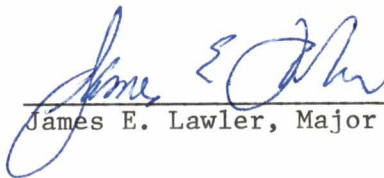
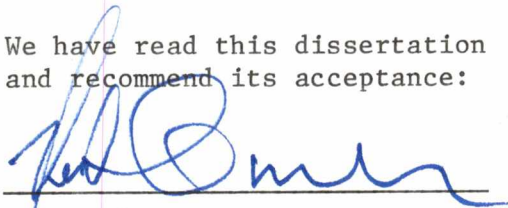


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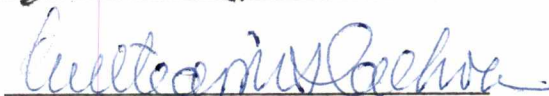

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HEMODYNAMIC PATTERNS AT REST AND DURING AVERSIVE CLASSICAL
CONDITIONING IN NORMOTENSIVE AND BORDERLINE HYPERTENSIVE
RATS: EFFECTS OF INTRACEREBROVENTRICULAR CLONIDINE

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

John Walter Hubbard

December 1983

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DEDICATION

To my parents for always giving me encouragement and support.

To my wife Jeanne, your love and faith in my abilities is a constant source of inspiration, that motivates me to accomplish my goals.

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In the course of my dissertation, many individuals generously gave of their time and expertise to insure the completion and quality of the project. My deepest appreciation and gratitude are extended to the chairman of my committee, Dr. James Lawler, for his constant encouragement, instruction, and friendship over the past five years. I would like to thank Dr. Robert Schaub and Dr. Thomas Chen for their guidance and tutelage during the course of my graduate training. I would also like to thank Dr. Neil Greenberg and Dr. Joel Lubar for their advice during several phases of the research, and Dr. William Calhoun for his support of my efforts.

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ABSTRACT

Recent studies using the first filial (F_1) offspring of female spontaneously hypertensive rats (SHR) and male Wistar-Kyoto (WKY) normotensive rats have found that this animal develops spontaneous borderline hypertension (systolic BP = 150 mmHg) which can be driven to pathologically hypertensive levels (systolic BP = 185 mmHg) using psychological stress.

The present study sought to characterize and compare the acute hemodynamic and sympathetic-adrenal medullary response patterns of WKY rats with borderline hypertensive rats (BHR), and to determine the role of central α_2 -adrenoreceptors in the mediation of these responses using intracerebroventricular infusion of the antihypertensive agent clonidine.

The studies utilized male BHR and WKY rats 18-28 weeks of age. The animals were implanted with intraventricular (i.v.t.) cannulae and exposed to 5 consecutive days of aversive classical conditioning (200 trials). Arterial catheters and electromagnetic flow probes around the ascending aorta were implanted and hemodynamic measurements were obtained from conscious animals at rest (baseline) and during 20 trials of aversive classical conditioning. The animals received an i.v.t. infusion of 4 μ l of normal saline or clonidine (8 μ g). Blood samples were taken at the end of each recording period for radioenzymatic assay of plasma catecholamine levels (epinephrine and norepinephrine).

It was found that the hypertension in the BHR was the result of an elevated total peripheral resistance index (TPRI) with a normal cardiac

index (CI). The BHR showed a greater pressor response to the psychological stress than did the WKY rats. The pressor response was the result of an increase in TPRI and CI for both strains; however, the increase in resistance was greater for the BHR. In addition, the BHR showed a greater increase in plasma norepinephrine in response to the stress than did the WKY rats.

Intracerebroventricular infusion of clonidine produced a significant reduction in mean arterial BP, which was due to a drop in TPRI, while CI remained unchanged. The clonidine treated animals also had significantly lower plasma epinephrine levels in response to the stress period than did the saline treated subjects.

The role of the sympathetic nervous system in the development of circulatory responses to psychological stress is discussed.

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CHAPTER I

INTRODUCTION

It has been estimated that 90-95% of all reported cases of hypertension lack any traceable physiological cause, such as tumors of the adrenal gland, diseases of the renal system, or advanced arteriosclerosis (Laragh, 1972; Pickering, 1972). This physiological anomaly is called essential or primary hypertension and has been a source of controversy among researchers and clinicians for several decades (Pickering, 1972).

During the developmental stage of essential hypertension, individuals generally have marginal elevations in blood pressure (BP) (Messerli, DeCarvalho, Christie & Frohlich, 1978), characterized by sphygmomanometer readings that fluctuate between 150-160 mmHg systolic and 90-100 mmHg diastolic (Julius & Esler, 1975). This condition is referred to as "borderline" hypertension and is defined according to the following criteria: (1) arterial BP levels that fall between the normotensive and the hypertensive range; (2) occasional normotensive readings; and (3) lack of any target organ damage (Julius, 1977). Some of these people become normotensive as they mature, while others show progressive increases in arterial BP leading to sustained essential hypertension (Julius, 1977). Although this malady is not associated with any identifiable physiological cause, chronic elevations in BP over several years increase the probability of coronary heart disease, left ventricular hypertrophy, congestive heart failure, cerebrovascular

infarction, and/or renal insufficiency (Cohn, 1977; Freis, 1979; Kannel, McGee & Gordon, 1976).

Characteristic changes in cardiovascular function seem to be associated with the shift from lability to permanency in some forms of borderline essential hypertension. In a follow-up study of 68 labile hypertensives, Eich, Cuddy, Smulyan, and Lyons (1966) found that patients under 50 years of age had an elevated cardiac index (cardiac output/square meter of body area) with normal total peripheral resistance (TPR) as compared to normotensive individuals. It was found that over the 50.4 month observation period these patients demonstrated a gradual shift in hemodynamic pattern to a normal cardiac index (CI) and increased TPR which maintained the elevated arterial blood pressure.

Abnormalities in sympathetic and parasympathetic nervous system regulation contribute to the basal elevation in cardiac output (CO) according to Julius, Pascual, and London (1971). The hemodynamic transition to an elevated TPR and reduced CO has been ascribed to a "total body autoregulation" in the major vascular beds (Coleman, Granger & Guyton, 1972; Guyton, Hall, Lohmeier, Jackson & Kastner, 1981). A decreased sensitivity in arterial baroreceptor reflex control (Takeshita, Tanaka, Kuroiwa & Nakamura, 1975) may allow the arterial BP of borderline hypertensive individuals to fluctuate excessively in response to environmental stimuli, thus contributing to the myogenic contraction of the vascular smooth muscle. Chronic hemodynamic perturbations such as these have been shown to cause structural changes in the vasculature resulting in an increased wall to lumen ratio, elevated TPR,

and sustained hypertension (Folkow, Hallbäck, Lundgren, Sivertsson & Weiss, 1973).

Essential hypertension, a "disorder of regulation" (Folkow, 1975), may result from the exacerbation of some genetic physiological abnormality in BP control (Murphy, 1973). The vascular smooth muscle of both humans and rats with essential hypertension has been shown to display a heightened sensitivity or reactivity to vasopressor substances (Lais & Brody, 1978; Meier, Weidmann, Grimm, Keusch, Glück, Minder & Ziegler, 1981). This deficit in vasomotor control coupled to an elevated sympathetic tone (Frohlich & Pfeffer, 1975; Schramm & Barton, 1979) and depressed reflex regulation of compensatory depressor systems (Takeshita et al., 1975) may play a major role in the etiology of essential hypertension.

The spontaneously hypertensive rat (SHR), an animal model of essential hypertension (Trippodo & Frohlich, 1981), displays an exaggerated sympathetic drive in response to environmental perturbations (Hallbäck & Folkow, 1974). Heightened vascular responsivity to norepinephrine prior to the development of sustained hypertension (Mulvany, Aalkjaer & Christensen, 1980) and reduced baroreflex sensitivity to elevations in arterial BP (Ricksten & Thorén, 1981) also characterize the pathophysiology of the SHR.

Further research on the borderline hypertensive condition in humans or in animals will help to elucidate the mechanisms which are responsible for "triggering" the physiological anomalies that contribute to the onset of essential hypertension (Julius, 1976). A greater

understanding of these causal mechanisms will expedite treatment regimens which in turn might block the transition from labile to established hypertension.

Neurogenic Mechanisms in Hypertension

The role of the autonomic nervous system in the control of circulatory responses that precede the development of pathological hypertension has become a major focus of research within the last two decades (Abboud, 1982; Axelrod, 1976; Frohlich & Pfeffer, 1975; Julius & Esler, 1975; Reid & Hamilton, 1980). One area of concentration has involved the identification of cardiovascular control centers in the central nervous system (CNS), and associated afferent and efferent projections from and to the heart, vasculature, kidneys, and other pressure regulating organs (Loewy & McKellar, 1980).

Investigators have observed that the afferents from the heart and blood vessels of hypertensive subjects display a reduced sensitivity to elevations in arterial BP (Abboud, 1979; Tarazi, Fouad & Ferrario, 1983). Consequently, the arterial and cardiopulmonary baroreceptors fail to make the necessary adjustments in heart rate (HR), vascular resistance, renin release, and vasopressin secretion to offset the hypertensive response (Zanchetti, 1979).

In the following sections, the role of arterial and cardiopulmonary baroreceptors in normal and abnormal BP regulation will be discussed. An analysis of neural centers and their modulatory influence on these reflex patterns will follow. Finally, the role of brain catecholamines in these regions will be discussed with specific reference to the

antihypertensive effects of the central α -adrenoreceptor agonist, clonidine.

Arterial and Cardiopulmonary Baroreceptors

Baroreceptors are specialized nerve endings that innervate the adventitia and portions of the media of vascular smooth muscle. These receptors respond to deformation or strain, induced by changes in intravascular pressure (Brown, 1980). The two major arterial baroreceptor populations are located bilaterally in the carotid sinuses, and in the aortic arch.

Afferent impulses emanating from the carotid sinus are carried via the nerve of Hering to the glossopharyngeal nerve, and synapse in the nucleus tractus solitarii (NTS) of the caudal medulla, while impulses emanating from the aortic arch ascend to the NTS by afferent vagal fibers (Berne & Levy, 1977). The pseudounipolar cells carried in the glossopharyngeal and vagus nerves have their cell bodies in the petrosal and nodose ganglia, respectively. Efferent pathways from the NTS project to the nucleus ambiguus (NA), paramedian reticular nucleus (PRN), lateral reticular nucleus (LRN), nucleus gigantocellularis (GRN), nucleus parvocellularis (PvRN), raphe nuclei (RN), dorsal nucleus medulla oblongata centralis (d-MOC), and intermediolateral cell column of the spinal cord (Antonaccio, 1977; Loewy & McKellar, 1980; Smith, 1974).

Cardiomotor vagal efferents from the NA descend via the vagus nerve and innervate the nodal tissues, atria, and limited regions of the ventricles of the heart (Randall & Armour, 1977). Sympathetic

cardiomotor and vasomotor efferent fibers leave the PRN, LRN, and the NTS, and descend the spinal cord in the dorsolateral funiculus (DLF) (Ciriello & Calaresu, 1977). These fibers synapse with preganglionic sympathetic neurons whose cell bodies are located in the intermedio-lateral gray matter of the spinal cord. Myelinated preganglionic fibers leave the spinal cord through the thoracolumbar ventral roots, join spinal nerves, and form the white rami (Wurster, 1977). The fibers then synapse in the sympathetic chain ganglia, or exit via the mixed nerve and synapse in peripheral ganglia, such as the stellate (Randall, 1977). Postganglionic noradrenergic sympathetic fibers project to and innervate vascular smooth muscle, nodal tissues of the heart, ventricular myocardium, and limited regions of the atrial myocardium (Randall & Armour, 1977).

In general, the aortic and carotid baroreceptors regulate arterial BP through a negative feedback system which operates at a specific gain and set point to minimize dramatic fluctuations in BP during normal activity (Norman, Coleman & Dent, 1981; Smith, 1974). Consequently, under normal conditions, if BP rises above the set point, aortic and carotid baroreceptor discharge will increase, vagal tone will increase to the heart, and sympathetic drive will decrease to the heart and vasculature, resulting in bradycardia and vasodilation. Alternatively, if BP drops below the set point, the carotid baroreceptors will decrease their rate of discharge, reducing vagal inhibition of HR and augmenting sympathetic drive to the heart and vasculature (Shepherd, 1980). This response pattern results in tachycardia and vasoconstriction. In

addition, it has been demonstrated by Ammons, Santiesteban, Koyama, and Manning (1980) that changes in carotid pressure elicit reciprocal changes in renal sympathetic nerve activity which regulates the secretion of renin from the juxtaglomerular apparatus. Thus, the carotid and aortic arch baroreceptors act tonically to regulate arterial BP by sympathetic and parasympathetic tone.

This negative feedback system may utilize inhibitory interneurons in the NTS and associated vasomotor regions which can be modulated by bulbar or suprabulbar structures (Antonaccio, 1977; Thomas & Calaresu, 1974). Studies employing sinoaortic deafferentation have demonstrated that removal of this tonic inhibitory sensory input to the NTS results in exaggerated BP lability (Norman et al., 1981) and an acute elevation in sympathetic activity (Alexander, Velasquez, Decuir & Maronde, 1980), which may contribute to the development of sustained hypertension in some animal models (Carey, Dacey, Jane, Winn, Ayers & Tyson, 1979; Laubie & Schmitt, 1979).

The heart contains many types of mechanoreceptors which respond to both cardiac filling and contraction (Armour, Wurster & Randall, 1977). These receptors are located in the atria, venous atrial junctions, and ventricles, and project their afferent fibers through the vagus nerve to the NTS (Shepherd, 1980). Electrophysiological studies with rats have demonstrated the presence of two basic types of atrial receptors (Thorén, Norell & Ricksten, 1979a). One type of receptor increases its rate of discharge in association with atrial contraction (A type) while the other increases its rate of discharge in response to atrial

filling (B type). Both of these receptors are mainly subserved by myelinated vagal afferents with fast conduction velocities. However, there are also many unmyelinated vagal afferents (C fibers), with slow conduction velocities, which respond to changes in cardiac filling (Thorén, Donald & Shepherd, 1976).

The cardiopulmonary receptors with myelinated afferents respond to changes in atrial distension, and regulate sympathetic drive to the sinoatrial node in order to maintain a constant heart volume (Linden, 1973). Cardiopulmonary receptors with unmyelinated afferents exert a tonic inhibitory effect on vasomotor centers in the medulla (Thorén et al., 1976). Distension of the atria results in both reduced sympathetic nerve activity, through a bulbar reflex arc, and decreased secretion of renin from the kidneys (Ricksten, Norell & Thorén, 1979). In addition, it appears that cardiopulmonary receptors exert a tonic inhibition on vasopressin secretion which is mediated by ascending noradrenergic pathways from the A1 (ventrolateral) cell group in the medulla (Linden, 1979; Sawchenko & Swanson, 1982; Thames & Schmid, 1979).

Thus, the arterial and cardiopulmonary baroreceptors are able to synergize during conditions such as hemorrhage to elicit maximal vasoconstriction in all vascular beds, while hypotension caused by depressed cardiac function (congestive heart failure) elicits an increased discharge in vagal C fibers which offset the vasoconstrictor response of the arterial baroreceptors (Thorén et al., 1976). Consequently, these interactions between the arterial and cardiopulmonary

baroreceptors provide for an integrated regulatory system which can differentially alter cardiac chronotropic and inotropic performance, vascular resistance, and fluid electrolyte balance in the face of various stressors which might perturb BP (Abboud, 1979).

Several investigators have reported defective baroreflex function in human borderline hypertension (Eckberg, 1979) and in various animal models of hypertension (Brown, Saum & Tuley, 1976; Gordon, Matsuguchi & Mark, 1981; Thorén, Noresson & Ricksten, 1979b, 1979c). The "resetting" of the baroreceptor set point to a higher threshold and depressed reflex sensitivity both characterize the anomalies seen in hypertensive subjects (Zanchetti, 1979). Baroreflex resetting may precede the development of hypertension (Gonzalez, Krieger & Sapru, 1983), or occur as the result of structural changes in the arteries or heart which reduce distensibility and occur as a consequence of hypertension (Sapru & Krieger, 1979). Andresen and colleagues (1980) have reported that young (5 weeks of age) SHR possess an intrinsic structural abnormality in aortic distensibility which occurs independently of the elevated BP, and is responsible for a mismatch between receptor and vessel wall properties. This type of structural abnormality (reduced distensibility) has also been observed in the atrial myocardium of the SHR (Ricksten, Yao, Ljung & Thorén, 1980) and may be responsible for resetting of the cardiopulmonary baroreceptors (Ricksten et al., 1979). Consequently, myocardial and vascular abnormalities in collagen synthesis and deposition prior to, or in the absence of, hypertension in the SHR (Cutilletta, Erinoff, Heller, Low & Oparil,

1977) are responsible, in part, for baroreceptor dysfunction in this animal model. The gradual increase in arterial BP seen in the developing SHR exacerbates this genetic anomaly and is responsible for sustained structural resetting of the baroreceptor elements (Andresen & Brown, 1980).

Resetting of baroreceptors in the SHR has also been ascribed to ionic disequilibrium which depresses axonal conduction and appears to result from a defect in the $\text{Na}^+ - \text{K}^+$ electrogenic pump (Brown, 1980). This abnormality is also present in young SHR prior to the development of pathological hypertension, and may act in concert with the aforementioned metabolic abnormality to blunt arterial baroreceptor activity.

Although the cardiopulmonary baroreceptors are reset to a higher pressure threshold in the SHR (Thorén et al., 1979b, 1979c) the reflex inhibition of sympathetic activity in the splanchnic nerves in response to volume expansion is augmented when compared to normotensive Wistar-Kyoto (WKY) rats (Ricksten & Thorén, 1980). A hyperactivity of the cardiopulmonary baroreceptors in human borderline hypertension has also been observed by Mark and Kerber (1982). In their investigation, the role of cardiopulmonary baroreceptors in the control of forearm vascular resistance during exposure to lower body negative pressure was compared in normotensive and borderline hypertensive individuals. The latter group displayed an augmented vasoconstrictor response to lower body negative pressure (-5, -10, and -20 mmHg) when compared to normotensive control subjects.

Mark and Kerber (1982) suggest three possible explanations for the augmented cardiopulmonary baroreceptor response of the borderline

hypertensive subjects: (1) the reduced sensitivity of arterial baroreceptors might synergize to enhance the vasoconstrictor response from the cardiopulmonary afferents; (2) the higher cardiac filling pressure (elevated central venous pressure) would increase the basal discharge of the cardiopulmonary receptors, which might result in an augmented response to lower body negative pressure; (3) the elevated left ventricular pressure might exert a tonic inhibitory effect on central vasomotor centers by way of left ventricular unmyelinated vagal afferents, which could result in a heightened vasoconstrictor response to unloading induced by lower body negative pressure. Of these possible explanations, the authors speculate that the enhanced cardiopulmonary vasoconstrictor response in borderline hypertension is due to both impaired arterial baroreceptor function and/or increased left ventricular pressure.

