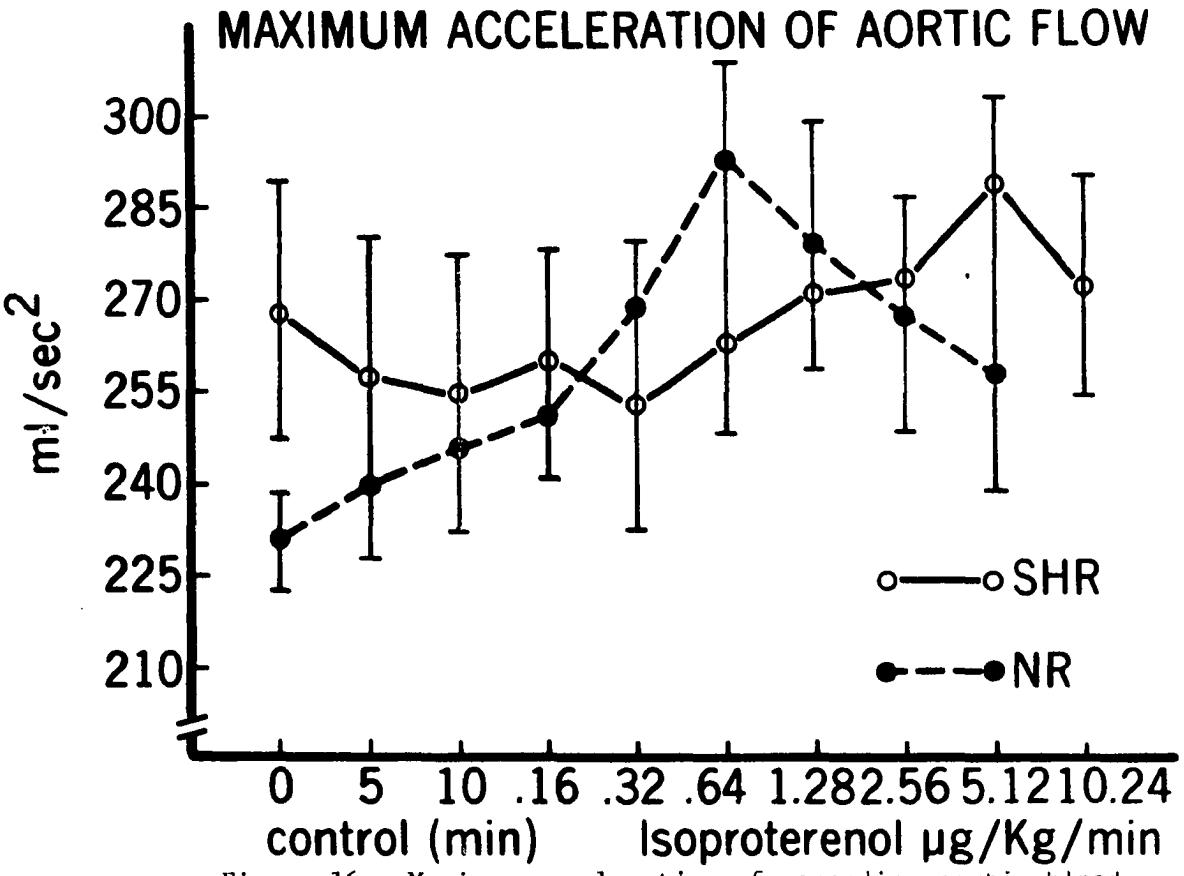

Figure 16. Maximum acceleration of ascending aortic blood flow responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean $\pm$ 1 standard error of mean values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2 minute infusions of increasing doses of isoproterenol. Time represented on the abscissa applies only to the control period.

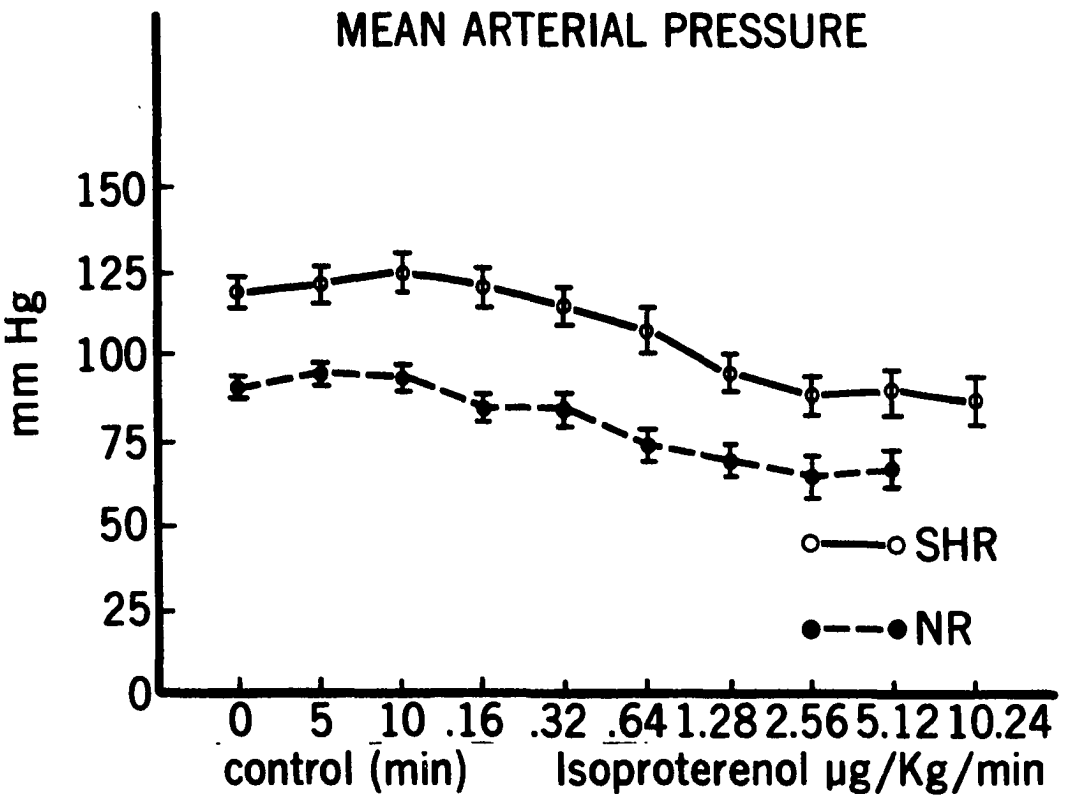

Figure 17. Mean arterial pressure responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to increasing doses of isoproterenol. Presented are mean $\pm$ 1 standard error of mean values for each control and dose level measurement. The first three points on the left are control measurements which are followed by successive 2 minute infusions of increasing doses of isoproterenol. Time represented on abscissa applies only to the control period.

0.32 μg/kg/min infusion level, mean arterial pressure of the NR and SHR rats had decreased by 9 and 8 mm Hg, respectively. These falls in arterial pressure of both groups paralleled each other with a maximum fall of 27 mm Hg observed in both groups at the 5.12 μg/kg/min dose level. Therefore, the significant difference in pressure between the NR and SHR groups was maintained throughout the entire dose range of isoproterenol.

Norepinephrine

The effects of increasing doses of norepinephrine on arterial pressure are shown in Figure 18. At the lowest dose (0.5 μg/kg/min) mean arterial pressure was increased by 4 mm Hg in both groups; these increases were statistically not significant. Nevertheless, administration of this dosage of norepinephrine to NR was associated with increased heart rate (356 to 378 beats/min $p<.01$), cardiac index (237 to 253 ml/min/kg, $p<.05$) and a measurable but statistically insignificant increase in maximum acceleration of aortic flow (252 to 264 ml/sec^2); total peripheral resistance remained unchanged (.990 to .979 mm Hg/ml/min). The SHR rats responded in a hemodynamically different manner to this same dosage of norepinephrine and 4 mm Hg mean arterial pressure rise (Figure 19). These SHR rats failed to increase heart rate (399 to 400 beats/min), cardiac index (247 to 238 ml/min/kg), or maximum acceleration of aortic flow (259 to 252 ml/sec^2), and total peripheral resistance significantly increased (1.428 to 1.503 mm Hg/ml/min $p<.05$).

At the next higher infusion rate (1.0 μg/min/kg) mean arterial pressure increased above pre-infusion levels by 10 mm Hg (92 to 102 mm Hg, $p<.001$) and 9 mm Hg (127 to 136 mm Hg, $p<.05$) in the NR and SHR groups,

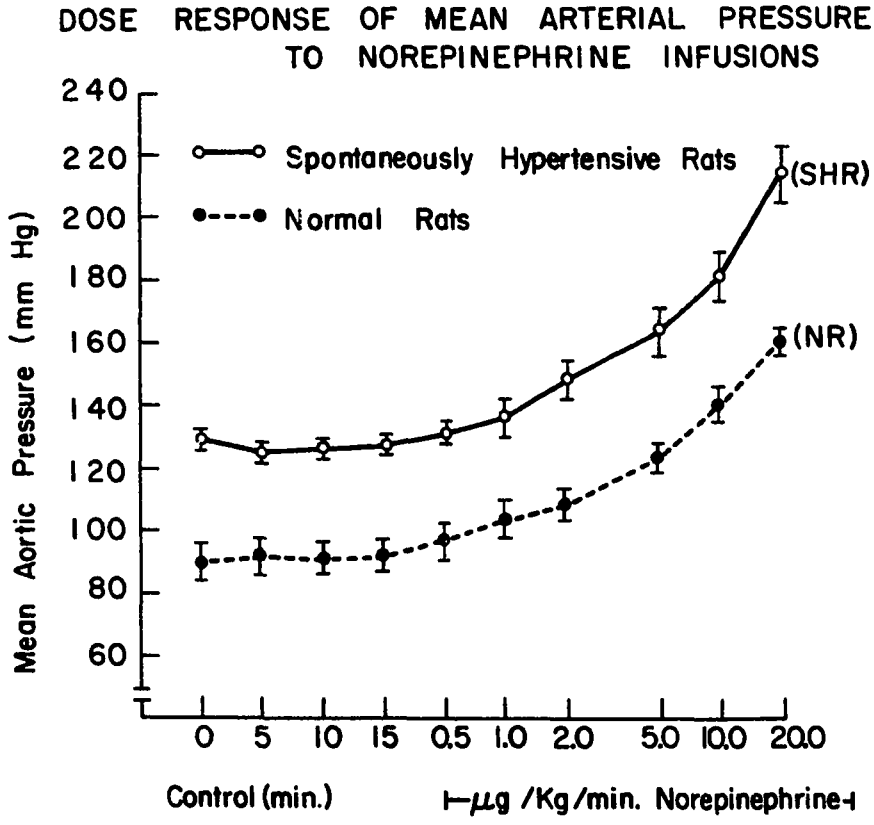

Figure 18. Relationship of doses of norepinephrine infusions to mean arterial pressure responses in 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats. Values of mean $\pm$ 1 standard error of mean for each group are given at each control and dose level. Measurements taken during the control and infusion period at a 5 minute and 40 second intervals, respectively.