Resetting of arterial and cardiopulmonary baroreceptors may also occur as the result of central modulation from bulbar and suprabulbar loci (Korner, 1980; Manning, 1980). Electrical stimulation of the cerebellar fastigial nucleus and the hypothalamic "defense area" has been shown to augment renal vascular resistance and renin release in response to carotid sinus activation and to inhibit reflex bradycardia following activation of the arterial baroreceptors (Achari, Al-Ubaidy & Downman, 1973; Koyama, Ammons & Manning, 1980; Thomas & Calaresu, 1974). Within this region of the hypothalamus, activation of sites in the lateral hypothalamus (LH), median forebrain bundle (MFB), and H fields of Forel (H) in anesthetized dogs has been shown to elevate the

equilibrium pressure of the carotid sinus and increase the gain of the baroreflex (Kumada, Schramm, Altmansberger & Sagawa, 1975).

Differential regulation of the myocardial (chronotropic and inotropic) and vascular components of the reflex, following stimulation of the hypothalamic defense area in anesthetized cats, produced an integrated cardiovascular response characterized by an increased CO for a given neurogenic drive to the heart, decreased left ventricular work, and increased muscle blood flow (Djojosingito, Folkow, Kylstra, Lisander & Tuttle, 1970; Kylstra & Lisander, 1970).

Elevated sympathetic discharge, coupled with a reduction in baroreceptor-induced vagal activation, appears to be responsible for this hemodynamic pattern (Gebber & Snyder, 1970; Thomas & Calaresu, 1974). It has been suggested by Smith and Nathan (1966) that this descending hypothalamic pathway courses through the inferior olivary nucleus (ION) before reaching more caudal medullary cardiovascular centers. Stimulation of this nucleus attenuated the heart rate deceleration and depressor response associated with the carotid sinus reflex.

Central modulation of the baroreflexes occurs during natural defense behaviors associated with muscle activation and exertion, feeding, and sleep (Stephenson, Smith & Scher, 1981). However, afferent feedback from the periphery to the CNS is necessary for the elaboration of an integrated cardiovascular response pattern. Disruption of afferent input to the CNS by sinoaortic denervation severely compromises cardiovascular adjustments to natural agonistic

behavior and emotional responses (Baccelli, Albertini, Del Bo, Mancía & Zanchetti, 1981; Baccelli, Albertini, Mancía & Zanchetti, 1976). However, cardiovascular responses to exercise, following sinoaortic denervation in the dog, do not appear to be significantly different from the intact animal (McRitchie, Vatner, Boettcher, Heyndrickx, Patrick & Braunwald, 1976). Recently, Walgenbach and Donald (1983) reported that the carotid baroreflex serves a dual purpose during dynamic exercise in dogs. First, the carotid sinus reflex serves to maintain arterial BP in the presence of metabolic vasodilation in the mildly exercised muscle; and second, the reflex acts to inhibit hypertensive responses during severe exercise, which is caused by elevated sympathetic drive to the systemic resistance vessels.

Taken together, these observations on the role of arterial baroreflexes during muscular activity associated with emotional behavior or purely as the result of dynamic exercise strongly suggest that "central command" modulates these two response patterns differently. However, it is apparent that afferent signals from arterial baroreceptors are necessary for the optimal utilization of cardiac output and the regulation of regional vascular resistance during both behaviors. In support of this observation, Smith, Astley, DeVito, Stein, and Walsh (1980) have reported that selective hypothalamic lesions in baboons eliminate the cardiovascular component of a conditioned emotional response (CER), but leave cardiovascular adjustments to dynamic exercise intact.

The pathophysiological implications of central modulation of the baroreflexes in the etiology of hypertension come from the observations

of Judy and Farrell (1979). They have suggested that central resetting in the SHR is responsible for a progressive debuffering of sympathetic nerve activity and mean arterial BP during the maintenance phase of hypertension. Yamori and Okamoto (1969) demonstrated that knife cuts between the posterior hypothalamus (PH) and mesencephalon reduced arterial BP in adult SHR to a significantly greater extent than normotensive control rats. Both groups of animals showed a significant decrease in HR following hypothalamic-mesencephalic transection. The results of this study and the observations of other investigators (Buñag, Eferakeya & Langdon, 1975; Takeda & Buñag, 1978) suggest that in the SHR there is a tonic elevation in sympathetic drive emanating from the hypothalamus, which is at least partly responsible for the elevated arterial BP seen in this animal model.

Neural Centers and Central Command

The classical view of neural control over the cardiovascular system, first proposed by Dittmar in the 1870's (Loewy & McKellar, 1980), revolved around the concept of a "vasomotor center" located in the ventrolateral reticular formation. Since that time, a plethora of studies have demonstrated that myocardial and circulatory responses can be elicited from all levels of the nervous system, and that these neural centers are organized to produce integrated response patterns (Hilton, 1975).

The localization of these areas within the CNS and their efferent and afferent pathways have relied heavily upon electrophysiological (Nathan, 1972b) and neuroanatomical (DeVito & Smith, 1982) techniques.

In addition, the use of selective lesions (West, Blessing & Chalmers, 1981) to eliminate the function of specific nuclei and knife cuts (Hartle & Brody, 1982) to transect the associated pathways have elucidated the functional role of these central cardiovascular systems. Ultimately, this information will extend our understanding of neural control of the circulation and the pathophysiology of neurogenic hypertension.

Several regions in the medulla oblongata participate in both reflexive and integrative control over the cardiovascular system (Doba & Reis, 1973; Iwamoto, Kaufman, Botterman & Mitchell, 1982). Electrolytic lesions of the NTS complex in rats have been shown to produce a lethal fulminating hypertension, as a consequence of sympathetic-adrenal medullary hyperactivity and subsequent elevation in TPR (Doba & Reis, 1974; Snyder, Doba & Reis, 1978). More discrete lesions of the NTS in cats, localized in the medial region of the complex, resulted in a chronic elevation of arterial BP and HR compared to sham lesioned control animals (Nathan & Reis, 1977). In addition, these animals displayed an increased lability in blood pressure responses to grooming, orienting, and augmented pressor responses to aversive classical conditioning (Nathan, Tucker, Severini & Reis, 1978).

The ventromedial and ventrolateral medulla have also been shown to exert a tonic influence on cardiomotor and vasomotor tone (Blessing & Reis, 1982; Kuo, Hwa & Chai, 1979). Blessing, West, and Chalmers (1981) found that lesions of the ventrolateral medulla (A1 catecholamine cell group) in rabbits elicited a rapid increase in mean arterial BP with an

associated bradycardia. The hypertension in these animals was the result of an elevated TPR and was mediated by a hypersecretion of vasopressin from the posterior pituitary gland (Blessing, Sved & Reis, 1982).

Stimulation of the LRN in anesthetized cats has been shown to produce a tachycardia and pressor response through an increase in sympathetic nerve activity (Thomas, Ulrichsen & Calaresu, 1977). Lesions of the LRN significantly reduced pressor responses elicited by stimulation of the posteromedial hypothalamus (PMH), carotid sinus nerve, and sciatic nerve (Ciriello & Calaresu, 1977). From these observations, it has been suggested that the LRN may represent a medullary region involved in somatomotor, cardiovascular, and sympathetic integration for the elaboration of pressor responses to exercise (Mitchell, Kaufman & Iwamoto, 1983). In support of this contention, Iwamoto and his colleagues (1982) have shown that lesions of the LRN abolish BP and HR increases to static exercise in cats.

The fastigial nucleus of the cerebellum has been shown to play an integral role in cardiovascular regulation during perturbations in orthostasis (Doba & Reis, 1974). Electrophysiological studies by Nathan (1972a) have indicated that this response pattern is mediated by way of the NTS and PRN of the medulla. Dormer and Stone (1976) found that electrical stimulation of the rostral fastigial nucleus in anesthetized dogs produced a rapid elevation in arterial BP and tachycardia which was the result of increased sympathetic nerve activity. However, these investigators also noted that the HR response was immediately buffered

by the baroreceptor reflex. In a follow-up study, Dormer, Foreman, and Ohata (1982) determined that the efferent fastigiobulbar fibers project bilaterally and activate ipsilateral sympathetic pathways in the DLF. Reis, Doba, and Nathan (1973) also found that stimulation of the rostral fastigial nucleus in conscious cats produced elevated arterial BP and HR in conjunction with feeding and predatory attack behavior. Thus, this nucleus serves both to regulate cardiovascular responses associated with movement and posture, and to modulate emotional and visceral responses in the carnivore.

In a series of studies, Ward and Gunn (1976a; 1976b) examined the role of the locus coeruleus (LC) complex, a structure in the rostral pons, in the control of BP. It was found that electrical stimulation of the LC in anesthetized cats produced a profound pressor response with an associated bradycardia, and that this response was dependent upon the integrity of the ventrolateral medulla. In addition, Ward and Gunn (1976b) demonstrated that the LC can attenuate depressor responses originating from the rostral RN and aortic depressor nerve, but exerts no inhibitory influence on depressor responses elicited from carotid sinus nerve stimulation. Although stimulation of the LC increases arterial BP, its role in the maintenance of hypertension appears to be minimal (Kawamura, Gunn & Frohlich, 1978).

It has been suggested by Mraovitch, Kumada, and Reis (1982) that the nucleus parabrachialis (NPB) is actually the region responsible for the pressor response observed by Ward and Gunn (1976a; 1976b). This study found that stimulation of the NPB in anesthetized cats produced a

stereotyped cardiovascular response pattern characterized by a rise in arterial BP, tachycardia, increase in CO, and elevation in TPR. The greatest degree of vasoconstriction occurred in the superior mesenteric vascular bed, followed by the renal and femoral arteries, respectively. The NPB may act to integrate forebrain and hindbrain input into an organized cardiovascular response pattern. However, the exact relevance of the NPB to a specific behavior associated with this cardiovascular response remains to be determined.

The term "central command" has been extensively utilized in the exercise literature (Mitchell et al., 1983) to describe forebrain excitation of medullary and spinal centers which serve to regulate cardiovascular function during static and dynamic exercise. However, the term may also be applicable to cardiovascular response patterns generated by emotional arousal. These CER are dependent upon the integrity of limbic and cortical structures within the CNS (Smith et al., 1980). Thus, central command from the forebrain prepares the animal for a physical challenge by activation of specific sympathetic pathways which govern myocardial performance and systemic vascular resistance.

The role of the hypothalamus in the control of circulation and as a neurogenic locus for hemodynamic responses which may trigger hypertension has been a principle area of cardiovascular research for several decades (Zanchetti, 1976). Stimulation of specific regions in the hypothalamus, i.e., the perifornical region, MFB, LH, and PMH have been shown to elicit cardiovascular responses which approximate those seen in

animals actively engaged in agonistic or defensive behaviors (Folkow & Rubenstein, 1966). This hemodynamic pattern consists of an increase in BP, CO, HR, stroke volume (SV), contractile force, muscle blood flow, and adrenergic vasoconstriction in the visceral vascular beds (Cobbold, Folkow, Lundgren & Wallentin, 1964; Fiegl, Johansson & Löfving, 1964; Folkow, Lisander, Tuttle & Wang, 1968; Rosén, 1961).

It has also been demonstrated that chronic stimulation of the hypothalamic defense area in conscious rats produces a moderate hypertension which diminishes after the stimulation period has ended (Folkow & Rubenstein, 1966). The authors suggest that repeated exposure to "mental stress" may mimic the sympathetic activation seen during hypothalamic stimulation, and act as a neurogenic trigger in the development of hypertension. It is important to emphasize that Folkow and Rubenstein (1966) used normotensive rats without a predisposition to hypertension, and that a chronic hypertension following hypothalamic stimulation might have resulted if a genetically predisposed animal were used. In support of this contention, Buñag and associates (1975; 1976) found an enhanced pressor responsiveness to PH stimulation in SHR when compared to normotensive Wistar rats. The biphasic PH pressor response appears to be mediated initially by an increase in sympathetic vasomotor tone, and secondly by an increase in circulating catecholamines from the adrenal medulla (Eferakeya & Buñag, 1974). Both electrolytic lesions of the PH regions (Buñag & Eferakeya, 1976) and transection of PH-mesencephalic pressor pathways (Yamori & Okamoto, 1969) have been shown to reduce the arterial BP of the SHR. These observations tend to

suggest that sympathetic hyperresponsiveness in the SHR (Yamaguchi & Kopin, 1980) is regulated in part by the PH excitation.

The paraventricular (PAH) and supraoptic (SON) nuclei of the hypothalamus have also been shown to participate in central cardiovascular regulation. Stimulation of the PAH and SON in anesthetized cats inhibits reflex vagal bradycardia following carotid sinus activation, elevates arterial BP, and increases HR through reciprocal changes in sympathetic and parasympathetic nervous activity (Ciriello & Calaresu, 1980). Neuroanatomical studies have demonstrated the presence of extensive connections between the PAH, NTS, and thoracic preganglionic sympathetic neurons (Ricardo & Koh, 1978; Saper, Loewy, Swanson & Cowan, 1976). Thus, in addition to their osmoregulatory function, these two nuclei exert a tonic inhibitory influence on vagal control of heart rate during carotid sinus nerve stimulation and may serve to coordinate neuroendocrine secretion of vasopressin with reflex cardiovascular control.

Several studies (Falcon, Phillips, Hoffman & Brody, 1978; Mohring, Kintz & Schoun, 1978) have indicated that the central pressor effect of angiotensin II (AII) is the result of both sympathetic activation and vasopressin release, and is dependent upon the integrity of several structures in the anteroventral portion of the third ventricle (AV3V) (Hartle, Lind, Johnson & Brody, 1982). Electrolytic lesions in this anterior periventricular tissue have been shown to prevent the development of hypertension in various animal models (Brody, Fink, Buggy, Haywood, Gordon & Johnson, 1978). The specific neuroanatomical

structures destroyed by the AV3V lesion include the periventricular (PVN) and preoptic (PON) nuclei of the hypothalamus, organum vasculosum of the lamina terminalis (OVLt), and the nucleus medianus (NM).

While AV3V lesions have been shown to prevent the development of renin-dependent, nonrenin-dependent, and salt-sensitive forms of hypertension, the lesion fails to prevent or even attenuate the development of hypertension in the SHR (Gordon, Haywood, Brody & Johnson, 1982). Phillips (1983) has argued that the results of this study do not eliminate the possibility that central AII may be involved in the etiology of hypertension in the SHR. However, the evidence presented is inconclusive and the criticisms raised are adequately dealt with by Gordon et al. (1982). Consequently, different neural mechanisms appear to be involved in these various animal models of hypertension.

The anterior hypothalamus (AH) exerts an inhibitory effect on sympathetic outflow and augments vagal tone during baroreceptor stimulation (Hilton & Spyer, 1980). It was found that discrete electrical stimulation of the PON and the AH in anesthetized cats resulted in a profound depressor effect with a concomitant bradycardia (Hilton & Spyer, 1971). In addition, it appears that this region tonically regulates sympathetic discharge to the adrenal medulla (Gauthier, Reis & Nathan, 1981). Nathan and Reis (1975) found that bilateral lesions of the AH in rats lead to the development of hypertension, tachycardia, hyperthermia, and increased locomotor behavior. It was determined that the hypertension was the result of an elevated TPR, and that bilateral adrenalectomy, adrenal demedullation, or adrenal denervation completely blocked the development of this response pattern.

In a similar study, Suarez and associates (1981) replicated the findings of Nathan and Reis (1975), but also measured plasma catecholamine levels in these animals. They found that plasma epinephrine and norepinephrine were significantly higher in lesioned rats compared to sham lesioned control animals. Thus, the AH exerts an inhibitory effect on sympathetic drive to the adrenal medulla and cardiovascular system, and facilitates vagal influences on heart rate during baroreceptor activation.

Other limbic structures, such as the septum and amygdala, have been shown to regulate emotional and autonomic response patterns. Calaresu and Mogenson (1972) found that two distinct cardiovascular response patterns could be elicited by electrical stimulation of specific sites in the septum of anesthetized rats. Stimulation of the lateral septum (LS) resulted in hypotension and bradycardia, while stimulation of the medial septum (MS) lead to hypertension and bradycardia. Using a combination of stimulation, lesion, and silver degeneration staining techniques Calaresu, Ciriello, and Mogenson (1976) attempted to identify the critical cardiovascular pathways mediating these divergent septal response patterns. It was found that the MS pressor response was dependent upon the integrity of the medial forebrain bundle (MFB), while the LS depressor and bradycardia responses were mediated via the stria medullaris (SM). Calaresu and his colleagues (1976) speculate that the MS and LS may participate in the integration of environmental stimuli and subsequent adjustments in cardiovascular function by the organism.

When considering the role of the septum in the regulation of emotional behavior (Lubar & Numan, 1973), it is conceivable that the

emotional hyperreactivity following septal lesions and cardiovascular reactivity might be carried by the same pathway.

While there is no information available on the cardiovascular pattern of the "septal rage syndrome," it has been noted that the behavioral hyperreactivity of these animals is dependent upon an intact MFB (Albert & Richmond, 1976) and that stimulation of the MFB in anesthetized rats elicits an elevation in arterial BP (Buñag, Riley & Takeda, 1981). Thus, it appears that the behavioral and autonomic responses seen following perturbations of the septum require an intact MFB and associated connections with the LH and PH.

Depressor and bradycardia responses have also been observed following electrical stimulation of the medial, lateral, central, and basal nuclei of the amygdala in anesthetized rats (Faiers, Calaresu & Mogenson, 1975). It has been suggested that cardiovascular hyperreactivity in the SHR is mediated by the central amygdaloid nucleus (Galeno, van Hoesen, Maixner, Johnson & Brody, 1982). Folkow and associates (1982) tested this hypothesis by lesioning the amygdala complex in the SHR. They found that these lesions produced a significant, but modest, reduction in the mean arterial BP of the SHR when compared to sham lesioned control animals. In addition, there was a significant reduction in pressor responsiveness to intense "environmental stress" (loud noise and vibrations) in the lesioned animals. The authors conclude that the amygdala complex may serve to enhance defense reactions in the SHR and exacerbate hypertensive responses, but is not the site of origin which is critical for triggering the development of hypertension in the SHR.

Consequently, it appears that limbic structures may coordinate behavioral and cardiovascular responses to environmental stressors via connections with the hypothalamus. This central command can then modulate baroreceptor reflexes, vagal tone, and sympathetic drive through brainstem and spinal connections to maximize myocardial performance and regulate regional blood flow in response to physical and metabolic demands.

Brain Catecholamines and Blood Pressure Regulation

Distribution and function of the cell groups. The anatomy and physiology of norepinephrine (NE), epinephrine (E), and dopamine (D) containing perikarya in the CNS have been extensively investigated using histochemical fluorescence and pharmacological techniques (Moore & Bloom, 1978; Moore & Bloom, 1979). These studies have revealed that catecholamine pathways project to and innervate structures which are known to regulate HR and arterial BP (Chalmers, 1975; Loewy & Neil, 1981).