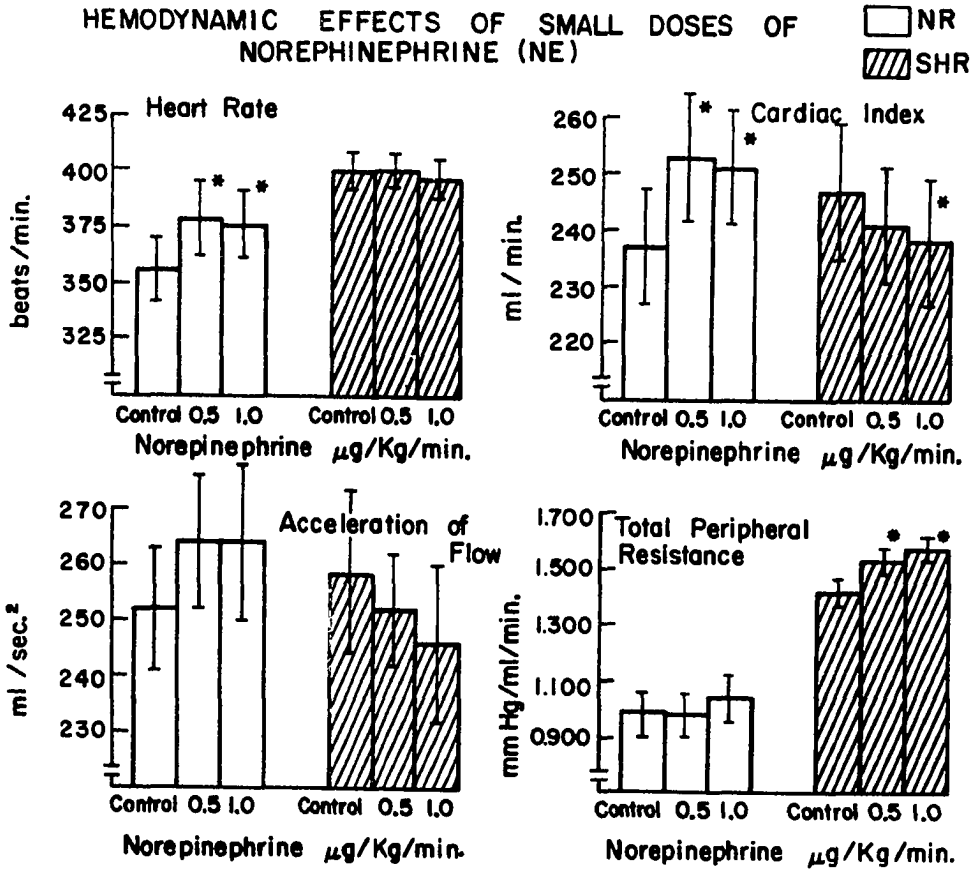

Figure 19. Hemodynamic responses of 10 normotensive (NR) and 10 spontaneously hypertensive (SHR) rats to low doses of norepinephrine. The small pressure increases of both groups were the same (see text). Shown are group means $\pm$ 1 standard error of mean. *$p<.01$, difference from pre-infusion to response with norepinephrine.

respectively. Still heart rate (356 to 376 beats/min $p<.05$) and cardiac index (237 to 250 ml/min/kg $p<.05$) remained elevated in the normotensive rats although total peripheral resistance failed to increase significantly. In continued contrast SHR rats further increased calculated total peripheral resistance (1.427 to 1.572 mm Hg/ml/min $p<.001$) because the cardiac index actually declined (from 247 to 238 ml/min/kg $p<.01$). The cardiac responses to higher doses of norepinephrine were difficult to interpret because the vasoconstrictor effects of this agent significantly increased arterial pressure and thus elicited baroreceptor reflexive cardiac inhibition.

Elevation of Arterial Pressure - Methoxamine

In contrast to these norepinephrine responses, cardiac responses to methoxamine infusion were qualitatively similar in the two groups of rats. Thus, as mean arterial pressure was increased by sequentially increasing steady-state infusion doses of methoxamine, heart rate (Figure 20) maximum acceleration of flow (Figure 21) and cardiac index (Figure 22) of both NR and SHR rats reflexively decreased. However, quantitatively these responses differed greatly. The increase in mean arterial pressure was significantly greater in the hypertensive rats (Figure 23), and most important, the mean arterial pressure level at which reflexive cardiac responses to the progressively increased afterload were first observed was different (Figures 20, 21 and 22). The average mean arterial pressure measured just prior to the consistent fall in heart rate or maximum flow acceleration (defined by a decrease in these variables by at least one standard deviation from control levels) was 120 and

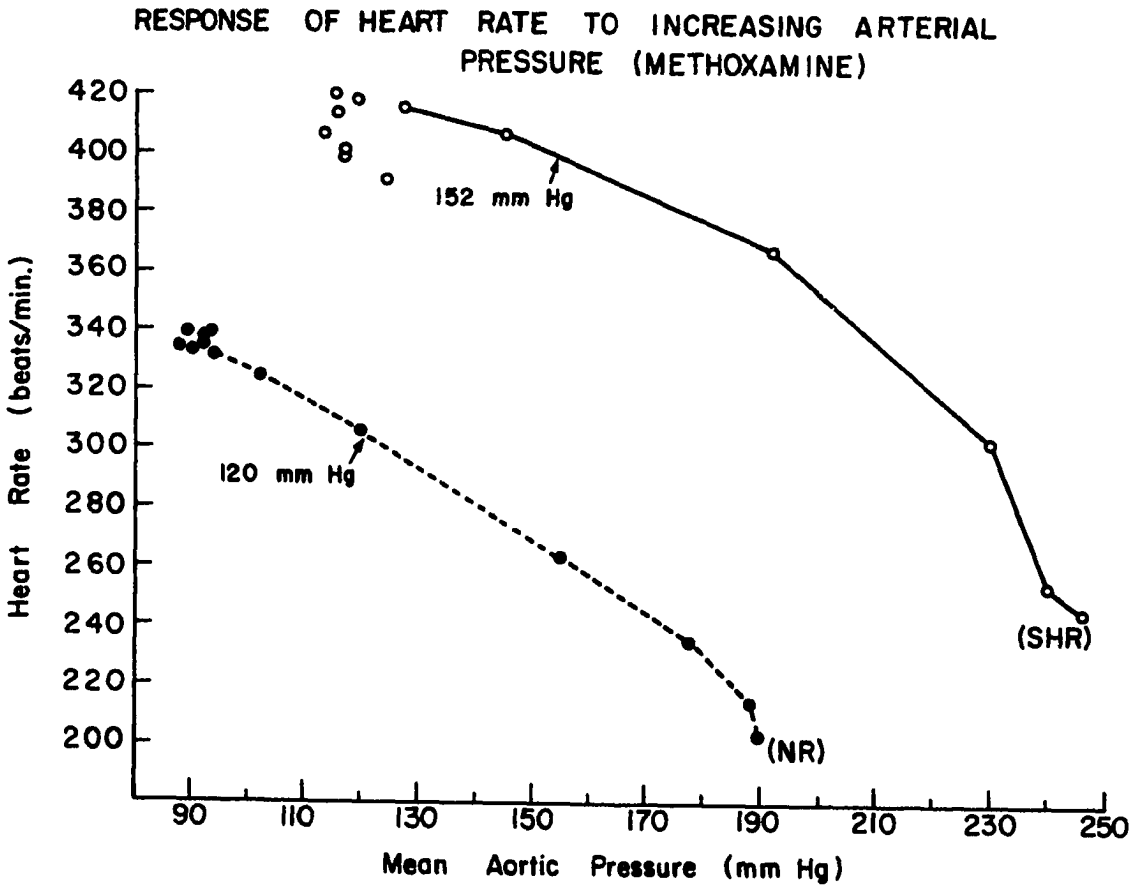

Figure 20. Heart rate responses of normotensive (NR) and spontaneously hypertensive rats (SHR) to acute pressure elevations during methoxamine infusions. Each circle represents the mean of 10 rats' responses. Pressures indicated by arrows represent the last measured mean arterial pressure before significant reductions in the plotted variable were observed (see text). The clustered circles represent repeated control measurements for each group.