De Jong (1974) infused NE directly into the NTS in anesthetized rats resulting in an immediate depressor response and bradycardia. In addition, pretreatment with phentolamine, an α -adrenergic blocking agent, completely blocked the cardiovascular response to centrally administered NE. These results suggest that NE innervation to the NTS exerts an inhibitory effect on BP and HR which is mediated by α -adrenergic receptors.

Snyder, Nathan, and Reis (1978) found that selective destruction of the catecholamine innervation to the NTS in rats, using the neurotoxin 6-hydroxydopamine (6-OHDA), produced a dose-dependent effect on HR and BP. Administration of 2 μ g directly into the NTS failed to elicit significant changes in HR or BP, while 4 μ g produced a transient elevation and increased lability in arterial BP without a concomitant change in HR. Finally, 12 μ g produced a fulminating hypertension and associated tachycardia. The authors observed that baroreceptor reflex sensitivity was depressed following the 4 μ g infusion, and conclude that catecholamine (primarily NE) innervation to the NTS serves to modulate baroreceptor activity, which in turn stabilizes arterial BP.

The NTS receives extensive catecholamine input from the A1 and A2 NE-containing cell groups, and via the more rostral C1 and C2 E-containing cell groups (Chalmers, Blessing, West, Howe, Costa & Furness, 1981). Talman, Snyder, and Reis (1980) found that electrolytic lesions of the A2 catecholamine cell group of the dorsomedial medulla in rats produced a marked increase in BP lability, an increase in BP response while the animals engaged in natural behaviors, and a reduction in the cardiovagal component of the baroreflex. The authors suggest that this response pattern is due to selective removal of NE input to the NTS.

Electrolytic lesions of the ventrolateral A1 cell group in rabbits lead to an acute hypertension, which is the result of an elevated TPR (Blessing et al., 1981), and severely attenuate the sensitivity of the baroreceptor vasoconstrictor component of the reflex (West et al., 1981). Consequently, it would appear that NE input from the A2 region

selectively modulates the activity of cardiovagal neurons in the NTS, while NE input from the A1 group tonically regulates sympathetic vasomotor elements of the baroreceptor reflex. The A1 projections also appear to exert a facilitatory effect on cardioinhibitory neurons which are activated by increased baroreceptor discharge (West et al., 1981).

Bilateral knife cuts at the level of the obex and lateral to the NTS, in anesthetized rats, result in an acute hypertension with no change in the NE levels of the NTS, A1, or A2 cell groups (Palkovits, De Jong, Zandberg, Versteeg, Van der Gugten & L  r  n  th, 1977). However, there was a significant elevation in E content in the nucleus commissuralis (NC) of the caudal NTS, which has been shown to contain axo-axonic synapses that may presynaptically regulate baroreceptor activity (Chiba & Doba, 1976). The E innervation to the NC of the NTS may be from both the C1 and C2 cell groups (Chalmers et al., 1981). Thus, both NE and E input to the NTS from the ventrolateral and dorsomedial cell groups serve to modulate baroreceptor sensitivity, by differentially affecting sympathetic drive to the vasculature and vagal tone to the heart.

The rostral metencephalon (pontine region) contains the A5, A6, and A7 catecholamine cell groups which have been shown to project to the spinal cord; however, only the A5 group appears to innervate the intermediolateral cell column (Loewy, McKellar & Saper, 1979). This observation suggests that the A5 region may regulate sympathetic vasomotor tone via a direct action on preganglionic neurons (Loewy & Neil, 1981). Electrical stimulation of the A5 cell group in

anesthetized rats resulted in an immediate pressor response (Loewy et al., 1979). Thus, the A5 catecholamine cell group may represent an integral link between rostral pontine structures, such as the NPB, and the intermediolateral cell column of the spinal cord for the elaboration of sympathetic vasomotor control (Loewy & McKellar, 1980). However, specific neuroanatomical and neurophysiological evidence is needed to support this contention.

Elimination of baroreceptor input to the CNS (by the use of sinoaortic deafferentation) has been shown to alter the absolute levels of catecholamines and the activity of synthesizing enzymes in various myelencephalic and diencephalic regions (Nakamura & Nakamura, 1981). Chalmers, Petty, and Reid (1979) found that there was a significant increase in tyrosine hydroxylase (TH) activity in the NTS, parahypoglossal nucleus (NPH), LC, and PH at 1 week following denervation. The level of NE in the LRN and NPH was significantly elevated over control levels 1 week after denervation, but significantly lower in the PH and AH when compared to sham operated control animals. Four weeks after the denervation, there were no differences in TH activity or NE content in any of the brain regions between the two groups of rats. However, TH levels were reduced in the spinal cord of denervated rats during this period. In contrast, phenylethanolamine-N-methyl transferase (PNMT) activity was reduced in both the PH at 1 week following denervation and the PH and AH at 4 weeks following denervation. In addition, there was a significant increase in PNMT activity in the spinal cord at 4 weeks postdeafferentation. The authors suggest that increased NE activity in

the aforementioned brain regions may participate in the initiation of hypertension in these animals, while decreased adrenergic activity in the hypothalamus may play a more critical role in maintaining the elevated BP in the sinoaortic denervated preparation (Chalmers et al., 1979).

The hypothalamus is richly innervated by NE, DA, and E containing neurons (Palkovits, 1981). The DA-synthesizing cells are located in the arcuate nucleus (AN), nucleus dorsomedialis subthalamicus zona incerta corner (ZI), PON, and PVN. These regions correspond to the A12 (AN), A13 (ZI), and A14 (PON and PVN) catecholamine cell groups. The two intrinsic DA pathways, i.e., the tuberoinfundibular and incerto-hypothalamic, serve to regulate neurosecretion from the pars distalis, pars intermedia, and pars nervosa of the pituitary gland (Moore & Kromer, 1978). The extrinsic DA mesocortical and nigro-neostriatal pathways originate at the level of the substantia nigra (cell groups A8, A9, and A10) and project fibers through the MFB to interconnect limbic structures and the corpus striatum with the mesencephalic tegmentum (Lindvall and Björklund, 1978). The role of hypothalamic DA neurons in blood pressure control remains unclear; however, recent evidence has suggested that projections from the A14 cell group descend to the spinal cord and may serve to regulate sympathetic drive to the cardiovascular system (Chalmers et al., 1981; Day, Blessing & Willoughby, 1980).

In contrast to DA, NE and E within the hypothalamus appear to originate from cell groups in the medulla (A1, A2, C1, and C2), pons (A5 and A6), and mesencephalic reticular formation (A7) (Palkovits, 1981).

The major pathways to the hypothalamus include the dorsal and ventral periventricular noradrenergic bundles, and the ventral noradrenergic bundle (within the MFB). Benarroch, Balda, Finkielman, and Nahmod (1983) found that bilateral destruction of the ventral noradrenergic bundle, in rats with 6-OHDA, resulted in an immediate increase in HR and arterial BP. These animals had significantly lower NE levels in the AH/PO area when compared to saline infused control rats, and displayed a hypersensitivity to the hypotensive effects of intrahypothalamically infused NE and clonidine. Ganglionic blockade with pentolinium initiated a rapid drop in arterial BP and HR, suggesting that the observed hypertension and tachycardia were the result of augmented sympathetic nervous system activity. In addition, destruction of serotonin (5-HT) containing neurons (projecting through the MFB to the hypothalamus) with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) completely blocked the development of hypertension in 6-OHDA treated animals. Recent evidence (Benarroch, Pirola, Alvarez & Nahmod, 1981) has also indicated that NE and 5-HT innervation to the AH/PO area is necessary for the central pressor action of AII. Thus, NE in the AH/PO exerts a tonic inhibition on sympathetic drive which is mediated by α_2 -adrenoreceptors, and a subsequent reduction in excitatory serotonergic activity (Benarroch et al., 1983; Kuhn, Wolf & Lovenberg, 1980).

Central catecholamine neurons have been implicated as the primary sites instigating the development of various forms of hypertension (Erinoff, Heller & Oparil, 1975; Wijnen, De Kloet, Versteeg & De Jong,

1980). Examination of brain catecholamine levels and synthesizing enzyme activity in the SHR has provided a substantial amount of information regarding the role of these transmitters in the pathogenesis of hypertension in this animal model. Tables 1 and 2 present the results of several studies which examined developmental changes in brain catecholamine levels (Isobe, Ohara, Kosaka & Aoki, 1980; Versteeg, Palkovits, Van der Gugten, Wijnen, Smeets & De Jong, 1976; Wijnen, Palkovits, De Jong & Versteeg, 1978; Yamori, Lovenberg & Sjoerdsma, 1970) and biosynthetic enzyme activity (Gianutsos & Moore, 1978; Nagaoka & Lovenberg, 1977; Saavedra, Grobecker & Axelrod, 1978) in the SHR, compared to normotensive WKY and (NIH) Wistar rats. The major points that can be gleaned from these studies are:

1. The SHR shows reduced NE levels and decreased dopamine- β -hydroxylase (DBH) activity in several hypothalamic nuclei (AH and PVN) shown to regulate arterial BP.
2. The SHR shows elevated E levels and increased PNMT activity in the hypothalamus (PAH and PVN) and medulla (NTS and A2 cell group).

Thus, it appears that reduced noradrenergic activity in the hypothalamus, specifically the AH area (Wijnen, Spierenburg, De Kloet, De Jong & Versteeg, 1980), parallels the development of hypertension in the SHR. In addition, there is a progressive increase in adrenergic activity in the A2 cell group which may reflect an adaptive change by the nervous system to offset the decreased NE in the hypothalamus.

Table 1

Developmental Changes in Brain Catecholamine Levels in the SHR Compared to WKY Rats

Study	Age	Region	DA	NE	E
Wijnen et al., 1978	2 weeks	AI cell group	--	--	elevated
	4 weeks	AI cell group	--	--	elevated
		PVN	--	--	elevated
		NTS	--	--	elevated
Saavedra et al., 1978		NIST	--	reduced	--
		AH	--	reduced	--
		PVN	--	reduced	--
		PAH	--	reduced	--
		DMH	--	reduced	--
Wijnen et al., 1978	7 weeks	PVN	--	--	elevated
Gianutsos and Moore, 1978	9 weeks	hypothalamus	--	--	elevated
Yamori et al., 1970 ^a	10 weeks	hypothalamus	--	reduced	--
		brainstem	--	reduced	--
Wijnen et al., 1978		PVN	--	--	elevated
		NTS	--	--	elevated
		PAH	--	--	elevated
Wijnen et al., 1980		AH	--	reduced	--
Isobe et al., 1980	14 weeks	hypothalamus	--	reduced	elevated

Table 1 (continued)

Study	Age	Region	DA	NE	E
Saavedra et al., 1978	14 weeks	NIST	--	reduced	--
		AH	--	reduced	--
		PVN	--	reduced	--
		PAH	--	reduced	--
		DMH	--	reduced	--
Gianutsos and Moore, 1978	15 weeks	hypothalamus	--	--	elevated
Versteeg et al., 1976	16 weeks	frontal cortex	elevated	--	--
		NIST	elevated	--	--
		PAH	--	--	elevated
		A7 cell group	--	elevated	--
		A5 cell group	--	elevated	--
		RNM	--	elevated	--
		A2 cell group	elevated	elevated	elevated
		A1 cell group	--	elevated	--
		RF	--	elevated	--

^aComparisons were made with normotensive Wistar rats.

Table 2

Developmental Changes in Brain Catecholamine Biosynthetic Enzyme Activity
in the SHR Compared to WKY Rats

Study	Age	Region	TH	DBH	PNMT
Nagaoka and Lovenberg, 1977	3 weeks	thalamus	--	increased	--
Saavedra et al., 1978	4 weeks	NIST	--	decreased	--
		PVN	--	decreased	--
		A1 cell group	--	--	increased
		A2 cell group	--	--	increased
Nagaoka and Lovengerg, 1977	5 weeks	hypothalamus	increased	increased	--
		thalamus	--	increased	--
Saavedra et al., 1978	6 weeks	A1 cell group	--	--	increased
		A2 cell group	--	--	increased
Nagaoka and Lovenberg, 1977	8 weeks	striatum	increased	--	--
		thalamus	--	increased	--
		pons-medulla	--	increased	--
Saavedra et al., 1978		A2 cell group	--	--	increased
Gianutsos and Moore, 1978	9 weeks	NTS	--	--	increased
			--	--	increased
Saavedra et al., 1978	10 weeks	A2 cell group	--	--	increased
			--	--	increased
			--	--	increased
			--	--	increased
	14 weeks	NIST	--	decreased	--
		AH	--	decreased	--
		PVN	--	decreased	--
		VMH	--	decreased	--

Table 2 (continued)

Study	Age	Region	TH	DBH	PNMT
Gianutsos and Moore, 1978	15 weeks	NTS	--	--	increased
Nagaoka and Lovenberg, 1977		thalamus	--	increased	--
Gianutsos and Moore, 1978	20-30 weeks	NTS	--	--	increased

The observation that aberrant catecholamine metabolism in the brain may serve to trigger the onset and maintenance of hypertension has led to the development of several centrally acting pharmacological agents which reduce sympathetic drive (Scriabine, Clineschmidt & Sweet, 1976).

The central antihypertensive effect of clonidine. Clonidine, 2-(2,6-dichlorophenylamino)-2-imidazoline, is a potent α -adrenergic partial agonist with both peripheral and centrally-mediated cardiovascular effects (Laubie, Delbarre, Bogaievsky, Bogaievsky, Tsoucaris-Kupfer, Senon, Schmitt & Schmitt, 1976). Systemic administration of clonidine is generally characterized by a biphasic response in arterial BP (Boissier, Giudicelli, Schmitt & Schmitt, 1968). Initially, intravenous (i.v.) infusion of clonidine leads to a slight elevation in blood pressure via a direct activation of α_1 -adrenoreceptors (post-synaptic) in the arteriolar wall (Kobinger, 1982). This pressor activity is followed by a delayed, longer duration depressor response, which results in hypotension and bradycardia, and is the result of more central mechanisms (Finch, Buckingham, Moore & Bucher, 1975; Scriabine, Sweet, Ludden, Stavorski, Wenger & Bohidar, 1977). It is this ultimate depressor activity which contributes to the effectiveness of clonidine in the treatment of essential hypertension.

Bolme, Forsyth, Ishizaki, and Melmon (1975) compared the hemodynamic effects of systemic versus central administration of clonidine in conscious monkeys. The primates were given either one of two doses i.v. (5 μ g/kg or 15 μ g/kg), or a single dose intraventricularly

(2 $\mu\text{g/kg}$, i.v.t.). Regional hemodynamic measurements, as well as CO, BP, and HR were determined 15 minutes, 1, 3, and 5 hours following the i.v. infusion, and 30 minutes, 1, and 3 hours following i.v.t. infusion. The results of the investigation indicated that the reduction in arterial BP to both i.v. (15 $\mu\text{g/kg}$) and i.v.t. clonidine was the result of a progressive reduction in CO. Blood flow to the kidney and liver were increased, while skeletal muscle, skin, and brain blood flow was reduced. In addition, both groups showed a significant reduction in HR, although the bradycardia was greater for the i.v. group. This difference was ascribed to a direct peripheral action of clonidine on α_2 -adrenoreceptors (presynaptic) of postganglionic sympathetic neurons innervating the heart.

Muir, Burton, and Lawrie (1969) studied the circulatory effects of i.v. clonidine (150 μg) on hypertensive patients at rest and during moderate exercise. They found that the hypotensive action was due to both a reduction in CO and TPR. A concomitant reduction in HR was also observed which minimized changes in SV. During exercise, systemic arterial BP, HR, SV, and CO increased while TPR decreased. These hemodynamic responses were observed both before and after clonidine administration. The discrepancy between the effect of clonidine on TPR observed in this study versus that seen by Bolme et al. (1975) can be reconciled by the work of Onesti, Schwartz, Kim, Paz-Martinez, and Swartz (1971). They found that no change in TPR occurred in response to clonidine if hypertensive patients were supine. However, when patients were shifted to an upright position, there was a significant reduction

in TPR in response to clonidine. The use of chair-restrained monkeys by Bolme and associates (1975) may have obscured any changes in TPR that would have been observed in a freely moving primate.

In addition to these cardiovascular effects, clonidine has also been shown to inhibit the secretion of renin, adrenocorticotrophic hormone, and vasopressin (Kimura, Share, Wang & Crofton, 1981; Rudolph, Kaplan & Ganong, 1980). These endocrine and cardiovascular responses have all been ascribed to the action of clonidine on α_2 -adrenoreceptors in both the CNS (Ganong, Rudolph & Zimmermann, 1979) and in the periphery (Pettinger, Keeton, Campbell & Harper, 1976).

It has been suggested that α_2 -adrenoreceptors are localized presynaptically, while α_1 -adrenoreceptors are located on the post-synaptic cell (Rand, Majewski, Medgett, McCulloch & Story, 1980; Starke & Altmann, 1973). However, other investigators have questioned this anatomical classification of α -adrenoreceptors, by demonstrating the presence of α_2 -adrenoreceptors on postsynaptic sites (Kobinger, 1982; Kobinger & Pichler, 1976). Consequently, a pharmacological classification has been proposed based on the affinity of the ligand for the α -adrenoreceptor. The α_2 -adrenoreceptors have a high affinity for "clonidine-like" compounds, while the α_1 -adrenoreceptors have a high affinity for "prazosin-like" compounds (Kobinger, 1982).

Berthelsen and Pettinger (1977) have divided α -adrenoreceptors on a functional basis. According to their system, activation of α_1 -adrenoreceptors facilitates calcium (Ca^{++}) influx into target cells (such as vascular smooth muscle) and results in a direct excitatory effect. In

contrast, stimulation of α_2 -adrenoreceptors antagonizes the influx of Ca^{++} into terminal buttons of both peripheral and central neurons (presynaptic effect), and depresses the activity of adenylate-cyclase in the postsynaptic cell (Gross, 1983; McAfee, Henon, Horn & Yarowsky, 1981). Both of these effects result in a direct inhibition of neuronal activity. Blockade of Ca^{++} influx at the terminal button leads to a subsequent inhibition of transmitter release due to the blockade of exocytotic vesiculation. This type of presynaptic inhibition involves a negative feedback loop whereby NE or E (native ligands for α_1 - and α_2 -adrenoreceptors) at high concentrations in the synaptic cleft may bind to α_2 -adrenoreceptors on the presynaptic cell and depress transmitter release (Langer, 1974; Rand et al., 1980).

The antihypertensive effect of clonidine has been attributed to activation of postsynaptic α_2 -adrenoreceptors in the CNS, which leads to a reduction in sympathetic drive (Baum & Shropshire, 1977; Timmermans, Schoop, Kwa & van Zwieten, 1981). Guyenet and Cabot (1981) demonstrated that clonidine reduces sympathetic discharge via direct action on sympathetic preganglionic neurons in the intermediolateral horn of the spinal cord. However, the principal areas in the CNS which appear to be responsible for the cardiovascular effects of the drug are in the medulla (Laubie et al., 1976; Sharma, Sandrew & Wang, 1978) and in the hypothalamus (Struyker-Boudier & van Rossum, 1972).