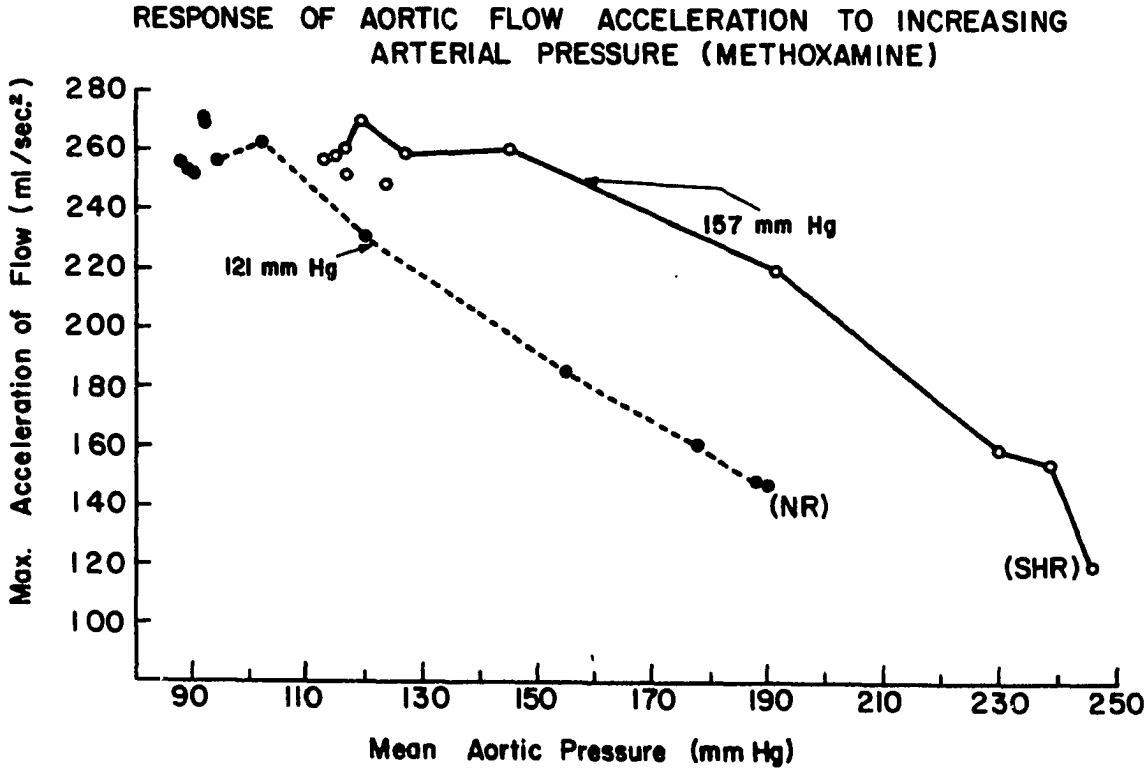

Figure 21. Maximum acceleration of aortic blood flow responses of normotensive (NR) and spontaneously hypertensive rats (SHR) to acute pressure elevations during methoxamine infusions. Each circle represents the mean of 10 rats' responses. Pressures indicated by arrows represent the last measured mean arterial pressure before significant reductions in the plotted variable were observed (see text). The clustered circles represent repeated control measurements for each group.

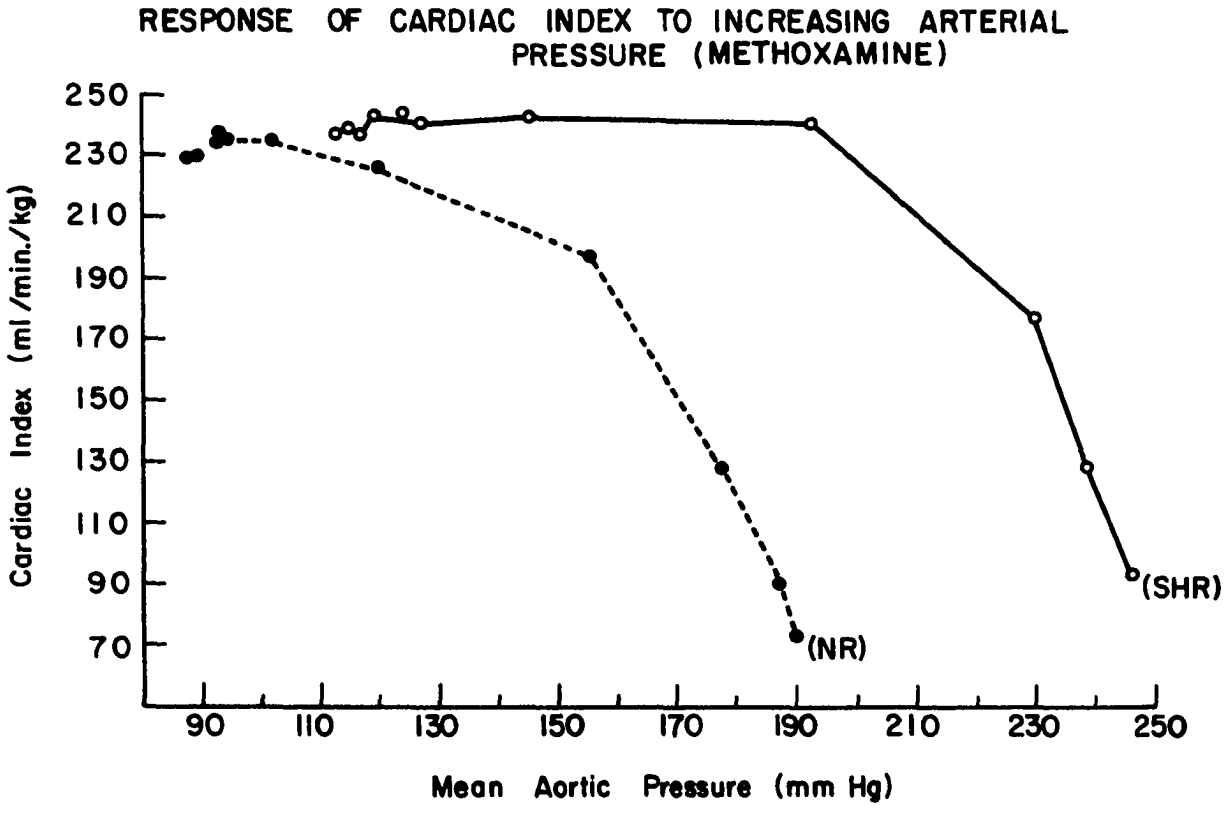

Figure 22. Cardiac index responses of normotensive (NR) and spontaneously hypertensive rats (SHR) to acute pressure elevations during methoxamine infusions. Each circle represents the mean of 10 rats' responses. The clustered circles represent repeated control measurements for each group.

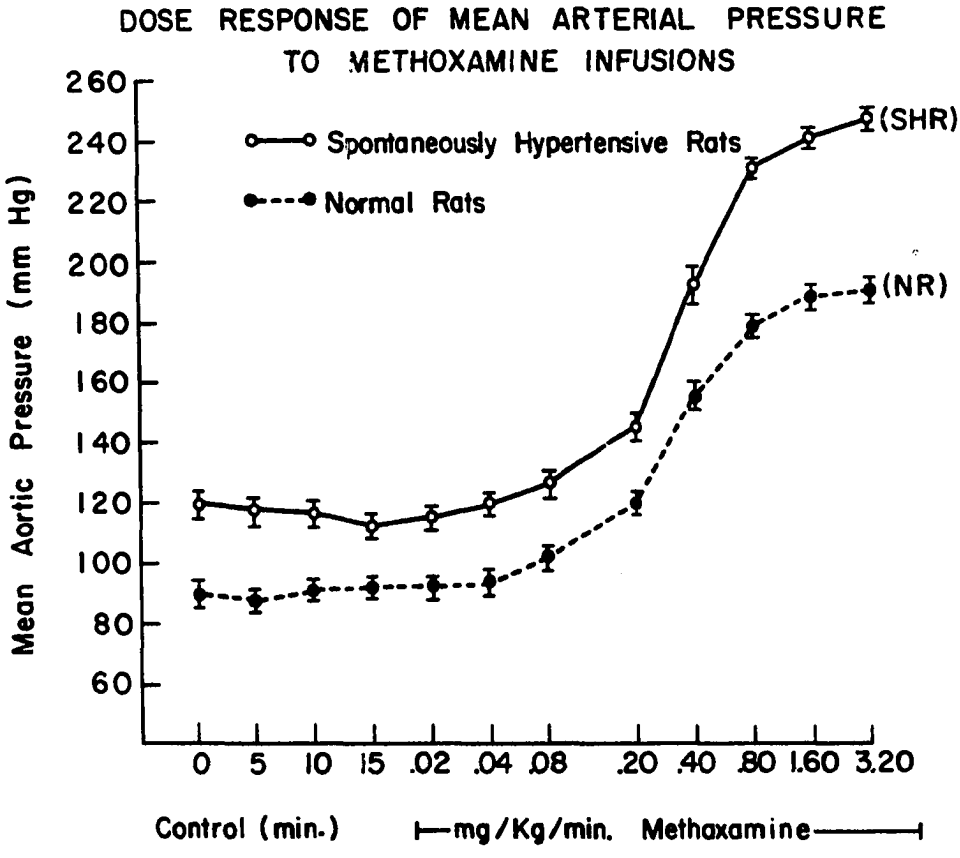

Figure 23. Relationship of doses of methoxamine infusions to mean arterial pressure responses in 10 normotensive and 10 spontaneously hypertensive rats. Values of mean $\pm$ 1 standard error of mean for each group are given at each control and dose level. Measurements taken during the control and infusion period are at 5 minute and 40 sec. intervals, respectively.

154 mm Hg in the NR and SHR groups, respectively ($p<.002$). In addition, the reflexive fall of cardiac index also occurred at a significantly higher mean arterial pressure in the SHR rats (133 and 182 mm Hg in the NR and SHR groups, respectively $p<.002$).

CHAPTER IV

DISCUSSION

This dissertation was undertaken to compare and contrast the hemodynamic characteristics and cardiac performance of age-matched normotensive and spontaneously hypertensive rats during the latter group's natural progression of hypertensive vascular disease. Towards this end it was necessary to develop techniques which permitted measurement of cardiac output and arterial pressure and means by which the contractile performance of the myocardium could be assessed in large numbers of these small animals. In addition, various pharmacological means of inhibiting and stimulating myocardial performance were employed to gain further insight into the nature of myocardial function during the natural development of systemic arterial hypertension.

The methods described herein, utilizing an electromagnetic flowmeter for the measurement of ascending aortic blood flow in open-chest, anesthetized rats, was chosen to achieve the above goals. This method was used because it provided a continuous measurement of flow as well as the phasic ejection characteristics of the left ventricle without necessitating the withdrawal of even small amounts of blood. Although the anesthesia and surgical preparation required for these measurements certainly altered the resting hemodynamics, meticulous care was always

taken to obtain the measurements of these hemodynamic variables in these NR and SHR rats under precisely similar conditions. Each facet of this study: comparison of three commonly employed rat anesthetic agents in normotensive rats; hemodynamic characteristics of various age groups of NR and SHR rats; selective pharmacological autonomic inhibition; and various pharmacological stresses on the cardiovascular system will be discussed individually. Finally, the concepts derived will be integrated to a more unified conclusion about the pathophysiology of SHR hypertension.

Anesthesia Comparison

Although the rat has been used with increasing frequency in cardiovascular studies, phasic aortic blood flow has not been investigated systematically. The present study demonstrates that phasic aortic blood flow can be quantified and characterized in open-chest, anesthetized rats using an electromagnetic flow probe placed around the ascending aorta. The preparation described remained stable for one hour in rats anesthetized with pentobarbital or ether. However, there were significant increases in heart rate and maximum acceleration of aortic flow during this observation period in those rats anesthetized with urethan. Giles, Quiroz and Burch have reported increased heart rate with time in dogs anesthetized with urethan; they also have shown progressive reductions in cardiac index and stroke volume during the five hour observation period (167). During the one hour observation period reported herein, cardiac index remained stable although there was a continuous decline in stroke volume. Therefore, it is probable that the cardiac index could have fallen also if the observation period of the present study had been extended.

Cardiac function (as determined by cardiac index, peak flow velocity, maximum flow acceleration and left ventricular ejection rate) was poorest in rats anesthetized with pentobarbital and best in ether anesthetized rats. Vidt, et al., using an indicator-dilution technique with ^{86}Rb, found that the cardiac output of ether-anesthetized rats was 1.5 times greater than in those anesthetized with pentobarbital (168). In the present study the cardiac output of the ether-anesthetized rats was also 1.5 times as great as the output of the pentobarbital group.

Cardiac output is believed to be augmented during ether anesthesia by increased circulating catecholamine levels (169-172). Although all indexes of cardiac function assessed in this preparation were highest with ether anesthesia, mean aortic pressure, total peripheral resistance index, and heart rate were no greater than the values obtained in rats anesthetized with pentobarbital. Hence, the former findings cannot be explained only by increased circulating catecholamines. In addition, the cardiac index of 214 ml/min/kg obtained by the electromagnetic flowmeter technique using ether anesthesia closely approximates the cardiac index reported by Popovic and Kent (173) in unanesthetized rats using the direct Fick method (286 ml/min/kg). Table 1 from that same study lists the individual values of cardiac index for seven male rats anesthetized with pentobarbital (40 mg/kg); the average of these animals is 176 ml/min/kg as compared to 139 ml/min/kg in the open-chest, pentobarbital-anesthetized rats of the present study. Furthermore, the findings in the ether-anesthetized group (214 ml/min/kg) are in close agreement with the cardiac index values of 210 ml/min/kg reported by Bullard (174) using an indicator-dilution technique (T-1824 dye), also in ether-anesthetized rats.

Although the electromagnetic flowmeter method measuring cardiac output has been used in the rat, the full potential of this technique for determining acute phasic changes in blood flow and assessing cardiac performance has not been realized heretofore. Thus, Ross et al. (175) measured cardiac output in five rats anesthetized with pentobarbital, reporting the electromagnetically determined range of flow as 90 to 200 ml/min/kg. Ledingham and his co-workers have used ascending aortic electromagnetic flow recordings to follow changes in cardiac output in rats over periods of weeks (176,177). Though such measurements were semiquantitative, they have been of great value in following hemodynamic changes in experimental renal hypertension (178-180). More recently, Lee, Karpeles, and Downing have used a preparation very similar to the one described in the present investigation to measure cardiac output in the rat. Again, however, full advantage of the phasic characteristics of ascending aortic blood flow was not utilized in assessing cardiac performance (181).

Hemodynamic Comparison of Normotensive and Spontaneously Hypertensive Rats

The hemodynamic studies of various age groups of NR and SHR rats reported herein, provide evidence to support the concept of a sequential progression from a hyperkinetic circulatory state early in the development of systemic hypertension to a normal cardiac output and a markedly increased vascular resistance later in the disease. Eich et al. have demonstrated that the high cardiac output, which was observed in several labile hypertensive individuals (182), later developed hemodynamic characteristics of a normal output with increased vascular resistance once

fixed diastolic hypertension was established (183). Other workers have also shown that cardiac output is elevated and total peripheral resistance normal in patients with labile or mild essential hypertension (184-192). However, Frohlich et al. emphasized that the vascular resistance in this high output state of labile hypertensive man is not normal, but inappropriately normal, for even though the calculated resistance values of normotensive subjects and labile hypertensive patients were not significantly different, if the cardiac output of the normals increased to the level of the labile or mild essential hypertensive patients, vascular resistance would decrease (184).

Several authors have speculated on how a high cardiac output state could lead to the development of sustained hypertension with a normal output and increased vascular resistance. Thus, Ledingham postulated that a cardiac output which was high in relation to the metabolic demands of body tissues, could produce a generalized vasoconstriction and thereby an increased vascular resistance (193). Indeed, Coleman, Granger and Guyton have demonstrated the existence of such a "whole-body autoregulatory mechanism" in areflex dogs (decapitated and spinal cord destroyed). They showed that an elevation of arterial pressure, initially produced by increasing cardiac output, was maintained by a gradual increase in vascular resistance as the increased flow returned towards normal (194). In addition, Conway has reported that acute blood volume expansion in unanesthetized dogs initially increased the cardiac output and arterial pressure, but within several hours cardiac output returned to normal and the increased pressure was maintained by an increased total peripheral resistance (66).

Such autoregulatory or adaptive phenomena have been evoked to describe the hemodynamic transition from an increased to normal cardiac output during the development of several forms of experimental renal hypertension. Thus, Ledingham and Pelling have demonstrated an initially increased cardiac output in rats made hypertensive by renal artery constriction and contralateral nephrectomy (178) and also in renoprival rats (179). Subsequently, the output of these hypertensive rats returned to normal as the elevated arterial pressure was maintained by an increased vascular resistance. Coleman and Guyton have produced hypertension in dogs by reducing renal mass by 70 per cent and increasing the salt and water intake of the animals (68). During the first week of this type of experimental hypertension the elevated arterial pressure was associated with increased cardiac output and normal total peripheral resistance (68); however, by the second week, cardiac output returned to normal and the elevated pressure was maintained by an increased peripheral resistance. In addition, Ferrario, Page, and McCubbin followed resting hemodynamics in dogs made hypertensive by wrapping one kidney in cellophane and removing the contralateral kidney. In these studies the early elevation of arterial pressure was initiated by an increased cardiac output. Later, the predominant factor in sustaining the hypertension was the increased total peripheral resistance (195). It, therefore, appears that an increased cardiac output is an identifiable hemodynamic abnormality early in these forms of experimental renal hypertension as well as in clinical labile hypertension.

Similarly, the present investigation of the Okamoto-Aoki strain of spontaneously hypertensive rats, very much akin to essential hypertension

in man, provides strong evidence for this type of hemodynamic transition in a laboratory model of naturally-developing hypertension. Thus, the results of this study demonstrate that young (Group I) SHR rats have a hyperkinetic circulation characterized by increased heart rate, cardiac output (as well as cardiac index), stroke work, peak flow velocity, and maximum acceleration of aortic flow, with normal stroke volume and total peripheral resistance. Hence, these hemodynamic findings in young (Group I) SHR rats are very similar to those of labile hypertensive man in whom the elevated arterial pressure is also associated with an increased heart rate, cardiac index and left ventricular ejection rate and a normal stroke volume and total peripheral resistance (184).

Moreover, the findings obtained in the large group of mature (Group II) SHR rats were also similar to hemodynamic observations in man with fixed diastolic hypertension of moderate severity of cardiovascular disease. Thus, the elevated arterial pressure in this group of hypertensive rats was associated with an increased total peripheral resistance and a cardiac output similar to their age-matched normotensive controls. Therefore, it appears that, in the natural progression of SHR hypertension, there indeed is an early hyperkinetic circulatory stage which precedes the more expected hemodynamic profile of normal cardiac output and increased vascular resistance. These findings underscore the importance of the concept that the prime pathogenetic mechanisms in spontaneously hypertensive rats, and possibly essential hypertension, are characterized by a system which produces a hyperkinetic circulatory state.