The functional integrity of the NTS, GRN, and LRN of the medulla has been shown to be requisite for the cardiovascular actions of clonidine. Rockhold and Caldwell (1980) found that the mediocaudal NTS

in the rat was highly sensitive to microinjections of clonidine. Concentrations from 1.25-40 nmol (.22-7.12 μ g) produced a dose dependent hypotension and bradycardia which could be blocked by the α_2 -adreno-receptor antagonist yohimbine.

Lipski, Przybylski, and Solnicka (1976) found that bilateral electrolytic lesions of the NTS in Wistar rats produced a neurogenic hypertension which was not attenuated by i.v. clonidine. As a control procedure, a separate group of rats received bilateral parasympathectomies (section of the vagosympathetic trunk) and occlusion of the right and left carotid arteries. This surgical manipulation yielded a neurogenic hypertension which was similar in magnitude to that seen following lesions of the NTS. However, an equivalent dose of clonidine (30 μ g/kg) evoked a rapid and profound reduction in arterial BP.

In a similar study, Rockhold and Caldwell (1979) compared the hypotensive effects of i.v. clonidine (30 μ g/kg) on SHR and WKY rats prior to and following electrolytic lesions of the NTS. The clonidine elicited a profound reduction in HR and arterial BP in conscious SHR before the NTS lesion. The WKY rats exhibited a biphasic response to the clonidine, characterized first by a short term hypertension and second by a moderate decrease in arterial BP and HR. Bilateral lesions of the NTS in both SHR and WKY rats resulted in a fulminating hypertension. These lesions completely abolished the effects of clonidine on HR and BP in the SHR, but did not alter the hypotensive effect of clonidine in the WKY. It should be noted that the lesion did block the clonidine-induced bradycardia seen in intact WKY rats.

It has been suggested that the bradycardia observed following clonidine administration is the result of central facilitation of baroreceptor impulses at the level of the NTS, which augments vagal tone to the heart (Laubie, Schmitt & Drouillat, 1976). Antonaccio and Halley (1977) have found that lesions of the NTS in chloralose-anesthetized cats failed to block the hypotensive effects of i.v. clonidine (10-30 $\mu\text{g}/\text{kg}$), but did significantly reduce the bradycardia effect of the drug. The authors suggest that clonidine affects HR via α_2 -adrenergic mechanisms in the NTS, but that the depressor effects of the drug are localized in other areas of the medulla.

In support of this observation, Chan and Koo (1978) reported that lesions of the medial medullary reticular formation (RF) in rats abolished the hypotensive action of i.v. clonidine (10 $\mu\text{g}/\text{kg}$). In an attempt to delineate a specific nucleus in the RF which mediates this effect, Chen and Chan (1980) examined the role of the GRN as the central site of clonidine action. They instigated experimental hypertension in anesthetized cats by sustained bilateral occlusion of the common carotid arteries. Microinjection of clonidine (.5 $\mu\text{g}/\text{kg}$) directly into the GRN elicited a significant drop in BP and HR. Bilateral electrolytic lesions of the GRN completely eliminated the bradycardia and depressor effects of intravenous clonidine.

Bilateral microinjections of clonidine (75 ng/kg) into the LRN of anesthetized cats were shown to induce a significant reduction in BP and HR (Bousquet, Feldman, Bloch & Schwartz, 1981). In addition, Bloch, Feldman, Bousquet, and Schwartz (1977) found that bilateral

microinjections of clonidine into the ventrolateral medulla attenuated the pressor effects of PH stimulation in anesthetized cats. These studies suggest that the LRN is the most sensitive region of the medulla to the α_2 -adrenoreceptor agonistic effects of clonidine, and that activation of these receptors can modulate sympathetic outflow to the heart and vasculature emanating from the PH (Barman & Gebber, 1982).

From the preceding research investigations, it can be concluded that the anatomical integrity of three regions (NTS, GRN, and LRN) in the medulla is necessary for clonidine to exert a central inhibitory influence on sympathetic outflow to the cardiovascular system and central facilitatory effect on cardiovagal neurons of the baroreflex. Bulbar centers such as these play a critical role in reflex myocardial and vascular adjustments. However, more integrative cardiovascular responses originate from the prosencephalon.

It has been suggested that clonidine may exert some of its circulatory and endocrine effects through subrabulbar structures such as the hypothalamus (Tadepalli & Mills, 1978). For example, Struyker-Boudier and van Rossum (1972) found that local microinjection of clonidine (1-20 μg) into the PH of anesthetized rats produced a dose dependent reduction in HR and BP. In another investigation, Struyker-Boudier, Smeets, Brouwer, and van Rossum (1974) found that microinfusions of clonidine (1.5-100 nmol or .27-17.8 μg) into the AH/PO area of anesthetized rats also elicited a bradycardia and hypotension. This response pattern could be antagonized by pretreatment with the α -adrenoreceptor antagonist phentolamine. The authors also noted that

clonidine mimicked the cardiovascular effects of centrally applied NE, but showed a greater physiological potency. Struyker-Boudier and associates (1975) have observed that E exerts a greater affinity for α_2 -adrenoreceptors in the AH/PO area than does NE.

The hypothalamus has been shown to regulate the secretion of renin from the kidney (Zehr & Feigl, 1973) which is mediated by the renal sympathetic nerves (Wilson, Ninomiya, Franz & Judy, 1971). It has been suggested by Ganong et al. (1979) that the suppression of renin secretion by clonidine may be due to an inhibition of both noradrenergic and serotonergic neurons. Evidence has been accrued that clonidine can inhibit the activity of 5-HT containing neurons by a direct inhibition of noradrenergic neurons in the CNS (Svensson, Bunney & Aghajanian, 1975). Microinjections of 5-HT into the AH/PO have been shown to elicit pressor responses in both anesthetized normotensive and hypertensive rats (Smits & Struyker-Boudier, 1976; Wolf, Kuhn & Lovenberg, 1981).

In support of these observations, Korner and Head (1983) demonstrated that both NE and 5-HT contribute to the centrally mediated cardiovascular effects of clonidine, and that these effects are modulated at both the bulbar and suprabulbar levels of the nervous system. Thus, clonidine may reduce BP, HR, and renin secretion by a stimulatory effect on postsynaptic α_2 -adrenoreceptors in the AH/PO area of the hypothalamus. This action would inhibit descending serotonergic neurons and result in reduced sympathetic drive to the kidney and cardiovascular system.

It is possible that abnormal catecholamine metabolism in the AH area (Wijnen et al., 1980), coupled with an anomaly in α -adrenoreceptor

regulation (i.e., an imbalance between excitatory and inhibitory receptors) may play a pivotal role in the etiology of essential hypertension (Cantor, Abraham & Spector, 1980; Morris, Devynck, Woodcock, Johnston & Meyer, 1981). The actions of clonidine in the hypothalamus and/or the medulla act to rectify this imbalance in catecholamine activity by stimulating inhibitory α_2 -adrenoreceptors. The ultimate depressor effect of the drug is the result of both a reduction in sympathetic outflow and an increase in vagal tone. The hemodynamic consequences of this change in neurogenic activity are a drop in CO and TPR.

Hemodynamic Patterns in Essential Hypertension

Arterial BP represents the product of CO and TPR. Thus, a fundamental question in the study of hypertension is to determine which of these variables contribute to the elevation in BP. Three characteristic hemodynamic patterns have been observed in both man and animals with hypertension (Ferrario & Page, 1978): (1) an elevated CO with a "normal" TPR; (2) an elevated TPR with a normal to low CO; and (3) a transitional pattern characterized by an early rise in CO, followed by a progressive rise in TPR.

Circulatory Patterns in Borderline Hypertension

The observation that some patients with labile or borderline hypertension have an elevated CO during the developmental stages of the disease has been well documented (Eich et al., 1966; Finkielman, Worcel

& Agrest, 1965). However, this pattern has not been observed in all patients with borderline hypertension and may represent a subset of individuals within the entire population of essential hypertensives (Julius, 1977). It has also been reported that individuals with borderline hypertension demonstrate defective reciprocal adjustments in CO and TPR, which normally serve to maintain appropriate BP levels during various physical challenges (Julius, Pascual, Sannerstedt & Mitchell, 1971). In this investigation, it was observed that patients with borderline hypertension showed an elevated CO and "normal" TPR while in a recumbent position when compared to normotensive subjects. However, these same individuals displayed an elevated TPR in response to mild exercise with only a modest increase in CO. In contrast, the normotensive subjects demonstrated a significant reduction in TPR and significant increase in CO in response to this physical challenge. During volume expansion with dextran, which increases BP, the borderline hypertensive individuals failed to show a reduction in TPR to the same degree as the normotensive group. It was also observed that borderline hypertensive subjects showed a greater increase in TPR to postural adjustments, i.e., moving from supine to sitting, than did the normotensive control subjects.

From these observations, Julius et al. (1971) have concluded that in borderline hypertensive individuals there is an abnormal relationship between CO and TPR which results in hypertension. The interaction between structural resetting of resistance vessels (increased wall to lumen ratio) in borderline hypertensive subjects (Takeshita & Mark,

1980) and sympathetic nervous activity (either normal or elevated) may contribute to this stereotypical cardiovascular response pattern (Esler, Julius, Randall, Ellis & Kashima, 1975). In addition, Mancia, Ferrari, Gregorini, Parati, Ferrari, Pomidossi, and Zanchetti (1979) have reported that essential hypertensives show pressor responses to positive neck pressure (decreased carotid baroreceptor activity) which result from increased TPR with little or no change in CO. This same pressor reflex in normotensive individuals is mainly due to an increase in CO, with only a modest change in TPR. Consequently, it appears that patterned neurogenic drive to the heart and vasculature of borderline hypertensive individuals may be different from that seen in their normotensive counterparts during exposure to various stressors.

Hemodynamics in the SHR

Developmental patterns. The SHR and control strain WKY rat have contributed greatly to our understanding of central and peripheral circulatory control in the development of hypertension (Iriuchijima, 1982).

Early studies which attempted to assess hemodynamic function in the SHR employed electromagnetic flowometry in the anesthetized state (Iriuchijima, 1973a; Iriuchijima, Numao & Suga, 1975). These studies found that hypertension in the adult SHR (12-52 weeks) was the result of an elevated TPR compared to normotensive control rats. This increase in TPR was maintained by augmented sympathetic nervous activity (Iriuchijima, 1973b). In contradiction to these findings, Pfeffer and

Frohlich (1973) reported that young adult SHR (9-12 weeks) had an elevated arterial BP which was the result of a hyperkinetic circulatory state (Frohlich, Tarazi & Dustan, 1969), manifested by an elevated HR, CO, maximum aortic flow velocity, and peak flow velocity, compared to normotensive control rats. At this age there was no significant difference in TPR between the SHR and the normotensive rats. Hypertension seen in old SHR (18-33 and 62-97 weeks) was the result of an elevated TPR with a normal CO as compared to the normotensive control subjects. A possible explanation for the discrepancy between these studies may involve the use of two different anesthetics, i.e., sodium pentobarbital versus ether.

Smith and Hutchins (1980) examined the effect of different anesthetics on hemodynamic function in SHR and WKY rats chronically instrumented with electromagnetic flow probes and arterial catheters. This technique allowed direct comparisons to be made between the conscious and the anesthetized state for each strain. Ether produced a significant reduction in TPR and arterial BP, and a significant increase in HR and CI for SHR and WKY strains. Significant increases in SV and peak aortic flow velocity occurred only in the SHR under ether anesthesia. In contrast, sodium pentobarbital decreased HR, CI, SV, BP, peak aortic flow velocity, and minute work (MW) in SHR and WKY strains. The authors conclude that hemodynamic patterns in anesthetized SHR and WKY rats are significantly different from those in the conscious state. Conclusions extrapolated from studies employing anesthetized preparations cannot be extended to conscious animals.

In view of these findings, Smith and Hutchins (1979) conducted a developmental hemodynamic study with conscious and chronically instrumented SHR and WKY rats. The purpose of the study was to determine if hypertension in the SHR is characterized by a shift from elevated CO to increased TPR and at what age this occurs. Hemodynamic measurements were taken on age-matched SHR and WKY rats from 30-41, 79-83, and 118-123 days of age. The results of the study demonstrated the presence of elevated arterial BP in SHR compared to WKY rats at 32 days of age. From 32 through 41 days of age, this hypertension was characterized by an elevated CI. Heart rate and stroke index (SI) were significantly elevated over WKY rats from 34-41 days. Minute work was elevated in the SHR from 34-120 days. The hypertension seen in the SHR beyond 80 days was the result of an elevated TPR. Hence, this study demonstrates the presence of a hyperkinetic circulatory state in the young SHR which is characterized by an elevated HR, stroke work (SW), MW, and CI. As the hypertension becomes fixed, there is a transition to an elevated TPR and normal CI and HR. This response pattern is attributed to total body autoregulation (Smith & Hutchins, 1979).

Hemodynamic Function and Myocardial Hypertrophy

The development of left ventricular hypertrophy in hypertension is seen as a physiological adaptation by the heart in response to an elevation in afterload (Tarazi & Levy, 1982). Pfeffer, Pfeffer, and Frohlich (1976) studied the development of left ventricular hypertrophy in the SHR and resultant changes in cardiac pumping ability. Hemodynamic comparisons were made between anesthetized SHR and two control

strains (WKY and normotensive Wistar rats) at 11, 24, and 83 weeks of age. In each of the three strains a rapid increase in body weight was paralleled by concomitant left ventricular growth, which occurred between 11-24 weeks of age. Augmented pumping performance was directly related to the increase in heart size. However, hypertrophic growth of the left ventricular mass in the SHR exceeded that which would be predicted on the basis of normal body growth. In old SHR (82 weeks of age) a reduction in cardiac performance was observed, despite an extensively hypertrophied left ventricle. Consequently, the natural growth of the left ventricle in the SHR from 11-24 weeks of age allows the animal to maintain a normal CO in the face of an elevated arterial BP. However, this adaptation in ventricular mass becomes deleterious with increasing age, when the heart begins to decompensate and the SHR develops congestive heart failure.

Pfeffer, Pfeffer, Fishbein, and Frohlich (1979) have reported that distinct morphological changes in the left ventricular wall parallel the reduction in pumping ability seen in the adult SHR. As in the previous study, hemodynamic comparisons were made between anesthetized SHR, WKY, and normotensive Wistar rats at 13, 25, 52, and 90 weeks of age. In addition, changes in left and right ventricular morphology were characterized for all animals at each age. The results of this study replicated the findings of Pfeffer et al. (1976) by demonstrating that myocardial function in the SHR becomes severely compromised with increasing age (52 and 90 weeks of age), and is the result of concentric cardiac hypertrophy and extensive left ventricular fibrosis.

Peak pumping ability in all strains was determined by using peak SV and peak CO per gram of left ventricle during a rapid infusion of Tyrodes solution. These indices remained constant for normotensive rats throughout the observation period. However, both peak SV and peak CO were reduced in the SHR at 52 and 90 weeks of age. Geometric changes in vertical and horizontal external circumferences increased with age, which paralleled the increase in left ventricular weight for each strain. With increasing age, the normotensive animals displayed minimal change in either septal or left ventricular free wall thickness. However, there was a significant increase in free wall thickness without an accompanying increase in septal thickness in the SHR from 52-90 weeks of age. Hence, left ventricular growth occurs via hypertrophy of myofibers and hyperplasia of connective tissue in both the SHR and normotensive rats. These changes in left ventricular mass occur in proportion to changes in body weight associated with maturation in the normotensive rats and result in developmental eccentric ventricular hypertrophy. In contrast, the growth pattern of the left and right ventricles of the SHR increases beyond the normal growth curve and results in a pressure-induced concentric ventricular hypertrophy.

The adaptation of the left ventricle to an increased afterload necessitates the development of increased wall tension by the myocardium. The Law of Laplace ($\text{Wall Tension} = \text{Transmural Pressure} \times \text{Internal Radius}$) would predict that concentric left ventricular hypertrophy would provide a mechanical advantage in dealing with an elevated afterload, by a redistribution of increased wall tension over a larger number of

contractile elements. This response by the heart would allow for diffusion of the tension so that the force per unit of cross-sectional area of myocardium is shifted to a normal level (Pfeffer et al., 1979). Hence, myocardial wall stress ($\text{Wall Stress} = \text{Pressure} \times \text{Internal Radius} / 2 \times \text{Wall Thickness}$) is reduced by the evolution of concentric left ventricular hypertrophy (Tarazi & Levy, 1982). As the hypertension progresses, both the left and right ventricles become less distensible due to an increased deposition of intracellular collagen and myocardial fibrosis (Pfeffer et al., 1979; Zak, Kizu & Bugaisky, 1979). The final result of the disease is dilation of the ventricular cavities (especially the left ventricle) which leads to a greater wall stress and requires an increased myocardial tension to maintain a normal SV, given a fixed preload or filling pressure. An inability to preserve SV ultimately results in an increased end-systolic volume, reduced CO, and the evolution of congestive heart failure (Pfeffer & Pfeffer, 1983).

Hallbäck, Isaksson, and Norell (1975) have demonstrated that the development of concentric left ventricular hypertrophy in the SHR leads to a progressive shift in the Frank-Starling relationship, such that a higher end-diastolic filling pressure (or increased inotropy) is necessary to maintain a normal SV or SW. In a series of studies, Hallbäck-Nordlander, Norell, and Thorén (1979) compared the performance of isolated hearts from SHR or normotensive rats to a range of diastolic filling pressures and determined the stroke volume (Frank-Starling relation). It was found that with increasing age (6 weeks versus 30 weeks) the SHR hearts were significantly heavier than

the normotensive rat hearts, and required a higher pressure in the lower physiological range of diastolic filling pressures to maintain a normal SV. Compensation for this rightward shift in the Frank-Starling relation would necessitate an increased end-diastolic filling pressure and/or augmented contractility to maintain a normal SV. The effects of increased afterload were also examined in the isolated hearts from age-matched SHR and normotensive rats. As would be expected, the SHR hearts maintained SV at higher afterloads and produced more efficient external work than the normotensive animals for preloads above 10 mmHg. The results of these studies support the adaptive function of the myocardial hypertrophy seen in the SHR.