The hyperkinetic circulation could be produced by abnormal autonomic control which, by producing a relative increase in adrenergic

stimulation to the cardiovascular system and kidneys, and consequently disturb sodium homeostasis, capacitance and resistance vessels as well as cardiac function. Increased adrenergic involvement in SHR hypertension has been implicated by the work of Okamoto et al., who demonstrated midbrain participation in the elevated pressures of these rats since mesencephalic transection (cerveau isolé) did not reduce arterial pressure, whereas transection at the base of the brain (encéphale isolé) reduced pressures of NR and SHR to the same level (138). In addition, anti-adrenergic hypotensive agents seem to be more effective in controlling arterial pressure of SHR rats than other hypotensive drugs (196). Important in any consideration of increased adrenergic influences is the inter-relation between the renin-angiotensin system and adrenergic activity. In this regard, the central actions of angiotensin in decreasing vagal activity (42), increasing sympathetic cardiac vasomotor outflow (43), enhancing sympathetic vascular constriction (197), or releasing catecholamines from the adrenal medulla (198) may be even more important than the direct arteriolar constrictor effect of angiotensin. These so-called indirect effects of angiotensin (because their actions are produced by altering neural activity) could thus increase cardiac rate and contractility and venous return (via venoconstriction) and thereby increase cardiac output. Although these actions of angiotensin fit neatly into a possible mechanism that potentially can produce a hyperdynamic circulatory state, no evidence for any abnormality in this system has yet been presented in the SHR rats.

A hyperdynamic circulatory state can also be produced by stress. Brod demonstrated that the stress of simple arithmetic can

transiently raise arterial pressure (199). Folkow and Neil point out that the cardiovascular effects of the "defense or alerting reaction produce a great mobilization of the cardiovascular system" (14). Indeed, Folkow and Rubinstein have produced systemic hypertension in rats by repeated stimulation of the hypothalamic defense area (118). Such stimulation could drive the cardiovascular system by the neurohormonal response it evokes. Structural alterations in the anterior pituitary, adrenal cortex, and thyroid gland of young SHR rats demonstrated alterations which suggested hyperfunction of these endocrine organs (200). In addition, destruction or removal of these endocrine organs suppressed the development of hypertension in young SHR rats (161). Clearly, more work is required to elucidate the pathogenetic mechanisms responsible for the neuro-humoral imbalance which produces a hyperdynamic circulatory state in young SHR rats.

Hemodynamics: Aging

Normotensive Rats. The present hemodynamic studies demonstrated that in male rats both heart rate and cardiac index (cardiac output per kilogram body weight) decreased progressively with advancing age. It has been demonstrated previously that heart rate decreases with age in both human beings and rats (201, 202). However, Lee, Karpeles and Downing (181) in a preparation very similar to the one used in the present series of investigations failed to confirm a significant decrease in the cardiac output per kilogram of male Wistar rats with aging, although the age range they examined (6, 12, and 24 months) was even wider than the present investigation (approximately 3, 6, and 12 months). Without minimizing their contribution it appears that the flows they reported

(90 ml/min/kg) in their six month pentobarbital anesthetized group were greatly reduced for the rat, even with the anesthetic used. Thus, the lowest flows obtained in the present study (139 ml/min/kg) were obtained in rats anesthetized with pentobarbital. Comparing this value with the cardiac output of 220 ml/min/kg in ether anesthetized rats of the present study and 286 ml/min/kg in unanesthetized rats (173) emphasizes the necessity for evaluating several anesthetic agents prior to establishing any physiological observation (see discussion Anesthesia Comparison). In addition to their use of pentobarbital, Lee, Karpeles, and Downing also inserted a catheter into the left ventricle through the apex of the heart. This procedure requires a greater exposure of the heart and thoracic cavity, and in our experience contributes to the cardiac output reduction. Thus, with the very low cardiac output observed by them in young rats, it would be difficult for these workers to demonstrate significant reductions in flow with age, although their 24 month groups did have a slightly lower flow than the 6 month group.

In the present study, in which ether anesthesia and a more limited thoracotomy (exposing only the great vessels) was used, the cardiac index of the rat declined significantly with advancing age (Figure 8). This fall did not result from impaired myocardial contractile function in the aging normal rats since peak aortic flow velocity and maximum acceleration remained unchanged from Group II to III (Figure 10). In addition, Grodner, Pool and Braunwald support these findings having demonstrated no deterioration of contractile function of isolated cardiac muscle strips from 6, 12 and 24 month old male rats (203). The decrease in cardiac index could be related to the decrease in metabolic rate of

aging rats (204) since cardiac output correlates highly with body metabolism (205). Indeed, Brandfonbrener, Landowne and Shock have shown a gradual reduction in cardiac index with age in man (202). However, these studies were performed in chronologically debilitated aging men.

Spontaneously Hypertensive Rats: Left Ventricular Hypertrophy. Persistent systemic arterial pressure elevation presents the left ventricle with a continuous demand to produce more force in order to eject blood (compensatory hyperfunction). The heart adapts to this persistently increasing workload by increasing myocardial mass. This compensatory hyperfunction of the heart has been experimentally-induced by strenuous exercise (207), increased venous inflow (arteriovenous fistula and anemia) (208), or by pressure overload (outflow tract constriction) (209), and all have produced cardiomegaly. Meerson postulated that there were two physiologically different types of myocardial compensatory hyperfunction: isotonic hyperfunction, associated with an increased flow component and isometric hyperfunction, associated with increased pressure load (210). Isometric hyperfunction (that which would be expected in hypertension) is characterized by a more intense workload, leading to hypertrophy and a more rapid exhaustion of myocardial functional reserve (210). Increasing the pressure component of external cardiac work is more costly in terms of myocardial oxygen consumption than a proportional increase in the flow component of external work (211). Heretofore, of all the experimentally-produced compensatory hyperfunctions of the myocardium, the isometric type produced chiefly by creating aortic stenosis has been most analogous to the stress placed by chronic systemic (arterial) hypertension. However, even this type of experimental procedure

may not represent the findings in man with systemic hypertension or, for that matter, the SHR rat. In animals with experimental aortic stenosis or aortic coarctation, the increase in myocardial mass occurs over a more contracted time period than that developed with a progressive increase in arterial pressure. Furthermore, these previous acute experimental models do not produce the same pathophysiological developmental characteristics.

Meerson has described three stages in the adaptive myocardial response to compensatory hyperfunction (210). Initially, the increased work-load overcomes the normal reserve capacity of the myocardium and the force and velocity of myocardial contraction become reduced. In the second stage, the myocardium increases its mass to restore its function despite the increased work-load. This stage is referred to as stable hyperfunction. During this period the increased performance may be the result of the increased myocardial mass since the concentration of the energy-generating enzymes is not increased (212). Since this myocardial adaptability cannot continue indefinitely, in the third stage, there is a progressive deterioration of function in which the force and velocity of contraction decrease, and eventually cardiac failure supervenes. This terminal stage of function of the hypertrophied myocardium has been well-documented both clinically (213, 214) and experimentally (215-217). At this juncture, it is important to re-emphasize that cardiac hypertrophy is a progressive process in which the myocardium continuously attempts to adapt in order to sustain the chronically increased work-load demand (compensatory hyperfunction).

In systemic arterial hypertension the compensatory hyperfunction

imposed upon the heart is in response to the progressively increasing arterial pressure; the left ventricle responds to this increased work load by increasing cardiac mass. In the present study, the left ventricular weight of each age group of SHR rats was greater than that of matched NR rats. Furthermore, with age and progressively increasing arterial pressure, the SHR left ventricular weight continued to increase over and above the expected increase in weight related to body growth. This relationship was shown in the progressive and persistent increase in the ratio of left ventricular weight to body weight in the SHR rats, whereas this relationship remained relatively constant with growth in the NR rats.

Left ventricular contractility, as assessed by peak aortic flow velocity and maximum acceleration of aortic flow, reflected different functional characteristics in each of the age groups of SHR rats. Thus, contractility of the 9 to 12 week (Group I) SHR rats was greater than that of their respective NR, whereas no difference was found in the indices of myocardial contractility between the 18 to 33 week (Group II) NR and SHR rats. In the oldest SHR group (62 to 97 week, Group III), myocardial contractility was significantly reduced as compared to their age-matched normotensive controls. These observations demonstrate that the presence of increased myocardial mass in itself does not denote a cardiac functional impairment. Indeed, the increased myocardial mass is a natural adaptation to sustain chronic increases in work-load (adaptive hyperfunction). Since ventricular hypertrophy is so often associated with some impairment in cardiac performance, and even overt circulatory failure (213), it should not follow that a causal relationship exists between hypertrophy and failure. Indeed, the myocardial hypertrophy is

probably a cardiac adaptive mechanism which delays the eventual failure initially precipitated by that amount of increased systemic resistance which initiated the compensatory hyperfunction and increased work load. Thus, if a heart subjected to a chronic pressure overload did not adapt first by compensatory hyperfunction and then by increasing its mass, overt circulatory failure would supervene more rapidly. Although this increased ventricular mass does not necessarily indicate myocardial impairment, it does reflect an abnormal hemodynamic state which, if uninterrupted, eventually will produce cardiac failure.