A second mechanism for maintaining SV in the face of an altered Frank-Starling relationship requires an increased inotropic response by the heart. Noresson, Thorén, and Hallbäck-Nordlander (1982) examined the performance of isolated hearts from adult SHR and normotensive rats (4-6 months of age), where preload and afterload could be varied independently. The hearts were paced electrically to maintain a fixed HR with a constant preload, while afterload was sequentially elevated. This manipulation produced a significant step-wise increase in contractility (measured as maximum left ventricular dP/dt) for both strains. Addition of epinephrine to the perfusate increased HR and maximum dP/dt to the same extent for both the SHR and normotensive rat hearts. Thus, in the uncompromised heart, an elevated afterload increases the maximum velocity of myocardial contraction (maximum dP/dt). This intrinsic inotropic response (Anrep effect) has been

ascribed to "homeometric" autoregulation (Berne & Levy, 1977). Since there were no differences observed in the inotropic state of the SHR heart working against hypertensive pressures, as compared to the normotensive rat heart working against normal pressures, it can be concluded that structural changes in the myocardium compensate for the elevated afterload. Hence, the concentric myocardial hypertrophy allows a lower contractile state to be maintained in the face of an elevated arterial BP in the SHR (Norell et al., 1982).

In contrast to the findings presented above, Saragoca and Tarazi (1981) have reported that the hypertrophied left ventricular myocardium of the SHR (20-24 weeks of age) demonstrates an impaired contractile, chronotropic, and relaxation response to graded infusions of isoproterenol compared to age matched WKY control animals. The SHR required a higher threshold dose of isoproterenol to elicit a chronotropic response and the maximal HR increase was significantly less than that seen in the WKY rats. The contractility indices (maximum dP/dt and dP/dt/P) of the left ventricle for the SHR showed smaller increases to the isoproterenol than did the WKY controls. Finally, left ventricular relaxation responses by the myocardium, in response to isoproterenol, were significantly reduced in the SHR. The WKY rats showed no impairment in relaxation response to the isoproterenol.

In support of the observations of Saragoca and Tarazi (1981), Limas and Limas (1978) have reported a decreased number of myocardial β -adrenoreceptors in young SHR (5 weeks of age) compared to WKY control rats. The diminished number of β -adrenoreceptors was evident before the

onset of myocardial hypertrophy and persisted well into the established phase of hypertension (at 39 weeks of age). The ultimate result of this condition would be a reduced inotropic and chronotropic response for a given level of sympathetic stimulation. This might be one possible explanation for the findings of Saragoca and Tarazi (1981). In addition, Limas and Cohn (1977) have demonstrated that SHR show a reduced cyclic-AMP dependent Ca^{++} uptake by the sarcoplasmic reticulum compared to WKY rats. This defect in Ca^{++} sequestration would lead to impairment of isometric relaxation in the hearts of the SHR following sympathetic stimulation.

Procedural differences between the studies of Norell et al. (1982), and Saragoca and Tarazi (1981), i.e., an in vitro preparation versus a closed chest anesthetized in vivo preparation, may explain the discrepancy in their respective conclusions regarding inotropic responses to β -adrenoreceptor stimulation in the SHR.

It has also been noted by several investigators that myocardial hypertrophy can develop in the SHR independently of the hypertension (Cuttilletta et al., 1977; Frohlich and Tarazi, 1979). Destruction of either the central or peripheral nervous system with 6-OHDA or nerve growth factor antiserum, respectively, in young SHR (14 days old) completely blocked the development of hypertension (Oparil and Cuttilletta, 1979). No significant change in BP was observed in similarly treated WKY rats. Hemodynamic measurements in anesthetized SHR treated with 6-OHDA or nerve growth factor antiserum revealed a progressive reduction in left ventricular performance similar to sham

treated SHR. In addition, there was a progressive development of left ventricular hypertrophy in the treated SHR, despite a normal blood pressure. The authors conclude that a myocardial abnormality exists in the SHR, such that increased protein synthesis and collagen deposition occur independently of pressure overload. However, increased arterial BP in the developing SHR can exacerbate this genetic anomaly and precipitate the development of myocardial pathology.

It can be concluded from the aforementioned hemodynamic studies that the development of hypertension in the SHR proceeds through characteristic stages which resemble certain conditions seen in human essential hypertension (Trippodo & Frohlich, 1981). A "hyperkinetic" circulatory state, characterized by an elevated HR and CI, is seen in the young SHR during the labile phase of hypertension. This is followed by a fixed hypertension which is the result of an elevated TPR. The myocardium of the SHR responds to the elevated arterial BP by a compensatory increase in left ventricular mass, i.e., concentric left ventricular hypertrophy. Depressed myocardial responsiveness to β -adrenoreceptor stimulation (Kunos, 1978) coupled with an enhanced α -adrenoreceptor vasoconstrictor response (Bohlen, 1979) may explain the hemodynamic transition from an elevated CI to an elevated TPR hypertension in the SHR.

In addition, Hallbäck and Folkow (1974) have suggested that a pressor hyperresponsiveness to environmental stress (light, noise, and vibrations) in the SHR may serve to exacerbate a pre-existing cardiovascular abnormality. Lundin and Hallbäck-Nordlander (1980) have

reported that increased pressor responses to "mental stress" (a blast of air and noise) in young SHR (5 weeks of age) were the result of a large increase in CO and a slight increase in the TPR.

This "defense" response in the young SHR was characterized by an increase in sympathetic outflow to the heart and vasculature. Thus, environmental stress may aggravate the hyperkinetic circulatory state of the young SHR and augment the development of hypertension (Yamori, Matsumoto, Yamabe, and Okamoto, 1969).

In the following section, the role of environmental stress in the etiology of essential hypertension will be discussed. Cardiovascular and hormonal responses to psychological stress will be examined in both normotensive animals and in animals genetically predisposed to develop hypertension.

Cardiovascular and Hormonal Responses to Environmental Stress

It has been repeatedly observed that environmental stimuli are capable of producing a variety of cardiovascular adjustments by the organism (Cohen & Obrist, 1975; Randall & Smith, 1974). Stressful stimuli such as loud noise, electric shock, and antagonistic confrontation with a conspecific have been shown to elicit elevations in arterial BP and HR in experimental animals (Alexander, 1974; McCarty, Chiueh & Kopin, 1978; Perhach, Ferguson & McKinney, 1975).

A barrage of audio, visual, and motion stimuli presented over several weeks can instigate mild hypertension (systolic BP of 150 mmHg) in Sprague-Dawley rats. It was found that peripheral sympathectomy with

6-OHDA completely blocked the development of stress-induced hypertension in these animals (Smookler, Goebel, Siegel & Clarke, 1973).

Several investigators have suggested that it is the anticipation of the aversive event, whether it is predictable or unpredictable, and the mode of coping utilized by the organism which determines its pathophysiological impact on the system (Lawler, Barker, Hubbard & Bachuss, 1981; Weiss, 1970; Weiss, 1977). Hence, the "psychological" component of the stressor may prove to be more deleterious than the physical event. Folkow (1975) has stated that exposure to stressful environmental stimuli elicits a defense response by the organism which is characterized by an increase in sympathetic drive to the cardiovascular system. It has been postulated that repeated exposure to these stressors may precipitate the onset of essential hypertension and lead to "structural autoregulation" of the systemic resistance vessels (Hallbäck, Göthberg, Lundin, Ricksten & Folkow, 1976). This structural change in the arterioles results in a fixed hypertension which is maintained by an elevated TPR.

An array of aversive behavioral techniques has been employed by researchers to elicit sympathetic arousal in the organism and concomitant elevations in arterial BP. These stressful manipulations have included psychosocial stress, sensory isolation, and classical and operant conditioning paradigms.

Psychosocial Stress

Henry (1977) has stated that social interactions between conspecifics of a given species may result in emotional arousal and

activation of the sympathetic-adrenal medullary or pituitary-adrenal cortical systems. A social interchange, which results in the displacement or movement down the dominance hierarchy, is associated with behavioral depression, decreased gonadotropin levels, and activation of the pituitary-adrenal cortical system (Mason, 1972). The sympathetic-adrenal medullary system is activated during agonistic behavior while the organism is actively coping with a threat to dominance status (Mancia, Baccelli, & Zanchetti, 1972).

Henry, Meehan, and Stephens (1967) have examined the effects of chronic psychosocial stress on the development of hypertension in CBA mice. The animals were exposed to four different types of psychosocial stimulation lasting from 6-12 months; including (1) mixing male mice from different colonies; (2) exposing male and female mice to chronic threats from a predator; (3) crowding the mice together in a small area; and (4) subjecting male and female mice to chronic territorial conflict by means of an interconnecting box system. It was found that each of these techniques increased systolic BP to approximately 160 mmHg in male mice and 140-150 mmHg in female mice. Castrated males showed only small transient elevations in systolic BP. Critical periods with regard to early experience and duration of the stress were found to influence the severity of the hypertension. For example, mice which were group housed following birth were less susceptible to hypertension induced by territorial conflict than mice who were isolated (4-5 months) following birth and then exposed to this psychosocial stress. The authors concluded that territorial conflict exposed the mice to the greatest

degree of psychosocial stress and ultimately produced the greatest elevations in systolic BP.

Henry, Stephens, Axelrod, and Mueller (1971) extended these findings by examining the effect of chronic territorial conflict on adrenomedullary activity and systolic BP in male and female CBA mice. The mice were exposed to 6 months of psychosocial stress beginning at 4 months of age. The animals that were exposed to psychosocial stimulation showed a progressive increase in systolic BP to 170 mmHg, which was significantly greater than the subjects who remained in social isolation (126 mmHg). In addition, the adrenal glands of the stressed mice were heavier than the control animals. Concomitant elevations in NE and E levels, as well as increases in monoamine oxidase, TH, and PNMT activity were also observed in the adrenal glands of stressed mice. The results of this investigation suggest that sympathetic-adrenal medullary hyperactivity is responsible, in part, for the development of psychosocial hypertension.

It has also been demonstrated that psychosocial stimuli can elicit profound increases in plasma renin activity (Clamage, Sanford, Vander, & Mouw, 1976). The role of the renin-angiotensin system in the development of psychosocial hypertension was investigated by Vander, Henry, Stephens, Kay, and Mouw (1978). Male and female mice were either group housed or placed in social isolation after weaning. When the animals were 4 months old, male isolates and females that were group housed were placed together in large "population cages," which allowed extended social contact between conspecifics. The animals were maintained in

this environment for 6 months. Blood samples for the determination of plasma renin activity and systolic BP measurements were taken periodically throughout the 6 month period. It was found that plasma renin activity increased during the first day of territorial conflict, decreased over the following 3 weeks, and increased again during the last two months. Systolic BP and plasma renin activity were significantly greater than the control isolates from the 1st week throughout the psychosocial stress period. Vander et al. (1978) conclude that during its initial stages, psychosocial hypertension is a "high-renin" form of hypertension. During the middle stage of the hypertension (months 2-4) the elevated BP was thought to exert an inhibitory effect on renin secretion through a negative feedback loop and the activation of intrarenal pressure receptors. Finally, during the latter stages of the hypertension (months 5-6) plasma renin activity and systolic BP increased simultaneously. This increase in renin secretion was thought to result from secondary damage to the kidney and renal vasculature.

In a follow-up study, Webb, Johnson, Vander, and Henry (1983) examined the pressor responsiveness of hindquarter and renal vasculature from CBA mice which had been exposed to 2 months of psychosocial stress. This period was selected because it represented a time in which the animals had a "non-renin" dependent form of hypertension. Comparisons were made between these CBA mice and normotensive mice with regard to vascular responsiveness. The threshold for a vasoconstrictor response to AII, in the renal and hindlimb vasculature, was substantially lower

in the hypertensive mice than in the normotensive controls. In addition, the maximal pressor response to AII was greater in the hindquarters of hypertensive mice, but not in the renal vasculature when compared to control subjects. A similar response pattern was also observed for NE with regard to the hindquarter vasculature of hypertensive mice. However, renal vascular responsiveness was not different between the two groups. The authors conclude that this heightened vascular sensitivity to AII and NE in the resistance vessels serves to maintain the elevated arterial blood pressure in CBA mice during this stage of hypertension.

Henry, Stephens, and Santisteban (1975) have examined the development of myocardial and vascular pathology in CBA mice exposed to different periods of psychosocial stimulation. Male mice which had been isolated for 3.5 months were paired with female mice which had been group housed. As in previous studies, the animals were placed in a large interconnecting box system which allowed multiple social interactions. The mice were then randomly selected and terminated following periods of social interaction which ranged from 2 days to 9 months.

A significant relationship between the duration of the psychosocial stress and the severity of vascular and myocardial pathology was observed. Increases in arteriosclerotic lesions, heart weight to body weight ratios, and myocardial fibrosis were observed in animals exposed to a minimum of 6 months of psychosocial stress. The mice which were returned to isolation following 5 months of psychosocial stress showed a progressive reduction in systolic BP with no myocardial or vascular

damage. In contrast, animals exposed to 9 months of territorial conflict showed sustained hypertension, with associated vascular and myocardial pathology, despite being returned to an isolated environment.

Thus, it appears that chronic psychosocial stress in CBA mice produces a sustained hypertension which results in myocardial and vascular pathology. Augmented sympathetic-adrenal medullary activity and heightened vascular reactivity to AII play a critical role in the development of this form of stress-induced hypertension.

Sound Withdrawal Hypertension

Maintenance of adequate sensory input to the CNS has been shown to be extremely important in determining the emotional health of the organism (Bexton, Heron & Scott, 1954; Scott, Bexton, Heron & Doane, 1959). Generally, sensory isolation results in distortions of thought, hallucinations, and depression. However, it has been reported that removal of auditory input to physiologically predisposed rodents can result in the development of hypertension.

Lockett and Marwood (1973) have reported that chronic sound deprivation in inbred Wistar rats produces a gradual increase in mean arterial BP. Over successive generations the animals had been showing a progressive rise in BP, during chronic exposure to elevated ambient noise levels. Hence, these rats were accustomed to a great deal of auditory input. The females of the strain were transferred to the soundproof chambers at 11-12 weeks of age and were housed in pairs. The mean arterial pressure of these animals increased following 2 weeks of

isolation and reached a peak level at 4 weeks. The hypertension had stabilized by the 6th week of isolation with a mean arterial BP level of 180 mmHg.

It was found that both salt-maintained, adrenalectomized rats and hypophysectomized rats failed to develop the sound-deprivation hypertension. Sound withdrawal hypertension would develop in adrenalectomized rats given glucocorticoid supplements, but not in similarly treated hypophysectomized rats. Lockett and Marwood (1973) conclude that this form of hypertension is dependent upon an intact pituitary-adrenal cortical axis.

The integration of incoming sensory information has been shown to occur in various structures of the limbic system (Numan, 1978). Consequently, disruption of these connections should modify an organism's response to increased or reduced sensory input. In order to test this hypothesis, Marwood and Lockett (1973) placed an array of electrolytic lesions in the septal nuclei of female Wistar rats prior to sensory isolation. The lesions were made in three regions: (1) the hippocampal commissure (HC) and midline bed nucleus (MBN); (2) the anteromedial area of the LS; and (3) the MS. The lesioned animals and sham-operated controls were given 8 days of postoperative recovery, which was followed by 6 weeks of sound deprivation. Lesions of the HC, MBN, and MS produced hypertension independently of sensory isolation. This elevation in BP was comparable to the levels developed by sham-operated and unoperated control rats exposed to 6 weeks of sound deprivation. Lesions of the anteromedial LS also produced a moderate elevation in BP,

which was significantly greater than the BP of sham-operated and unoperated control rats maintained under normal sensory input. The authors suggest that the failure of lesioned animals to show an increased BP response to sensory deprivation indicates that these limbic structures play an important role in the development of sound withdrawal hypertension in the intact rat.

It is interesting to note that social or sensory isolation attenuates the development of hypertension in the SHR (Hallbäck, 1975; Lais, Bhatnagar & Brody, 1974). This antihypertensive effect is attributed to a reduction in sympathetic arousal and defensive cardiovascular responses. Thus, it appears that in the SHR a reduction in sensory input is prophylactic, while in normotensive Wistar rats (Bennett & Gardiner, 1979) and inbred borderline hypertensive Wistar rats, it leads to the development of moderate and severe hypertension, respectively.

These observations suggest that differences in sensory integration and/or neuroendocrine regulation in the SHR, compared to the two Wistar strains, may contribute to these divergent response patterns (Iams, McMurtry & Wexler, 1979; Sackler, Weltman, Pandhi & Vaidya, 1974).

Classical and Operant Conditioning Paradigms

Behavioral techniques employing classical (Pavlovian) or operant conditioning procedures have proven to be extremely useful in studying the cardiovascular responses of conscious animals to both appetitive and aversive stimuli (reinforcers). The hemodynamic responses elicited from a conscious animal under stimulus control can then be related to

analogous cardiovascular responses seen in the anesthetized animal during electrical stimulation of nerve trunks, ganglia, or nuclei in the spinal cord and brain. The combination of these behavioral and electrophysiological techniques allows more discrete analysis of neuroregulatory cardiovascular responses (Randall, 1977). Information obtained from these studies will ultimately contribute to the understanding of psychological stress and cardiovascular disease.

Randall and Smith (1974) examined the effects of emotional conditioning and exercise on cardiovascular responses in conscious, chair-restrained rhesus monkeys. The animals were conditioned to exercise when a discriminative stimulus (S^D) was presented. This behavior consisted of turning a wheel for 1 minute and was reinforced with a banana-flavored food pellet. Following the exercise response, aversive classical conditioning was employed to instigate a CER. A 1 minute tone was presented and served as the conditioned stimulus (CS). At the end of this period, an unavoidable electric shock was delivered which served as the unconditioned stimulus (UCS). The animals were then implanted with catheters and strain gauge arches to assess cardiovascular responses to these behavioral manipulations.

It was found that presentation of the CS elicited a sustained increase in both systolic and diastolic BP, left ventricular pressure, right ventricular contractile force, left ventricular contractile force, and left and right ventricular dP/dt . Heart rate increased with the presentation of the CS, but then returned to control levels by the last 20 seconds of the stimulus. The unconditioned response was characterized by a sudden increase in HR and decrease in arterial BP.

The cardiovascular response to exercise was similar to that of the CER. There was an immediate increase in HR, arterial BP, left ventricular pressure, right ventricular pressure, and myocardial contractility. Administration of propranolol, a β -adrenoreceptor antagonist, completely blocked the increase in HR and cardiac contractility observed during the behavioral tasks. These observations suggest that sympathetic activation, rather than withdrawal of vagal tone, plays a major role in regulating myocardial responses to both exercise and classical aversive conditioning in the nonhuman primate.

In another study, Randall, Cottrill, Todd, Price, and Wachtel (1982) examined changes in CO and blood flow distribution in conscious monkeys at rest and during aversive classical conditioning. Hemodynamic measurements were made using a dye-dilution technique with indocyanine green. It was found that the conditioned pressor response was the result of an increase in CO and a decrease in TPR. There was also a significant increase in HR to the CS. Blood flow increased to the muscles but decreased to the kidneys, liver, and other viscera during the aversive conditioning period.

Smith, Hohimer, Astley, and Taylor (1979) also examined changes in renal and hindlimb blood flow, as well as BP, HR, and oxygen consumption in conscious baboons during the performance of dynamic leg exercise and a CER. The animals were chair restrained on a daily basis and conditioned according to operant and classical techniques previously described (Randall and Smith, 1974). In addition, the baboons were conditioned to lever press in response to the presentation of a light

(S^D). The lever press behavior was maintained on a variable interval schedule, where the animal was reinforced once on an average of 15 seconds following the first response (VI 15). The baboons were then implanted with arterial and venous catheters, and electromagnetic flow probes around the left renal artery and lower abdominal aorta. Following a 3 week recovery period, cardiovascular measurements were made during each of the three behavioral conditions.