The Group I and II SHR rats had increased left ventricular mass and produced greater external cardiac minute work than their respective normotensive controls without any detectable impairment of myocardial contractility (Figure 10). Thus, these SHR rats appear to represent Meerson's stage two (of stable hyperfunction) (210); a stage during which the increased ventricular mass is believed to compensate for the increased work-load imposed by the vascular disease-hypertension. Aoki et al. have shown that the myocardium of 15 to 25 week SHR rats has the potential for increased mechanical work since the total myofibrillar ATPase activity is greater than that of normotensive rat hearts (218). This increased myofibrillar ATPase activity appears related to the increased cardiac mass since, when ATPase activity is expressed per unit myocardial protein, the NR and SHR rat enzyme activity did not differ. However, because of the increased mass of the SHR myocardium the total ATPase activity was absolutely increased. Similarly, it has been demonstrated in other models of ventricular hypertrophy that the activity (expressed per gram of heart) of respiratory enzymes (involved in ATP regeneration)

also was not increased; again, however, because of this absolute increase in myocardial mass the hypertrophic myocardia demonstrated an increased ability to regenerate ATP. Thus, it appears that during the stage of stable hyperfunction the adaptive increase in myocardial mass enhances the ability of the heart to utilize and regenerate usable energy and thereby increase external work.

Beznak has shown that as left ventricular weight of aortic-banded rats increased so did the ability of the heart to pump more fluid when the cardiovascular system was expanded with polyvinylpyrrolidone solution (219). This increased maximum cardiac output was interpreted as a measure of the "reserve force" of the heart and was used as evidence that the hypertrophied heart was a "stronger" heart. Alexander et al. have demonstrated that as the right ventricular mass of pulmonary artery banded dogs increased so did the maximal work the ventricle could perform (220). Thus, adaptive hypertrophy of the heart produces an increase in the functional capacity of the heart, although it is unknown whether the hypertrophied ventricle could sustain the increased work load.

Cardiac adaptations associated with the ever-persisting compensatory hyperfunction do not continue indefinitely and in Meerson's final stage (III) there is an exhaustion of the myocardium and gradual deterioration of cardiac function (210). In the present study, reductions in the indices of myocardial contractility were observed eventually in the oldest (Group III) SHR rats although age-matched NR exhibited no reductions. In addition, despite a further increased ventricular mass, the external minute work of the SHR myocardium was no greater than that of matched NR.

The reduced contractility observed in these animals (Group III SHR) suggests that the ventricle was no longer capable of compensating for the continually increasing work-load. Simon et al. have shown in man with chronic pressure loads from aortic stenosis, that the contractile state of the hypertrophied ventricle of older patients was reduced when compared with younger patients with similar left ventricular wall thickness and muscle mass per square meter of body surface area (221). Thus, in naturally developing hypertrophy in man and SHR rats, the contractile state of the hypertrophied ventricle appears to be age-related. These findings support the concept that the myocardial hypertrophic adaptation associated with compensatory hyperfunction becomes exhausted and the heart is no longer able to maintain the increased work-load.

Pharmacological Assessment of Autonomic Function

Parasympathetic Inhibition

Atropine is an alkaloid derivative of the belladonna plants, which is a competitive antagonist to acetylcholine at cholinergic receptors of the effector organ (222). Since parasympathetic influences upon myocardial function are mediated by postganglionic release of acetylcholine from the vagus nerve, sufficient doses of atropine can greatly reduce the response to vagal stimulation. The alteration of myocardial function by atropine should therefore reflect mainly the removal of tonic influences from the parasympathetic division of the autonomic nervous system. Hence, the greater the change in myocardial performance after atropine administration, the more significant were

the tonic parasympathetic influences during the preatropine period.

In the present study, administration of atropine to Group I and II NR significantly increased heart rate. Although the older Group II NR had lower preatropine heart rates, they also exhibited the greatest increase in heart rate after atropine, thereby indicating greater vagally-mediated tonic activity in Group II NR. Thus, the heart rates of the two groups after parasympathetic inhibition were no different. Adolph demonstrated that parasympathetic restraint on heart rate develops 16 to 20 days after birth in the rat, and he suggested that this development may be responsible for the slower heart rate in older rats (223). This progressively increasing participation of parasympathetic control with aging has been reconfirmed for other species, including man.

In sharp contrast, atropine did not increase the rates of either Group I or II SHR rats who already had faster heart rates. Since parasympathetic inhibition did not alter the SHR heart rate, it would appear that under the conditions of the present investigation, vagal activity was minimal in these two age groups. These findings may explain, in part, the increased heart rate of SHR rats when compared to NR. However, even after the parasympathetic component of heart rate in the NR was suppressed by atropine, the heart rate of the SHR rats was still significantly greater. This indicates either a major underlying increase in the intrinsic pacemaker firing or beta-adrenergically mediated cardioacceleration (infra).

Other important hemodynamic effects of cholinergic inhibition were observed in both NR and SHR rats. Atropine slightly reduced the mean arterial pressure and maximum acceleration of aortic flow in all

groups (NR and SHR) studied. In addition, the cardiac index of the youngest SHR rats (Group I) was also lowered, though significant changes were not observed in any other group. These alterations might reflect minor inhibition of sympathetic activity. Indeed, Marrazzi has demonstrated that postganglionic sympathetic discharge rate could be attenuated with atropine (224). Along these lines, it is noteworthy that atropine, though a highly selective antagonist of muscarinic parasympathetically mediated effects, can in high doses also antagonize the nicotinic action of acetylcholine (225). The latter effect would inhibit transmission at autonomic ganglia (226). Thus, high doses of atropine have also been shown to induce postural hypotension (227) and vascular pooling (228). These effects, then, may be produced by inhibition of pre to postganglionic sympathetic transmission which is also cholinergic. Admittedly, these slight reductions in arterial pressure and ventricular contractility following atropine are unusual. However, it is possible that they reflect some small degree of sympathetic inhibition (i.e., nicotinic antagonism at ganglia) along with the predominant parasympathetic inhibition, which is perhaps so noticeable in the SHR rats because of their high level of tonic sympathetic activity (infra). In any event all the qualitative differences observed prior to atropine between NR and SHR rats still existed after atropine, even though quantitatively the difference between the two groups was altered. Thus, even after atropine the SHR rats still had a greater heart rate and mean arterial pressure and the Group I SHR a greater cardiac index and maximum aortic flow acceleration than that of matched NR. These findings suggest that the differences in cardiac function may be attributed to intrinsic myocardial

differences or differences in beta-adrenergically mediated stimulation.

Combined Parasympathetic and Beta-adrenergic Inhibition

Jose has described a method of producing pharmacological denervation of the heart by the simultaneous administration of parasympathetic and beta-adrenergic inhibitors (166). This procedure greatly inhibits the extrinsic influences on myocardial function, such as parasympathetic and sympathetic neurotransmitters, (acetylcholine and norepinephrine) and circulating catecholamines (norepinephrine and epinephrine), and therefore provides a state in which the intrinsic performance of the myocardium may be evaluated. Conversely, by examining the effects of this autonomic blockade on myocardial function, it is possible to assess the contribution of autonomic influences on myocardial performance. In the present study intrinsic cardiac performance of NR and SHR rats was examined after inhibition of parasympathetic and beta-adrenergic influences with sufficient doses of atropine and sotalol, respectively. Sotalol was chosen to produce beta-adrenergic blockade because it does not have the nonspecific myocardial depressant effects (164, 229) that a more commonly used beta-blocking agent, propranolol, possesses (230, 231).

In evaluating intrinsic performance it is essential to produce maximal pharmacological inhibition of extrinsic influences or else the so-called intrinsic levels will be dose-dependent. In the present study 1.0 mg/kg atropine was given in two cumulative doses of 0.5 mg/kg. The first dose produced near maximal hemodynamic effects; doubling this dose produced only slight additional hemodynamic alterations. In addition, this dose of atropine was sufficient to prevent reflexive

bradycardia in NR until the mean arterial pressure exceeded 200 mm Hg (unpublished observations). Thus, this dose was sufficient to overcome natural vagal nerve stimulation induced by sudden increases in arterial pressure.

Adequacy of beta-adrenergic inhibition with sotalol was demonstrated by the prevention of hemodynamic responses to the specific beta-adrenergic agonist-isoproterenol. NR, treated with 40 mg/kg sotalol, only demonstrated chronotropic and inotropic increases when isoproterenol was infused at a rate of 0.64 mg/kg. This same dose, in the unblocked state, produced near-maximal increases (see discussion, Cardiac Stimulation) in the measured indexes of myocardial performance (unpublished observations).

The importance of employing sufficient doses of autonomic inhibiting drugs in evaluating intrinsic myocardial performance is underscored by the work of Walsh (232). He used atropine and sotalol to inhibit external autonomic influences on the heart to study effects of temperature on intrinsic heart rate of the rat. However, since only 8 mg/kg of sotalol (intraperitoneally) was used, tonic beta-adrenergic effects on heart rate were not completely inhibited. Using this lesser degree of inhibition he concluded that in the normal temperature range, the contribution of sympathetic activity to the resting heart rate of the rat is small. However, from the present study, it is obvious that even 10 mg/kg sotalol (intravenous) was insufficient to evaluate intrinsic function since higher doses reduced heart rate even further (Figure 11).

Following pharmacological cardiac denervation, heart rate of both Groups I and II (NR and SHR rats) fell to the same intrinsic level,

demonstrating that the observed pretreatment heart rate differences were the result of extrinsic autonomic influences. In addition, in the autonomically-blocked state the cardiac index of all groups of NR and SHR rats fell to precisely the same level. The increased cardiac index of the Group I SHR rats remained elevated after atropine, but following sotalol, it became similar to their matched NR, thereby strongly implicating a beta-adrenergic receptor mediated component in the hyperkinetic circulatory state of young SHR rats. These findings are suggestive of those patients reported by Frohlich et al. in mildly hypertensive patients (184, 233). Frohlich has also has demonstrated that the higher the pretreatment cardiac index, the more responsive the hypertensive patient was to antihypertensive therapy with beta-blocking drugs (234).

Although cardiac function of NR and SHR rats was similar following autonomic inhibition, the arterial pressures of the SHR rats remained significantly higher. These findings support the work of Julius Pascual and London, who have produced pharmacological cardiac denervation in mildly hypertensive patients having increased cardiac index and heart rate (235). They reported that, although autonomic inhibition eliminated the differences in heart rate and cardiac index between normotensive and hypertensive individuals, the arterial pressure of the hypertensive group remained significantly elevated. These findings, and those of the present study, suggest that the hyperdynamic circulatory state reported early in the natural development of hypertension may not, in itself, be directly responsible for the increased arterial pressure. However, it is possible that the hyperdynamic circulatory state reflects an altered autonomic function producing increased net sympathetic drive. Thus,

although it is not yet possible to directly evaluate sympathetic activity to vascular smooth muscle, the cardiac effects of altered sympathetic function are more readily evaluated. Only following cardiac denervation (pharmacological) was it apparent that the "inappropriately normal" resistance of the Group I SHR rats was actually increased since in the blocked state arterial pressure became elevated despite the reduction of cardiac index to NR levels.

Folkow, et al. have provided evidence that the increased systemic arterial pressure in SHR rats may eventually result in structural changes of resistance vessels. Thus, they reported that following maximal vasodilation (using papaverine), flow in artificially perfused systemic vascular bed of SHR rats was always less than in matched NR at the same perfusion pressure (154). This increased resistance has thus been explained as an adaptive medial hypertrophy of the vascular smooth muscle to the increased arterial pressure load. The thickened vascular wall encroaches upon the lumen as the wall-to-lumen ratio of the vessel increases and, therefore, even in the maximally relaxed state, this structural adaptation of the vessel produced increased resistance. In addition, Sivertsson has illustrated that the resistance increase, produced by the same extent of vascular smooth muscle shortening, was greater in vessels with increased wall-to-lumen ratios (236). Thus, even at similar degrees of vascular smooth muscle contraction, the resistance of the vessels which have adapted to the pressure overload was greater. Folkow, et al. have also shown, using intensive antihypertensive therapy (which maintained arterial pressure of the SHR rats within normotensive ranges for 8 weeks), that the resistance to flow at maximal dilation of the vasculature was

the same as untreated NR (237). Therefore, the hypertrophic vascular adaptation was related to the pressure load. Further, Fries et al. have shown that following withdrawal of antihypertensive therapy, the arterial pressure of SHR rats increased; however, pressure did not reach the level of untreated age-matched SHR rats but that level of pressure at which therapy was initiated (238). These findings are compatable with the concept of structural adaptations of resistance vessels to increased pressure load. Effective antihypertensive therapy, therefore, removed the stimulus for such structural changes; with discontinuation of the therapy, the structural changes in the vasculature continued to develop.

Intrinsic Cardiac Performance-Normotensive Rat. Following pharmacological cardiac denervation cardiac index, mean arterial pressure, and heart rate of Group I and II NR were reduced to the same level. However, both before and following the inhibition of extrinsic influences on myocardial contractility, the maximum acceleration of aortic flow of the Group I NR was significantly lower than the Group II NR. These differences suggest that during the maturation process of the rat (from 2 months to 6 months of age) the contractile myocardial machinery appears to develop. Whether this observation is typical of this developmental stage will require further investigation. However, it permits speculation that perhaps during the aging process there are two simultaneous processes: one, by which myocardial performance improves and the other, by which it deteriorates. Usually, organ function maintains a balance between these processes, but it is possible that during the rapid growth period intrinsic cardiac performance improves and later it stabilizes.

Cardiac Stimulation

Cardiac stimulation with low dose levels of beta-adrenergic receptor agonists (norepinephrine and isoproterenol) increased heart rate and inotropic indices in the normal heart. In contrast, these same low dosages failed to evoke an increased cardiac performance in the SHR. There are several possible explanations for this altered responsiveness of the hypertrophied myocardium to beta-adrenergic stimulation. The present study strongly suggests that the lack of SHR myocardial responsiveness to beta-adrenergic stimulation was not the hearts inability to respond to this stimulation, since isoproterenol increased heart rate and the same inotropic indexes to equivalent peak levels as NR. Indeed, higher dosages of these beta-adrenergic receptor agonists were required to achieve such responses.

It is possible that the failure of these low doses of beta-adrenergic agonists to stimulate the SHR myocardium was a result of a more rapid catecholamine reuptake (and conversely diminished receptor binding), and hence, agonist inactivation by the hypertrophied myocardium. Spann, et al. have reported that norepinephrine concentrations were markedly reduced in ventricles which had undergone rapid hypertrophic growth in response to outflow track constriction (239). Papillary muscle strips from these norepinephrine-depleted rat ventricles were also shown to be supersensitive to the positive inotropic effect of norepinephrine (239). In this respect Louis, et al. have shown that the endogenous norepinephrine levels of young SHR myocardiums were normal (143, 144). However, these studies were performed on young rats who had significantly elevated arterial pressures but without increased cardiac mass. Therefore, it

is still possible to speculate that the hypertrophied ventricle of the SHR could be norepinephrine-depleted. Thus, just as an unsaturated sponge rapidly soaks up water, the hypertrophied and (possible) norepinephrine-depleted myocardium might provide more rapid reuptake of exogenous norepinephrine to inactivate the hormone. However, this explanation does not seem to be a likely explanation for the observation that low doses of beta-adrenergic stimulants failed to increase inotropic and chronotropic responses of the SHR myocardium. Isoproterenol (which was also ineffective in low doses) is not inactivated by reuptake at the terminal adrenergic neurons (240).

Another possible explanation for this reduced responsiveness of the SHR myocardium to low levels of beta-adrenergic stimulation is that its tonic adrenergic drive may already be functioning at higher levels. Therefore, further increases in cardiac adrenergic activity could require greater amounts of exogenous beta-adrenergic stimulating agents. This possibility seems to relate to the findings of the present study (see discussion, Pharmacological Assessment of Autonomic Function) which demonstrated that not only was the beta-adrenergic contribution to myocardial function increased in the SHR, but also, parasympathetic activity was reduced. Levy has reemphasized the concept of accentuated antagonism in discussing the complex interrelationship between adrenergic and parasympathetic influences on myocardial function (241). This concept developed from observations that vagal stimulation (or cholinergic influences) can attenuate inotropic myocardial response to sympathetic stimulation (242). Utilizing this line of reasoning, the net adrenergic drive to the SHR myocardium could be increased by adrenergic factors as well as

reduced vagal influences. Thus, it may well be that higher levels of exogenous beta-adrenergic stimulating agents are required to produce measurable increases in myocardial performance in the SHR, because there already exists a greater net adrenergic drive.

Still another possible explanation for the altered SHR myocardial responsiveness may be the increased myocardial mass. Hence, a lesser concentration of the exogenous catecholamines reaches the receptor sites. Inherent in this assumption is the undocumented concept that the increased cardiac mass occurs without a proportional increase in the number of receptor sites. To the contrary, Spann et al. have shown that papillary muscle from hypertrophied ventricles was supersensitive to exogenous norepinephrine (239).

Other alternatives for these observations in SHR rats may involve an altered affinity of the beta-adrenergic receptor for its agonist. Thus, if receptor affinity were reduced, higher levels of the agonist would be required to evoke a response. However, once stimulated, normal increases in myocardial performance would be expected with this hypothesis.

Although the reasons for the altered responsiveness at this time are highly speculative, the implications are clear. There is altered myocardial autonomic function in the SHR and both autonomic inhibition and adrenergic stimulation studies have demonstrated qualitative differences in NR and SHR hemodynamic responses (although qualitatively they were similar). Again, the emphasis is on degree; NR myocardial function was reduced with beta-adrenergic blocking drugs, but not to the same extent as in SHR. Isoproterenol and norepinephrine did stimulate

cardiac function of the SHR to the same peak levels of the NR, but only after requiring much higher doses.

All this sums up to a single unifying concept: the altered function in the SHR is not one of a deficit of mechanism or inability to adapt, but a subtle difference in cardiovascular and/or autonomic regulation.

Elevation of Arterial Pressure - Methoxamine

Just as the responses to cardiac stimulation and inhibition showed subtle regulatory alterations in the SHR, so did the responses to vascular stimulation. Thus, methoxamine, an alpha-adrenergic agonist which is essentially free of direct myocardial effects (243-245), produced vasoconstriction with reflexive cardiac responses. Alterations in cardiac performance during methoxamine infusion were for the most part, indirect (or reflexive) arising as a result of the increased systemic arterial pressure rather than from direct myocardial effects. Increasing doses of methoxamine were employed to elevate progressively arterial pressure of NR and SHR rats. Over a wide pressure range the SHR consistently demonstrated higher arterial pressures than NR having the same cardiac index (Figure 22). In other words, for any given cardiac index arterial pressure (and therefore vascular resistance) is greater in the SHR. These findings are consistent with those of Folkow and coworkers, who demonstrated that at least part of the increased vascular resistance in the SHR resulted from morphological vascular alterations which increased the wall-to-lumen ratio (154). These same coworkers believe that much of the arteriolar thickening was produced by hypertrophic changes in the contractile portion of the vessel wall and not merely a "water-logging"

effect. This belief was substantiated by the observance that maximally-constricted perfused hindquarters of SHR rats demonstrated a 45 per cent greater resistance than NR (158). These findings suggest that at least part of the increased wall-to-lumen ratio must reflect an increase in the smooth muscle components of the vessel. In the present study, the maximum pressures and calculated total peripheral resistance achieved from high methoxamine doses was markedly greater in the SHR. Thus, the in vivo hemodynamic studies were similar to the perfusion studies in that at any given systemic flow level arterial pressure was higher in the SHR; and furthermore, the maximal pressures achieved with norepinephrine and methoxamine were also greater in the SHR.