The onset of the CS produced a marked increase in HR, BP, and aortic blood flow, with an associated reduction in renal blood flow compared to the control lever press period. Hohimer and Smith (1979) observed a similar hemodynamic pattern during dynamic leg exercise in the baboon. A combination of surgical denervation of the kidney and pharmacological blockade with propranolol revealed that the initial drop in renal blood flow was mediated via the renal nerves, but the secondary increase in renal vascular resistance was the result of circulating epinephrine from the adrenal medulla.

Similar hemodynamic changes in renal and muscle blood flow have been observed following stimulation of the AH in conscious, chair-restrained monkeys (Forsyth, 1970). It has also been found that selective lesions of the hypothalamus eliminate or modify aversively motivated cardiovascular responses in the baboon (Smith et al., 1980; Smith, Stephenson, and Randall, 1974).

Thus, the integrity of the AH, medial portion of the LH, dorso-lateral region of the ventromedial hypothalamic nucleus (VMH), and fornix (F), are necessary for the elaboration of the cardiovascular component of the CER in the nonhuman primate.

Anderson and Yingling (1978) have observed that classically conditioned pressor responses in dogs result from an increase in CO with a slight reduction in TPR. The increased CO was a function of both an elevated HR and augmented SV. Obrist, Howard, Lawler, Sutterer, Smithson, and Martin (1972) have also noted that sympathetic influences on the heart during aversive classical conditioning in dogs are more clearly reflected by changes in contractility rather than HR. Thus, in the dog, conditioned cardiac acceleration is the result of a withdrawal of vagal tone (unlike the nonhuman primate).

The effects of aversive classical conditioning paradigms on cardiovascular responses in rodents have been limited to measurements of HR and BP (Hatton, Wilkin, Francisco, Hoffman, Buchholz & Fitzgerald, 1979; LeDoux, Sakaguchi & Reis, 1982). Consequently, the relative contributions of TPR and CO to the conditioned BP responses must be assessed indirectly using selective pharmacological blockade.

Hatton, Buchholz, and Fitzgerald (1981) examined the development of HR and BP responses to classical aversive conditioning in male SHR and WKY rats. The animals were catheterized in the lower abdominal aorta and given 3 days of postoperative recovery prior to the conditioning period. All animals were restrained in plexiglass enclosures and exposed to 10 trials in which the CS (tone) was presented without the UCS (electric shock). This procedure allowed the investigators to observe the orienting response (OR). During the following 30 trials, the CS and the UCS were paired together with an interstimulus interval of 10 seconds. The UCS overlapped with the last .5 seconds of the CS.

The OR for both the SHR and the WKY rats consisted of a HR deceleration with a concomitant increase in BP. Both of these response patterns were exaggerated in the SHR. The conditioned response (CR) in the SHR also consisted of a cardiac deceleration coupled with a larger pressor response. The WKY rats showed a smaller pressor response to the onset of the CS and a significant increase in HR. Pharmacological blockade with propranolol or atropine (parasympathetic muscarinic antagonist) revealed that the HR deceleration in the SHR was vagally mediated, while the HR acceleration in the WKY was the result of increased sympathetic drive to the heart. Phentolamine pretreatment in the SHR eliminated both the BP and HR responses to the CS. In the WKY, phentolamine produced a depressor response and reduced the BP response to the CS, but did not alter HR acceleration. The authors suggest that the effect of phentolamine on the HR response in the SHR was an indirect effect, mediated by a central withdrawal of vagal tone, and instigated by the drop in arterial BP. The results of this study suggest that the exaggerated pressor response seen in the SHR during presentation of the CS is the result of an enhanced sympathetic drive to the vasculature.

Both appetitive and aversively-reinforced operant conditioning techniques have been employed in experimental animals to produce increases in arterial BP (Grignolo, Light & Obrist, 1980; Goldstein, Harris, Izzo, Turkkan & Keiser, 1981). Unlike classical conditioning, operant techniques allow the organism some "control" over the environment in order to avoid an aversive stimulus, or receive a positive reinforcer.

Forsyth (1971) examined regional blood flow and BP responses in 5 rhesus monkeys during 72 hours of stressful Sidman avoidance. In this procedure the animal must learn a temporal discrimination, and is not provided with an overt signal to cue the onset of the aversive event. The monkeys in this study were maintained in a primate chair and conditioned to press a lever in order to avoid an electric shock to the tail. Each response reset a 20 second timer which, if allowed to complete the cycle before another response, would result in a tail shock (20 second response-shock interval).

The animals were given 4-7 days postoperative recovery following implantation of arterial and venous catheters. The monkeys were then conditioned from 3-5 days prior to the cardiovascular measurements. Arterial BP, CO, TPR, HR, and regional blood flow measurements were determined prior to, after 20 minutes, and 4, 24, and 72 hours following the onset of the avoidance period.

Arterial BP increased significantly during the avoidance period. The initial pressor response during the first 4 hours was the result of an elevated CO. There was an increase in HR with little change in SV. During the 24th hour, the elevated BP was due to an increase in TPR and CO. Finally, by the 72nd hour, the hypertension was completely due to an elevated TPR. During this period CO and HR returned to baseline levels. There was a significant decrease in renal blood flow during the first 20 minutes of the avoidance period, with a concurrent increase in blood flow to the heart, skeletal muscle, and liver. Blood flow to the kidney, skeletal muscle, and gastrointestinal system was significantly

reduced by 72 hours. However, there was an increase in blood flow to the heart, spleen, pancreas, and liver. This shift in hemodynamic pattern from a high CO to an elevated TPR hypertension has been observed in some individuals with borderline essential hypertension and implicates a stress response as a contributory factor (Eich et al., 1966; Julius, 1977).

Lawler, Obrist, and Lawler (1975) measured cardiovascular responses in 4 dogs exposed to 10 days of Sidman avoidance. Hemodynamic measurements were made 1 hour per day during preavoidance, avoidance, and postavoidance periods. A distinct cardiovascular pattern emerged in two reactive dogs, characterized by significant increases in arterial BP during preavoidance, which was the result of an elevated TPR. In contrast, the pressor responses during avoidance were maintained by an increased CO while TPR dropped. The change in CO during avoidance was the result of an increase in both HR and SV. There was also a dramatic increase in aortic dP/dt during the avoidance period, which reflected augmented sympathetic influences on the heart. The authors noted that these two reactive dogs were under excellent schedule control and avoided shock with a high rate of success. On the other hand, the two least reactive dogs were under poor experimental control. These observations strongly suggest that the psychological component of the paradigm, rather than the shock itself, was responsible for inducing hypertensive responses in the reactive animals.

In a series of studies, Anderson and Brady (1976) and Anderson, Yingling, and Brady (1976) attempted to dissect out the relative

contributions of α - and β -adrenergic influences on BP and HR in dogs prior to and during Sidman avoidance. It was found that α -adrenergic blockade with phenoxybenzamine produced a significant reduction in systolic BP, but not in diastolic BP, during the first hour of preavoidance. The drop in HR which was observed during preavoidance was unaffected by the phenoxybenzamine. In contrast, α -adrenergic blockade attenuated both the HR and BP increases observed during the performance of the avoidance behavior. Infusion of propranolol did not alter the increase in BP and decrease in HR seen during preavoidance, but did reduce the tachycardia response during Sidman avoidance. The β -adrenergic blockade also failed to alter the increase in BP seen during the avoidance period. Taken together, these results suggest that preavoidance changes in HR are the result of vagal influences, while HR increases during avoidance are the result of an increased sympathetic tone. In addition, Anderson et al. (1976) suggest that increased sympathetic drive to the heart and vasculature is most clearly observed during avoidance, rather than preavoidance periods.

Another operant conditioning technique which has proven useful in eliciting elevations in arterial BP and HR involves signalled avoidance, in conjunction with various schedules of reinforcement. Herd, Morse, Kelleher, and Jones (1969) have reported that squirrel monkeys conditioned to avoid electric shock on either a fixed interval or fixed ratio schedule show profound increases in mean arterial BP. The monkeys were implanted with arterial catheters and trained to press a key to turn off a light (S^D) which had previously been paired with electric shock.

Monkeys trained on the fixed ratio schedule were conditioned to a green light which signalled the onset of electric shock. Pressing the key 30 times (FR 30) turned off the green light, turned out the light in the experimental chamber, and eliminated the presentation of the aversive reinforcement for 1 minute. At the end of this period, the S^D was presented and the animal had the opportunity to respond again. In the fixed interval schedule, electric shock was delivered after a white light had been on for either 2 or 4 minutes (FI 2 or 4). The first key press made by the monkey following one of these minimum periods turned off the S^D and resulted in a 1 minute time out period, as previously described.

It was found that mean arterial BP increased over the conditioning period and by the 4th month was elevated before, during, and after each session. These persistent elevations in BP were seen in 4 of the 6 monkeys who were under rigid stimulus control and demonstrated efficient responding. Thus, the hypertensive responses observed in these animals increased over time as the number of electric shock presentations decreased.

Operant conditioning techniques have also employed reinforcement of a physiological response rather than a behavior (such as key pressing) in order to produce elevations in HR, BP, and TPR. Harris, Gilliam, Findley, and Brady (1973) have reported large increases in HR and arterial BP in baboons operantly conditioned to raise diastolic BP in order to avoid shock and obtain food. During the conditioning period animals were required to maintain diastolic BP above a criterion level

(100 mmHg) during a 12 hour conditioning session. It was found that during the 12 hour conditioning-off period HR and BP returned to basal levels within 6-8 hours. Thus, over the 6 month conditioning period, the animals became very efficient at avoiding shock and receiving food by elevating diastolic BP; however, no sustained BP effect was observed during the conditioning off period. Goldstein et al. (1981) have reported that these operantly conditioned elevations in HR and BP are associated with a significant increase in plasma NE and E. Consequently, it appears that enhanced sympathetic activity is responsible for the increased HR and BP seen during this operant conditioning procedure.

Anderson and Yingling (1979) have demonstrated that cardiovascular response patterns in dogs exposed to signalled avoidance can be modified using operant conditioning procedures. Chronically instrumented dogs were trained to press a response panel following the presentation of an auditory stimulus in order to avoid electric shock. Once the animals had learned the response and were under stimulus control, a second tone was presented whenever TPR fell below a criterion level. The tone would remain on for 30 seconds, followed by an electric shock, unless the animal increased TPR over the criterion level. This response would turn off the tone and result in shock avoidance. It was found that TPR increased in these dogs over sessions of 5-16 hours in duration. The response pattern was characterized by a decrease in HR, CO, respiratory rate, and behavioral activity. There was a concomitant increase in arterial BP and SV. However, as with the previous study of Harris

et al. (1973), these changes in TPR were tied to the conditioning session and produced no sustained changes in cardiovascular function or in arterial BP.

Thus, while behavioral techniques have proven effective at inducing acute elevations in arterial BP, their chronic effects in the etiology of essential hypertension remain unresolved.

Buchholz, Lawler, and Barker (1981) have suggested that classical and operant conditioning techniques, which allow the organism to "predict" or "control" the onset of aversive events, respectively, may not be sufficiently stressful to initiate a sustained hypertension. In order to test this hypothesis, the researchers employed a shock-shock (avoidance-avoidance) conflict paradigm which reduced the ability of experimental animals to control the presentation of an aversive stimulus. The conflict schedule required male Long-Evans rats to turn a wheel following the onset of a tone in order to avoid 5 electric shocks to the tail (avoidance procedure); however, this response then led to the presentation of 1 inescapable electric shock (conflict). Consequently, the experimental animals were punished for making the appropriate response, but avoided receiving a greater shock density. Experimental and yoked control¹ animals were exposed to this paradigm for 8 consecutive weeks. Indirect measurements of systolic BP using tail plethysmography were performed each week for experimental, yoked control, and nonshocked restraint control animals.

¹ A yoked control was paired with a specific experimental animal and received the same pattern of tail shocks, but had no control over their presentation.

The results of this study demonstrated that the shock-shock conflict paradigm was indeed more stressful than avoidance alone; however, the degree of hypertension produced in the experimental and yoked control animals exposed to conflict only approached borderline hypertensive levels (150 mmHg).

Lawler, Barker, Hubbard, and Allen (1980) have suggested that normal animals may be "genetically protected" from the deleterious effects of psychological stress, and that a physiologically-predisposed animal may serve as a better model for the study of stress-induced hypertension. In an experiment designed to test this hypothesis, these authors (1980) examined the effects of 12 weeks of chronic shock-shock conflict on the development of hypertension in the first filial (F_1) generation of female SHR and male WKY rats. It was hoped that this genetic cross might yield offspring with an inherent susceptibility to develop hypertension, but without spontaneously developing pathological hypertension. The systolic BP of maturational control animals remained stable throughout the 12 week period at approximately 150 mmHg. In contrast, restraint control and experimental animals showed a significant increase in systolic BP, over the conflict period, to 165 mmHg and 185 mmHg, respectively. The elevation in systolic BP for the experimental group persisted for 10 weeks following the termination of the conflict schedule, and in all likelihood reflected a sustained hypertension. In contrast, the systolic BP of the restraint control animals returned to borderline hypertensive levels and was not different from the maturational control group. In addition, the stress-induced

hypertension seen in experimental animals instigated severe pathological changes in the myocardium, such as pressure-induced myocardial hypertrophy, myofibrillar degeneration, and fibrosis.

Friedman and Dahl (1975) and Friedman and Iwai (1976, 1977) have reported that Dahl salt-sensitive (S) rats, which develop sustained hypertension when given a high salt diet (4%-8%), also develop hypertension in response to a chronic approach-avoidance conflict schedule. The Dahl salt-resistant (R) rats show no such sensitivity to either salt or psychological stress.

The stress-induced hypertension developed by S rats fed a normal salt diet was rapid in onset, declined gradually over the stress period, and failed to result in sustained hypertension (Friedman & Dahl, 1975; Friedman & Iwai, 1976). A combination of a moderate salt diet (2%) and psychological stress has been shown to exacerbate this hypertensive response in the S rats; however, the pressures obtained were still significantly less than S rats fed a high salt diet. Hence, while the S rat shows a definite sensitivity to salt, its response to psychological stress is less intense and only results in a transient hypertension.

In contrast, the borderline hypertensive rat (BHR) utilized by Lawler et al. (1980) displays a definite predisposition to develop stress-induced hypertension which is slow in onset and results in myocardial pathology. Thus, the BHR would seem to be an excellent animal model for studying the role of genetics and its interaction with psychological stress in the etiology of essential hypertension,

especially in view of the recent findings of Hastrup, Light, and Obrist (1982). This study indicated that the male offspring of hypertensive parents show greater HR and systolic BP responses to psychological stress than the offspring of normotensive individuals, an analogous condition to that seen in the BHR (Lawler, Barker, Hubbard, Cox & Randall, 1984).

Statement of Rationale and Purpose

Psychological stress has been shown to instigate a cardiovascular response pattern by the organism in preparation for the aversive event. This defense response originates from several nuclei within the hypothalamus and limbic forebrain, and leads to an increased sympathetic drive to the heart and vasculature. It has been suggested that repeated activation of this defense response may lead to the onset of essential hypertension.

Studies examining the effects of environmental stress on cardiovascular and sympathetic-adrenal medullary responses in the SHR have demonstrated that this animal displays an exaggerated defense response compared to normotensive WKY rats. In addition, abnormal catecholamine content and metabolism in the hypothalamus of the SHR has been implicated as the central locus of this heightened sympathetic response pattern. However, despite its sympathetic reactivity, the SHR is of limited utility to the behavioral scientist because of its early and progressive development of hypertension, regardless of environmental input.

Consequently, the ideal animal model for the study of stress and its role in the development of hypertension would be an animal with a genetic predisposition to show exaggerated defense responses to environmental perturbations. For this reason, experiments were performed to examine the effects of psychological stress on sympathetic and hemodynamic responses in BHR and WKY rats.

The present study utilized an aversive classical conditioning paradigm to activate the sympathetic nervous system of BHR and WKY rats chronically instrumented with i.v.t. cannulae, arterial catheters, and electromagnetic flow probes around the ascending aorta. This instrumentation allowed assessment of hemodynamic variables such as systolic BP, diastolic BP, mean BP, CO, CI, TPR, TPR index (TPRI), SV, SI, SW, MW, maximum aortic dP/dt, and maximum aortic dP/dt/P at rest and during behavioral stress. Intracerebroventricular infusion of the central α_2 -adrenoreceptor agonist clonidine provided information regarding the role of these receptors in mediating cardiovascular function during the two behavioral states.

In addition, blood samples were drawn from the arterial catheters during all conditions, and assayed for plasma NE and E concentrations, as an index of sympathetic adrenal-medullary activity.

The following specific questions were of interest in this study:

1. Does an elevated TPR or CO contribute to the basal differences in arterial BP between BHR and WKY rats?
2. Do the BHR have higher resting plasma catecholamine levels than do the WKY rats?

3. Do the hemodynamic responses of BHR rats at rest and during aversive classical conditioning resemble those of WKY rats?
4. Do the BHR show a greater sympathetic adrenal-medullary reactivity to behavioral stress than do WKY rats?
5. Does i.v.t. clonidine reduce BP and alter hemodynamic patterns at rest to the same degree in BHR and WKY rats?
6. What effect does i.v.t. clonidine have on resting plasma levels of NE and E in these two strains?
7. Do BHR and WKY rats given clonidine show similar sympathetic adrenal-medullary and hemodynamic responses to aversive classical conditioning?

CHAPTER II

MATERIALS AND METHODS

Subjects

Eighteen male BHR and 12 male WKY rats 18-28 weeks of age were used in this study. The inbred parent strains (6 female SHR, 6 female WKY, and 6 male WKY rats) were obtained from Taconic Farms, Germantown, New York. Prior to breeding, indirect measurements of systolic BP, using tail plethysmography, were obtained on the parents to insure that the SHR were hypertensive and the WKY were normotensive. The female SHR and WKY rats were paired together 4 weeks prior to breeding to synchronize their estrous cycles. Two separate breedings were executed 4 weeks apart, utilizing half of the animals in each breeding. Each pair of females shared a common male WKY rat for 2 weeks. At the end of this period, the female rats of each strain were individually housed and the males were group housed 3 to a cage.

All BHR and WKY pups were born within 2 days of each other on the first breeding, and within 4 days of each other on the second breeding. At 4-5 weeks of age, all male pups were weaned, and the female pups were sacrificed. A total of 30 male pups, 18 BHR and 12 WKY were randomly selected from these litters. The animals were housed according to strain, 3 in each cage, until they were 9 weeks of age. At this point the animals were individually housed until they were used in the study.

Apparatus

Home Cages

The rats were housed individually in standard plastic laboratory cages under a 12-hour light/dark cycle, and were maintained on laboratory rat chow and tap water ad libitum.

Conditioning Cages

During conditioning and recording periods, each subject was placed in a plexiglass box 7.0 cm × 28.0 cm × 8.5 cm in size. A wheel 7.6 cm in diameter, consisting of two .6 cm thick plexiglass discs attached at the perimeter by 12 equally spaced 5.1 cm × .5 cm stainless steel bars, used during operant conditioning, was rendered immobile during the classical conditioning paradigm. The subject's tail protruded through a 1.2 cm wide slot in the rear of the box. The animal was then restrained by slipping a .9 cm i.d. × 1.9 cm o.d. wooden disc over the tail and securing it with tape. This prevented the animal from rotating around in the box and biting the two shock electrodes on its tail. The top of each box was also padded with 1.5 cm thick foam rubber to protect the animal's head and i.v.t. cannula from impact during the presentation of the aversive UCS (shock).

Two of these boxes were kept in a sound attenuating chamber, .3 m × .8 m × .25 m in size, which contained a house light, speaker, and ventilation fan. Four of these chambers were located in a 2 m × 3 m × 2.5 m experimental room.

Programming Instrumentation

A KIM-1 microprocessor (MOS Technology) with custom interfacing (Murray & Lawler, 1978) and software (Barker, unpublished) was used to control the onset and duration of tones (CS) and shocks (UCS) for the classical conditioning paradigm. This computer system was located in a small room across from the experimental room.

Cardiovascular Monitoring Equipment

Indirect measurements of systolic BP, using tail plethysmography, were taken on the parents of experimental animals prior to breeding, and on the experimental animals at 13-14 weeks of age and 2 days following i.v.t. cannula surgery.

Animals were placed in Narco Bio-Systems plexiglass restraints and warmed for approximately 10 minutes to insure adequate dilation of the tail artery. The surface temperature of heating plates, located beneath these restraints, was monitored using a telethermometer (Model 43TD, Yellow Springs Instruments) and maintained at 37°C by a regulated heating unit. The rat's tail was slipped through a 34 mm (length) × 13 mm (i.d.) tail cuff and attached to a Narco pneumatic pulse transducer. This signal was then fed into a Narco Bio-Systems electro-sphygmograph coupler (Model 7211) and amplifier (Model 7070) housed in a four channel Narco physiograph (Model PMP-4A).

Direct measurements of systolic BP, diastolic BP, and HR were obtained from each chronically instrumented rat by an arterial catheter attached to a Narco Bio-Systems RP 1500 pressure transducer. The transducer was connected to a Grass low-level D.C. preamplifier (Model

7P1F) and driver amplifier (Model 7DAF) in a Grass 8 channel polygraph (Model 7D). Heart rate was obtained from the pulsatile arterial BP signal via a Grass tachograph preamplifier (Model 7P4F) and driver amplifier (Model 7DAF).

A Carolina Medical Electronic square-wave electromagnetic flow meter (Model 501) in conjunction with an EP 107.5 flow probe was used to measure ascending aortic flow in each rat. The signal from the flow meter was fed into a Grass driver amplifier (Model 7DAF) in order to chart pulsatile flow on the polygraph. The output signal from the driver was fed into a Grass integrator (Model 7P10B) to obtain beat-by-beat values of SV.

The arterial BP, blood flow, and SV signals were also fed into a four channel Burr-Brown analogue to digital converter and SYM-1 microprocessor system (Synertekh Systems Corp.). Custom software developed in this laboratory (Lawler, unpublished) allowed second-by-second determinations of systolic, diastolic, and mean BP, maximum aortic dP/dt , maximum aortic $dP/dt/P$, HR, CO, SV, TPR, CI, and SW. The computer sampled the three input variables 14 seconds prior to and 14 seconds during the presentation of the CS, for 20 trials, over a 30 minute period. In addition, TPRI, SI, and MW were also calculated from values obtained by the microprocessor. At the end of a recording session the data was displayed on a MIME-I CRT terminal (Micro Term, Inc.) and then output to hard copy by an Integral Data Systems printer (Model IDS-440).

Procedure

General Description

Prior to the conditioning phase of the study, all animals were implanted with a single 22 gauge guide cannula in the anterior ventral portion of the third ventricle. Following 2 days of surgical recovery, the rats were conditioned for 5 consecutive days on a classical conditioning paradigm. On the 6th day the animals were implanted with either an arterial catheter (protocol 1) or electromagnetic flow probe (protocol 2) around the ascending aorta, and then given 3 or 4 days of postoperative recovery, respectively. Surgery was performed again on these subjects, either to implant an arterial catheter (for those animals which had previously received a probe), or to implant an electromagnetic flow probe (for those animals which had previously received a catheter). The subjects were then given an additional 3 days of recovery following the catheter surgery, or 4 days of recovery following the probe surgery.

Hemodynamic data and blood samples were then collected from these subjects at rest and during aversive classical conditioning, either with or without clonidine, during the following 2-3 days. At the end of this period, the animals were terminated, the probes removed, and the cannula placement verified.

Table 3 summarizes the information presented in the preceding paragraphs.

Table 3
Research Protocols

Strains ^a	Days													
	M	T	W	Th	F	S	Su	M	T	W	Th	F	S	Su
1. BHR WKY	S _I	-	C	C	C	C	C	S _C	-	-	S _P	-	-	-
				S _I	-	C	C	C	C	C	S _C	-	-	S _P
2. BHR WKY	S _I	-	C	C	C	C	C	S _P	-	-	S _C	-	-	-
			S _I	-	C	C	C	C	C	S _P	-	-	-	S _C

^aN = 2 for each strain
S_I = Intraventricular surgery
- = Recovery
C = Conditioning
S_C = Catheter surgery
S_P = Flow probe surgery
R = Recording

Intraventricular Cannula Surgery

Two animals were selected from the subject pool and food deprived for 24 hours. Each surgery lasted approximately 1 hour and consisted of the following protocol.

The animal was anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg, i.p.) and its head was mounted in a stereotaxic instrument (David Kopf). The scalp was incised 2.5 cm along the midline exposing the periosteum. The connective tissue and muscle were scraped from the skull and retracted laterally until bregma was clearly visible. A .5 cm square bone flap was then made over bregma and carefully removed to expose the midsagittal sinus. Three holes were trephined around this opening for the placement of bone screws used to secure the cannula. The dura around the sinus was carefully dissected so that the sinus could be retracted. A 22 gauge guide cannula (Plastic Products Co.) was then lowered 8.5 mm below the dura and .2 mm anterior to bregma (or 6.0 mm anterior to the interaural line) according to the coordinates of Pellegrino, Pellegrino, and Cushman (1979). The opening in the skull was filled with a small piece of gel foam and sealed with bone wax. Furacin topical antibiotic was applied to the incision. The guide cannula was then anchored to the skull with bone screws and cemented into place with cranioplastic cement (Plastic Products Co.). A 28 gauge obturator was inserted into the opening of the guide cannula and extended 1 mm beyond the tip of the guide. The animal was then given an intramuscular (i.m.) injection of 60,000 units of procaine penicillin (300,000 units/cc), returned to its home cage, and given 2 days of postoperative recovery prior to conditioning.

On the day of conditioning, indirect measurements of systolic BP were made on all animals which had previously received i.v.t. cannulae. This procedure was performed on the subjects prior to their exposure to the aversive conditioning paradigm. The systolic BP values obtained during this period were compared to those obtained when the rats were 13-14 weeks old. No significant changes in systolic BP were observed as a result of this surgical procedure.

Classical Conditioning

The animals were exposed to the aversive classical conditioning paradigm 1 hour per day for 5 consecutive days. The subjects were placed in the experimental boxes and shock electrodes were attached to the tail of each animal. The door to the sound attenuated chamber was closed, and the fans and house lights were turned on. An 87 db white noise was also turned on in the experimental room to mask extraneous sounds generated in the laboratory. The program was then initiated to begin the aversive classical conditioning paradigm.

A 2000 Hz, 75 db tone (CS) was presented 14 seconds before (and during) a .3-.4 mA, 1 second duration electric shock (UCS) was administered to the tail of each subject. The CS and UCS ended simultaneously. A fixed 75 second intertrial interval was used before the presentation of the next CS. Consequently, 40 trials were presented over the 1 hour conditioning period. At the end of the session, animals were removed from the experimental boxes and returned to their home cages.

This procedure was repeated on each of the 5 conditioning days. At the end of the 5th conditioning day, the subjects were food deprived for surgery on the following day.

Catheter and Flow Probe Surgery

During the course of the experiment two surgical protocols were employed. The first involved implantation of an arterial catheter in the abdominal aorta, followed 3 days later by a thoracotomy and implantation of an electromagnetic flow probe around the ascending aorta. The second protocol employed the thoracotomy first, which was followed 4 days later by the arterial catheterization. Regardless of the protocol used, all subjects received 7 days of postoperative recovery (a minimum of 4 days following the thoracotomy and 3 days following the laparotomy) prior to obtaining the hemodynamic data.

The first surgical schedule was staggered so that laparotomies could be performed on one set of animals, while another set of subjects received thoracotomies (see Table 3). The following aseptic surgical procedures were used on each animal in the study.

Catheterization of the lower abdominal aorta was accomplished using a laparotomy procedure. The rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a 4-4.5 cm incision was made along the midline of the abdominal cavity. The viscera were retracted laterally and blunt dissection techniques were used to separate the connective tissue between the left renal artery and vein, and the left ilio-lumbar artery and vein. The aorta was carefully dissected away from the

inferior vena cava, and an 8 cm 3-0 silk ligature was passed beneath it. A heparinized catheter constructed of 20 cm of PE 60 tubing (pulled down at the tip) joined with a silastic sleeve to a 2 cm section of teflon tubing (.38 mm i.d. $\times$.53 mm o.d.) was passed through the left body wall and tunneled subcutaneously to exit the back of the neck. The catheter was then attached to a 3 cc syringe and filled with heparinized saline (330 units/cc).

After inserting a 27 gauge needle into the aorta, a cotton tipped applicator was applied to the puncture until clotting occurred. The teflon tip of the catheter was then passed through the clot and advanced upstream to a level just below the renal arteries. Brief ligation with the 3-0 ligature was only implemented when hemostasis failed to occur at the site of the puncture. The catheter, secured to the psoas major muscle with two 3-0 ligatures, was externalized, clamped with hemostats, and plugged with a 20 gauge stainless steel obturator. The retractors were then removed and the viscera were returned to their original position within the body cavity. The muscle wall of the abdomen and skin were closed with 3-0 silk, and the catheter was anchored to the back of the neck with additional 3-0 ligatures. The animal was given an injection (i.m.) of 60,000 units of procaine penicillin (300,000 units/cc), slipped into a custom made rat jacket (see Figure 1), and returned to its home cage for a 3 day recovery period. The catheters were flushed daily with .25 ml of heparinized saline (330 units/cc) to maintain patency.

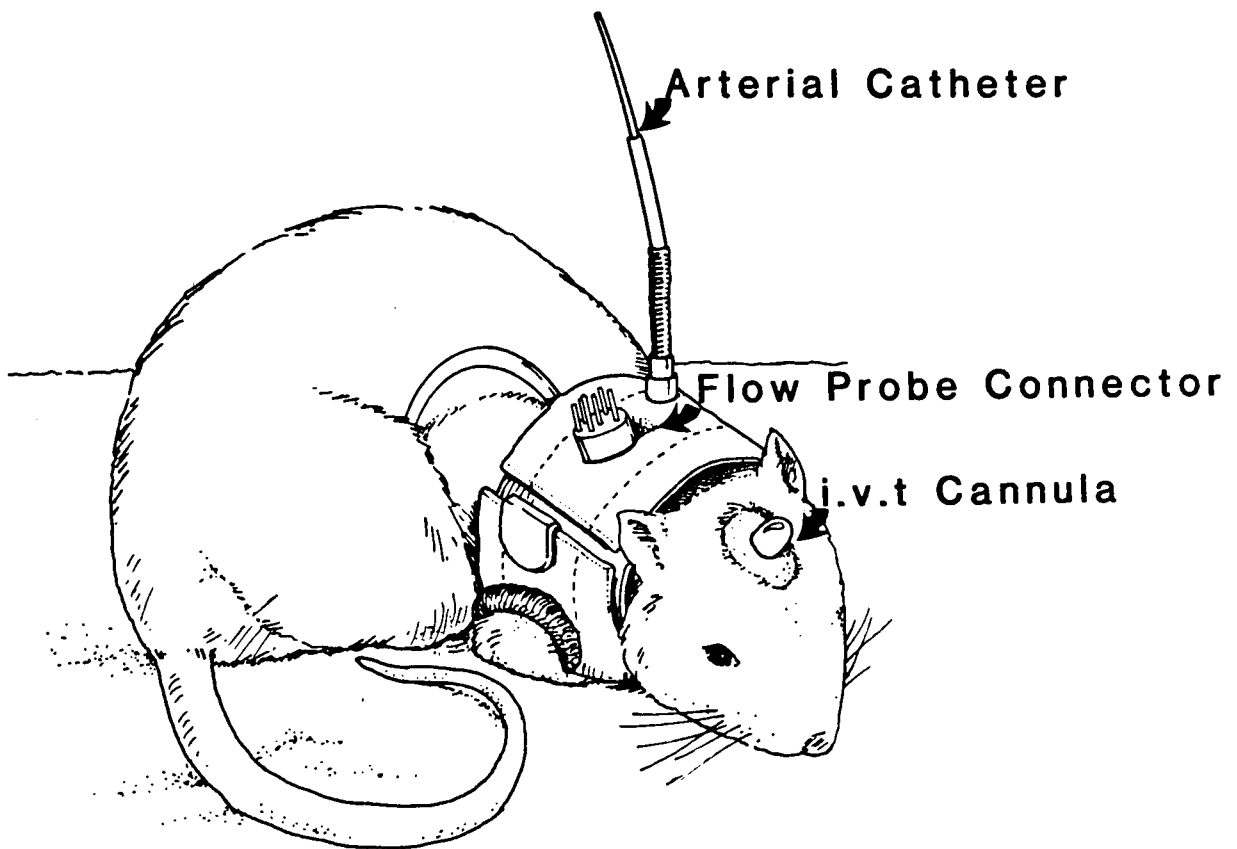


Figure 1. An instrumented rat in a custom jacket which prevented access to the arterial catheter, flow probe cable, and connector.

The electromagnetic flow probe (Carolina Medical Electronics, EP 100 series) was implanted according to the technique developed by Smith and Hutchins (1979, 1980), using probes were precalibrated in vitro and sterilized with ethylene oxide. The subject was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and intubated with PE 240 tubing. A 2.5-3.0 cm incision was made over the right third intercostal space, laterally from the sternum to the mid-axillary region, and the thoracic and associated ventral lateral muscle groups were separated and cut until the third right intercostal space was visualized.

The animal was attached to the respirator (Harvard Apparatus) and ventilated 50 times per minute on a 1:1 inspiratory to expiratory ratio with a pressure of 20-25 cmH₂O. The pleura was then punctured and the chest opened using lacrimal sac retractors. The right lung was packed with neurosurgical gauze soaked in normal saline (.87%) and four 3-0 ligatures were passed around the third and fourth ribs.

After the pericardium was opened and the aorta was carefully dissected free from the surrounding tissues above the curvature of the pulmonary trunk, two 3-0 ligatures were then looped under the ascending aorta. The aorta was carefully lifted and a 7.5 mm luminal circumference electromagnetic flow probe was placed around it 3-4 mm distal to the heart. A silastic key was then inserted into a slot in the probe to secure it around the aorta, the two ligatures were removed along with the neurosurgical gauze holding the right lung, and a 20 gauge needle, attached to an aspirator, was then carefully introduced into the thoracic cavity. The ribs were closed and tied to the flow

probe cable. Negative pressure was applied (12 inches of vacuum) to remove excess fluid and aid in the reinflation of the lungs. The thoracic muscles were then closed with 5-0 silk. The skin incision was closed with 3-0 silk and the flow probe cable and connector were externalized behind the right forelimb and secured by the rat jacket with a strip of velcro (see Figure 1). A ground wire was also passed subcutaneously at the level of the left pectoral muscle and attached to the spring guide of the rat jacket. The jacket was then fastened around the rat. The animal was given a 60,000 unit i.m. injection of procaine penicillin (300,000 units/cc) and returned to its home cage.

During postoperative recovery, the diet was supplemented with a mash made from rat chow, maple syrup, raisins, and nuts. This mixture stimulated the animal's appetite and improved recovery.

Hemodynamic Recording

Baseline period. Basal hemodynamic measurements were obtained from conscious, freely moving rats in a plexiglass cage 20.0 cm × 23.0 cm × 18.0 cm in size (similar to the home cage). This recording cage was kept in a small room (1.2 m × 2.5 m × 2.5 m) adjacent to the cardiovascular monitoring equipment. A small one-way mirror on one wall of the room allowed behavioral observations to be made during the 30 minute baseline recording period.

On the day of recording, the subject was weighed and a hematocrit was obtained to insure that the animal was not anemic. These values generally ranged from 30-45. Transfusions from donor rats of each

strain were given as needed. The rat was then transferred to the recording cage and allowed to habituate to the new environment, while the equipment was calibrated.

The RP 1500 pressure transducer was suspended over the recording cage and calibrated with a mercury column at 0, 100, and 200 mmHg. A Beckman voltmeter (Model HD100) was used to monitor the voltage equivalent for each of these pressure values. Adjustments for the baseline (0 mmHg) and sensitivity were made with the balance and gain controls of the preamplifier, respectively. The calibration signal was also fed into the analogue to digital converter and microprocessor system. A calibration program (Lawler, unpublished) provided a hexadecimal output for channel 1 of 0-1, 63-65, and C8-CA for each pressure value of 0, 100, and 200 mmHg, respectively. These values were displayed on the CRT and provided a very accurate calibration of ± 1 mmHg for the blood pressure channel. A 30 cm section of PE 90 tubing was then filled with heparinized saline (300 units/cc), attached to the pressure transducer and submerged in a beaker of water at the rat's midchest level. The balance control was used to adjust the baseline voltage so that the output of the CRT displayed 0-1 for 0 mmHg. This offset procedure was necessary to take into account the difference between the height of the transducer over the cage and the position of the rat's heart.

Following this calibration procedure for the measurement of arterial BP, the subject's catheter was attached to a Spalding Medical rodent swivel (Model 212) and then to the pressure transducer. A

flexible cable from the swivel was also attached to the flow probe connector on the rat's back (see Figure 1). This system allowed the subject free movement about the recording cage. In addition, a 28 gauge injector cannula, attached to a 2000 μ l glass syringe by a 45 cm section of PE 50 tubing, was inserted into the guide cannula on the rat's head. The syringe either contained normal saline or clonidine (2 μ g/ μ l) and was mounted into a Harvard Apparatus infusion pump (Model 940). The subject was then allowed to rest quietly while the flow meter and physiograph were calibrated.