Baroreceptor Function. It is generally held that reflexive control of cardiovascular function is altered to hypertension since bradycardia is not usually observed with persistently elevated arterial pressure (246). Indeed, increased heart rate was observed at each age (and severity) level in the SHR rats of the present study as well as in all types of hypertension in man (247). There are several possible mechanisms by which baroreceptor function may be altered. The "set-point" or the arterial pressure level about which reflexive control is evoked may be influenced by higher central nervous center interactions (248, 249). The afferent input may also be altered by some alteration of the peripheral baroreceptors (or mechanoreceptors), or a change in arterial distensibility. It is also possible that the efferent limb of the reflex is ineffective in modulating cardiac and vascular function.

Evidence has been provided from experimental renal hypertensive dogs that there is some reduction of mechanoreceptor function. Thus,

McCubbin, Green and Page demonstrated less carotid sinus nerve firing at three levels of intrasinus pressure (250); and Kezdi also reported less nerve traffic at any given level of intrasinus pressure (251). These results have been interpreted to represent a reduction of mechanoreceptor function, since at any given intrasinus pressure the hypertensive animals demonstrated less carotid sinus nerve activity.

It is also possible that the decreased mechanoreceptor firing could be caused by a reduced distensibility of the arterial wall and not an actual reduction of receptor function. Since the mechanoreceptors are stretch receptors, located in the arterial walls, a higher transmural pressure would be required to induce the same stretch in the less distensible arteries. Aars has provided evidence to support this concept in hypertensive rabbits (252). In his studies afferent nerve traffic of the hypertensive animals was less than in normotensives; however, when the intrasinus pressure was increased, the peak firing rate of the normal and hypertensive rabbits were the same. Thus, the afferent input from the mechanoreceptor could have the same activity; however, higher transmural pressures were required.

Our results confirm and extend the concept of altered baroreceptor function in SHR rats. The efferent limb of reflex alterations produced by increased pressure was appropriate. Thus, as pressure was progressively elevated with methoxamine, heart rate, maximum acceleration of aortic flow, and cardiac index of both NR and SHR rats fell reflexively. Nevertheless, this reflexive fall of these variables was shifted to the right (higher systemic pressure levels) in SHR (Figures 20, 21, 22). Admittedly, this means of evaluating cardiovascular reflex activity was

provided by an arbitrary and empirically-derived index; however, other workers have demonstrated by other methods that higher arterial pressure levels are necessary to elicit reflexive adjustments in SHR rats (253, 254). Thus, Nosaka and Okamoto have also reported this shift along the pressure axis in the firing rate of the aortic depressor nerve in SHR (253). These workers reported that the mean arterial pressure level about which aortic depressor nerve firing was changed the most was at 129 and 159 mm Hg for their control and SHR rats, respectively. In addition, the reflexive reduction of arterial pressure associated with elevations of carotid sinus perfusion pressure was greatest in SHR when the basal sinus perfusion pressure was raised from 160 mm Hg (254).

In the present study the average mean arterial pressure measured prior to the consistent reflexive decrease in heart rate and maximum flow acceleration was 120 and 154 mm Hg in the NR and SHR groups, respectively. These pressures are remarkably similar to the levels observed by Nosaka and Okamoto, using a different method of evaluating baroreceptor function (supra). Moreover, although both groups of rats required a 30 mm Hg pressure rise to produce measurable reflexive changes, the level of pressure obtained before reflexive bradycardia and withdrawal of sympathetic activity was observed was just above the control pressure level before thoracotomy (117 and 151 mm Hg, NR and SHR, respectively). Furthermore, altered reflexive function can be demonstrated even in the preinfusion state. Before methoxamine, the SHR had a greater heart rate; had the NR reached the preinfusion pressure levels of the SHR, the already slower heart rate of the NR would have exhibited reflexive bradycardia.

The present work provides the hemodynamic consequences for the shift in aortic depressor nerve firing levels reported by Nosaka and Okamoto. Thus, the alteration in baroreceptor activity in the SHR appears to be associated with an increased threshold level of arterial pressure required to elicit reflexive control of cardiovascular function. Thus, a higher pressure for "set point" was required in SHR to elicit reflexive cardiac responses to the elevated pressure. Hence, mechanoreceptor function in the SHR does not seem to be abnormal since it has been shown that aortic depressor nerve discharge frequency of SHR can equal and exceed that of NR (253). Hemodynamically, we have shown that with greater increases in arterial pressure the reductions in heart rate, maximum acceleration of aortic flow, and cardiac index were as great in the SHR as in NR. These reflexive differences in the NR and SHR provide further support for the concept of reset baroreceptor function in the maintenance of sustained systemic hypertension. It still remains to be seen what the contribution to altered baroreceptor function by arterial distensibility and central adjustment of the "set-point" are in this form of natural hypertension.

It would seem that the cardiovascular system of the SHR rats appears to be able to withstand excessive arterial pressure levels at least under these experimental circumstances with short term infusions of norepinephrine and methoxamine. However, these animals pay the price for their sustained arterial pressure elevations in the unstressed state; they have increased incidence of severe vascular and cardiac lesions and nephrosclerosis (255) which eventually results in an increased mortality rate (256).

CHAPTER V

SUMMARY

Hemodynamic studies were performed on 93 normotensive (NR) and 63 spontaneously hypertensive (SHR) male Wistar rats of three age groups (Group I, 9 to 12 weeks; Group II, 18 to 33 weeks; and Group III, 62 to 97 weeks). Each animal was anesthetized with ether and artificially ventilated with a positive-pressure respirator. Catheters were inserted into the carotid artery and jugular vein; following thoracotomy, an electromagnetic flow probe was placed around the ascending aorta. This instrumentation provided measurements of flow and pressure which permitted calculations of hemodynamic variables.

Using appropriate controls, alterations in hemodynamic characteristics produced by parasympathetic and combined parasympathetic and beta-adrenergic inhibition were examined to assess the contribution of the autonomic nervous system. In addition, beta adrenergic receptor agonists were infused in order to compare the cardiac responsiveness of NR and SHR to exogenous beta-adrenergic stimulation. Finally, arterial pressure of both NR and SHR rats was elevated with an alpha-adrenergic receptor agonist-methoxamine to evaluate reflexive cardiac inhibition.

These studies have provided answers to the questions posed on Page 35 of this dissertation, specifically:

1. The hemodynamic characteristics of the SHR rat depend on the age group studied. Thus, in young (10 week) SHR rats, the elevated arterial pressure was associated with an increased cardiac output; the total peripheral resistance was not significantly greater than in matched NR. With age, and progressive increases in arterial pressure, the cardiac output of the older SHR rats (24 and 72 weeks) was similar to matched NR. Therefore, at these ages the elevated arterial pressure was associated with an elevated total peripheral resistance.

2. Associated with the progressively increasing systemic arterial pressure of SHR rats was a dramatic increase in the left ventricular weight. This increase in myocardial mass was not related merely to body growth since the ratio of left ventricular weight to body weight, though stable in NR, continued to increase with age in SHR rats. Left ventricular contractility was assessed by comparing measurements of the maximum acceleration and the peak flow velocity of aortic flow. These measurements characterize the initial phase of ventricular ejection when the ventricle is said to be functioning as an impulse generator.

In all age groups, the SHR left ventricular weight was greater than that of aged matched NR. However, the indexes of left ventricular contractility demonstrated that the contractile performance of the hypertrophied heart decreased with age. That is to say, the maximum acceleration of aortic flow and the peak flow velocity were increased in the youngest SHR rats (Group I) and were no different in the Group II rats, but decreased in Group III SHR rats, when compared with their age-matched NR. Therefore, contractile performance of the hypertrophied left ventricle of the hypertensive animals progressively deteriorated

with age and increasing hypertrophy. This is in contrast to the aging process in the NR in which left ventricular contractility increased from Group I to II, and then remained stable even in rats over one year of age (Group III).

3. Under the conditions of the present investigation (ether anesthesia, and open-chest recording of hemodynamic function) tonic parasympathetic activity on cardiac function was markedly reduced in both age groups of SHR rats. On the other hand, beta-adrenergic mediated influences on cardiac function, although predominant in both NR and SHR, was greater in the latter. Therefore, an abnormal balance, manifested by reduced parasympathetic and increased beta-adrenergic activity, accounts for many of the differences in cardiac function (i.e., increased heart rate, left ventricular contractility, cardiac index) in the SHR rats. This difference results in a far greater net adrenergic response of the hypertrophied myocardium.

Similarly, low levels of beta-adrenergic receptor stimulation with norepinephrine or isoproterenol increased chronotropic and inotropic indexes in the NR myocardium, but failed to elicit these responses in the SHR myocardium. However, similar maximal peak responses were obtained when very high doses of these beta-agonists were administered to the SHR rats. It is possible that the failure of low doses of the beta-adrenergic agonist to stimulate the SHR myocardium is yet another way of reflecting this already increased net adrenergic drive.

4. The present investigation demonstrated that the SHR rats did exhibit appropriate reflexive bradycardia and decreased ventricular contractility when systemic arterial pressure was elevated with peripheral

arteriolar vasoconstriction. However, higher levels of arterial pressure were required to produce these reflexive adjustments in cardiovascular function. These studies support the work of other investigators that baroreceptor reflexive activity is "reset" to higher pressure levels in hypertension.

In conclusion, these studies provide pertinent physiological data concerning the development of left ventricular hypertrophy in systemic hypertension. Since the SHR represents the best experimental model yet developed for essential hypertensive man, and since no studies presently exist demonstrating the physiological progression of the cardiovascular deterioration with naturally progressing hypertension, the results reported herein provide new information concerning this important aspect of the pathophysiology of hypertension.

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