The pulsatile aortic blood flow signal was fed into channel 2 of the analogue to digital converter. The baseline was adjusted with the zero control on the meter so that a hexadecimal output of 39 was seen on the CRT. This value corresponded to 0 flow. The integrator was then balanced for a 0 flow signal. A 30 ml/minute flow signal was dialed in with the zero adjustment on the flow meter and integrated over a 1 second period. Two sets of values were then displayed on the CRT. The first values (4D) represented the baseline for 0 flow. The second values (59-5A) represented the average of 10 integrations which was equivalent to a SV of .500 ml. The flow meter was then readjusted to 0 flow. The probe factor was set to a specific value for each animal, based on the probe calibration data and the hematocrit of the animal. The meter was then nulled and balanced prior to the beginning of the recording period.

The data acquisition program used by the SYM-1 microprocessor was then initiated. This program was the same one previously discussed (see

Cardiovascular Monitoring Equipment), except that no distinction was made between the tone-off and tone-on periods, since no CS or UCS was presented during the baseline hemodynamic measurements. Thus, baseline data were obtained for 28 seconds during every 90 second interval, for a total of 30 minutes. Values obtained during the first 7 intervals represented a preinfusion baseline. At the end of the 7th interval, the infusion pump was started and run for 45 seconds. The pump had been precalibrated to deliver a 4 μ l volume over this period of time. The total dose of clonidine delivered was 8 μ g. Intervals 8-20 were considered to be the postinfusion period. At the end of the 20th interval (30th minute) a .5 ml blood sample was rapidly obtained via the arterial catheter and placed in an ice cold disposable polypropylene microcentrifuge tube containing 2.7 mg of EGTA and 1.7 mg of glutathione. The sample was spun down at 4°C and an aliquot of plasma was removed for radioenzymatic assay of catecholamines. The subject was then disconnected from the swivel and infusion pump, transferred to the conditioning box, and the data were retrieved from the computer and output to hard copy. Care was taken to insure that each animal was transferred to the conditioning box and the stress session begun within 1 hour of the respective infusion.

Stress period. The subject was placed in the conditioning box and secured as previously described (see Conditioning Cages and Classical Conditioning). The catheter and flow probe connector were accessible through a hole in the top of the box. The pressure transducer was mounted on the side of the box, at heart level, and allowed to stabilize

before the onset of the stress session. The flow probe connector was attached to a flexible cable from the flow meter in the adjacent room. All calibration procedures were the same as those conducted for the baseline period. The aversive classical conditioning paradigm was presented by the KIM-1, but was under the control of the SYM-1 micro-processor. The stress session was 30 minutes in duration, consisting of 20 trials, each lasting 90 seconds. All of the time intervals and intensity parameters for the CS and UCS were the same as those used during earlier aversive classical conditioning. At the end of the stress period, a second .5 ml blood sample was drawn from the arterial catheter and treated as previously described.

Catecholamine Assay

The technique utilized for the determination of plasma catecholamine levels employed a single isotope radioenzymatic-thin layer chromatographic (TLC) procedure (Cryer, Santiago, & Shah, 1974; Passon & Peuler, 1973) modified by Upjohn diagnostics (Kalamazoo, MI). The catecholamines were converted to their O-methylated derivatives by incubating the samples at 37°C for 60 minutes with [³H]-S-adenosyl-L-methionine (New England Nuclear, Boston, MA) and catechol-O-methyl transferase (extracted from rat liver using the procedure developed by Axelrod and Tomchick, 1958). The reaction was terminated at the end of this period by placing the samples in ice water and adding 50 µl of 1 M borate buffer, pH 11.1, containing 0.2 µmole of normetanephrine and metanephrine, to each sample tube. The O-methylated tritium labeled products were extracted with 2 ml of toluene-isoamyl alcohol (3:2, v/v)

taken up into 100 μ l of 0.1 N acetic acid, and washed a second time with 1 ml of toluene-isoamyl alcohol (3:2, v/v). The tritiated metanephrines and normetanephrines, corresponding to E and NE, respectively, were separated by TLC. The solvent system for this procedure consisted of tert-amyl alcohol, benzene, and methylamine (6:2:3, v/v). The areas on the silica plate corresponding to [^{3} H]-metanephrine and [^{3} H]-normetanephrine were visualized under ultraviolet light, scraped into 20 ml scintillation vials, and oxidized to [^{3} H]-vanillin by the addition of NaIO_4 . The samples were then radioassayed using liquid scintillation spectrometry. The intraassay coefficient of variation for E and NE was 5%.

Termination of Subjects

At the end of the last recording day for each run, the subject received a 4 μ l infusion of Evans Blue into the i.v.t. cannula, to verify the placement of the cannula within the third ventricle, which was followed by a lethal injection of sodium pentobarbital. The animal's chest was then opened along the sternum. Care was taken to avoid cutting the probe. When the chest was opened, the flow probe was carefully removed from the aorta, rinsed with distilled water, and allowed to dry. The animal was then perfused intracardially with normal saline, followed by 10% buffered formalin. The rat was decapitated and the brain was removed for examination. A midsagittal cut was made in the brain, exposing the ventricular system and the relative distribution of the Evans Blue. The pattern and area covered by the stain was noted and recorded for each subject. Figure 2 depicts a typical diffusion pattern of the Evans Blue localized in the ventral portion of the third ventricle and hypothalamus.

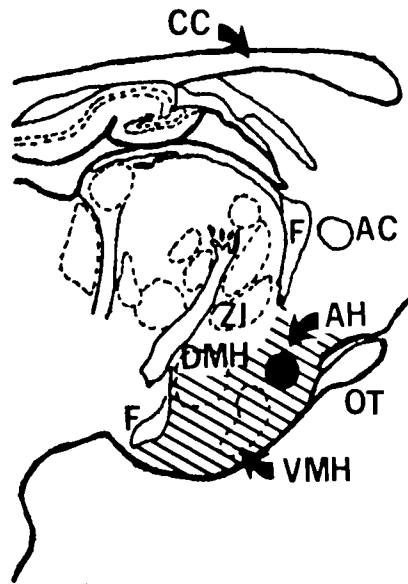


Figure 2. Midsagittal section through a rat brain according to the atlas of Pellegrino et al. (1979) indicating the tip of the i.v.t. cannula ●, and the diffusional pattern of a 4 μ l infusion of Evans Blue . CC, corpus callosum; F, fornix, AC, anterior commissure; ZI, zona incerta; AH, anterior hypothalamus; DMH, dorsomedial hypothalamic nucleus; OT, optic tract; VMH, ventromedial hypothalamic nucleus.

CHAPTER III

RESULTS

The data reported in this chapter were obtained from 9 male BHR and 8 male WKY rats 18-28 weeks of age. The remaining 13 animals were not used due to incomplete data, which was the result of equipment malfunction or the premature death of the animal. The most common cause of death for these rats was rupture of the ascending aorta. The animals on which baseline and stress (aversive classical conditioning) data were obtained, either with or without clonidine, were in good health and showed normal activity levels.

The hemodynamic data obtained during the pre-CS and CS periods were collapsed across trials to yield a single mean for each of the time intervals. In addition, the 14 second pre-CS period was reduced to a single mean and the 14 second CS period was collapsed into 2 second blocks. The data are presented as means $\pm$ S.E.M. and a $p < 0.05$ was chosen as the level for statistical significance.

The first section of the results will compare the hemodynamic and sympathetic-adrenal medullary responses of BHR and WKY rats during baseline and stress periods. The second section will then examine the depressor effects of clonidine in both BHR and WKY rats at rest and during exposure to the aversive classical conditioning paradigm.

Cardiovascular and Sympathetic Response Patterns
at Rest and During Stress

Table 4 presents baseline cardiovascular data for the BHR and WKY rats obtained while the animals were resting quietly, during the first

Table 4
Baseline Cardiovascular Data for BHR and WKY Rats

Variable	BHR ^a	WKY ^a
Body weight (g)	350 ± 9	355 ± 14
Hematocrit	40 ± 1	41 ± 2
Systolic BP (mmHg)	155 ± 4 ^b	135 ± 3
Diastolic BP (mmHg)	103 ± 2 ^b	88 ± 2
Mean BP (mmHg)	120 ± 3 ^b	103 ± 2
HR (bpm)	390 ± 13	381 ± 10
CO (ml/min)	100 ± 5	105 ± 6
SV (ml)	.261 ± .019	.278 ± .022
TPR (mmHg·min/ml)	1.22 ± .07 ^b	1.01 ± .07
CI (ml/min·100 g)	28.5 ± 0.9	29.5 ± 1.0
SI (ml/100 g)	.074 ± .004	.078 ± .004
TPRI (mmHg·min·100 g/ml)	4.25 ± .16 ^b	3.54 ± .16
SW (ml·mmHg)	31.5 ± 2.6	28.6 ± 2.0
MW (mmHg·ml/min)	12,085 ± 715	10,760 ± 489
Aortic dP/dt (mmHg/sec)	2,549 ± 278 (N = 7)	2,333 ± 279 (N = 5)
Aortic dP/dt/P (sec ⁻¹)	20.9 ± 2.3 (N = 7)	23.3 ± 3.0 (N = 5)

^aN = 8 per strain unless otherwise specified.

^bBHR and WKY rats differ at $p < 0.05$.

10-11 minutes of the home cage recording period. The BHR had a significantly higher resting systolic [$t(14) = 4.42$], diastolic [$t(14) = 4.77$], and mean BP [$t(14) = 4.79$], compared to WKY rats. This elevated blood pressure in the BHR was the result of higher vascular resistance, as indicated by TPR [$t(14) = 2.16$], and TPRI [$t(14) = 3.07$]. All other resting cardiovascular variables, weight, and hematocrit were not significantly different between the BHR and WKY rats.

Figures 3 and 4 provide sample physiograph data obtained during the baseline and aversive classical conditioning periods, for a BHR and WKY rat, respectively. The conditioned cardiovascular responses depicted in these figures consisted of an increase in HR, a biphasic increase in arterial BP, and a decrease in SV.

Figure 5 shows the increase in systolic and diastolic BP for BHR and WKY rats from baseline to the pre-CS and CS periods. A two way analysis of variance for systolic BP revealed a main effect of strain [$F(1/14) = 29.29$], time [$F(8/112) = 78.51$], and a strain by time interaction [$F(8/112) = 8.42$]. A similar trend was also seen for diastolic BP, with a main effect of strain [$F(1/14) = 25.21$], time [$F(8/112) = 67.70$], and a strain by time interaction [$F(8/112) = 2.47$]. These interactions, depicted in Figure 5 show a greater pressor reactivity for the BHR compared to the WKY rats from baseline to the stress period.

Subsequent Tukey (HSD) post-hoc comparisons made across time revealed that the BHR showed a significant increase in systolic and diastolic BP from the baseline to the pre-CS period, while WKY only showed a significant increase in diastolic BP across this time. The BHR

BHR

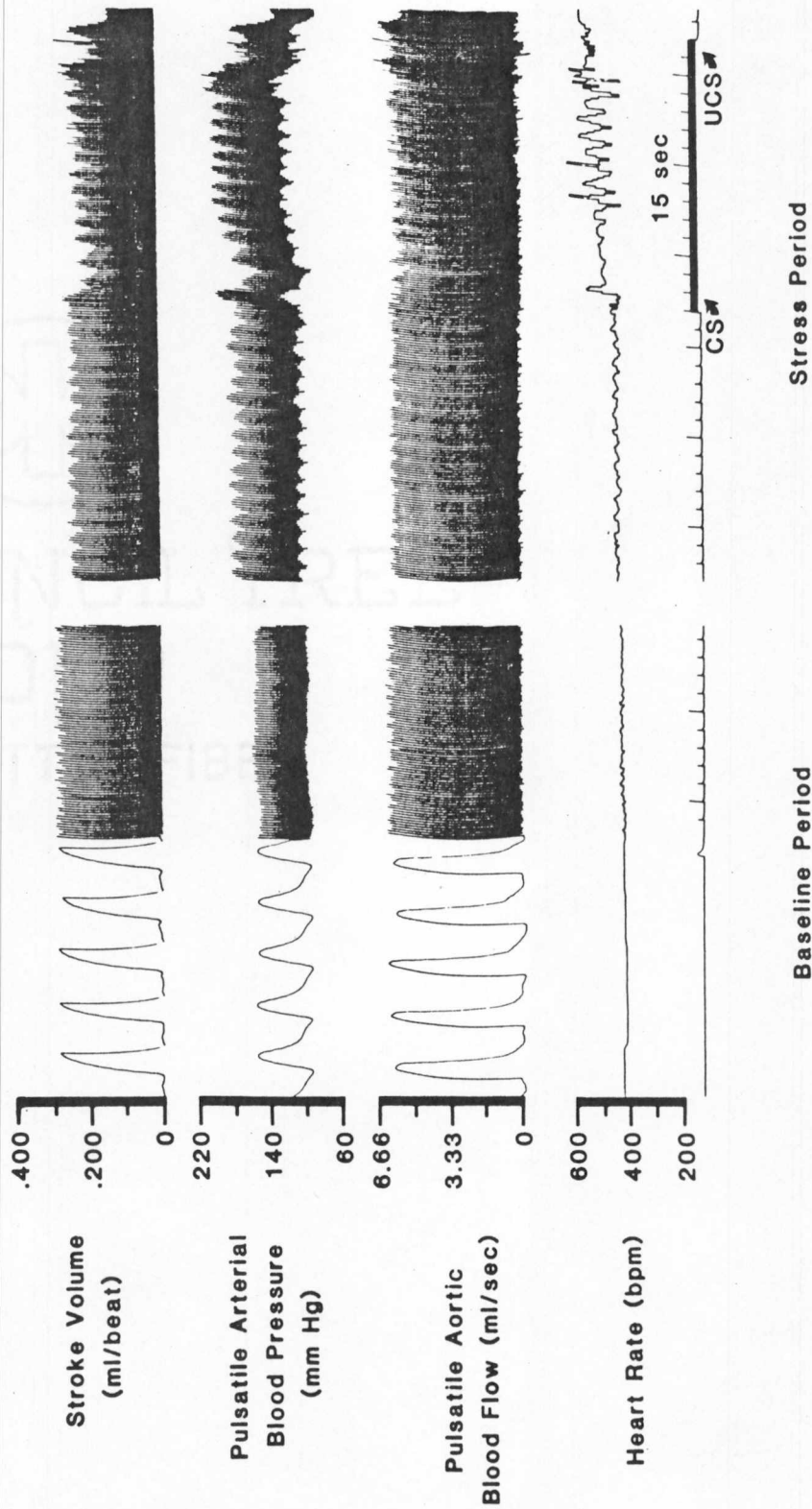


Figure 3. Baseline and conditioned cardiovascular response patterns for a BHR during a single trial in the stress period. The CS is a 15 sec tone and the UCS is a .3-.4 mA, 1 sec electric shock. The paper speed during the fast tracing is 100 mm/sec.

WKY

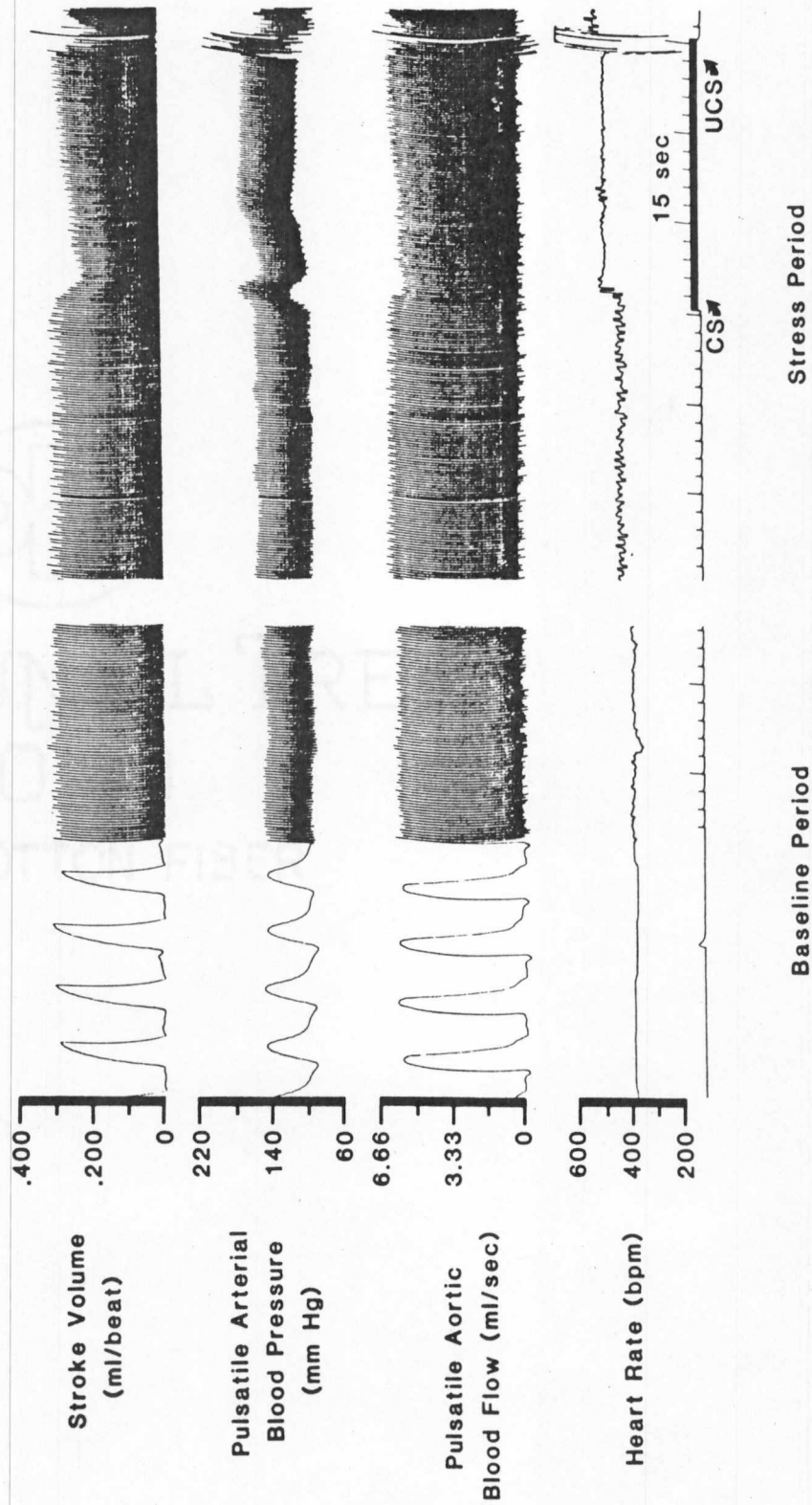


Figure 4. Baseline and conditioned cardiovascular response patterns for a WKY rat during a single trial in the stress period. The CS is a 15 sec tone and the UCS is a .3-.4 mA, 1 sec electric shock. The paper speed during the fast tracing is 100 mm/sec.

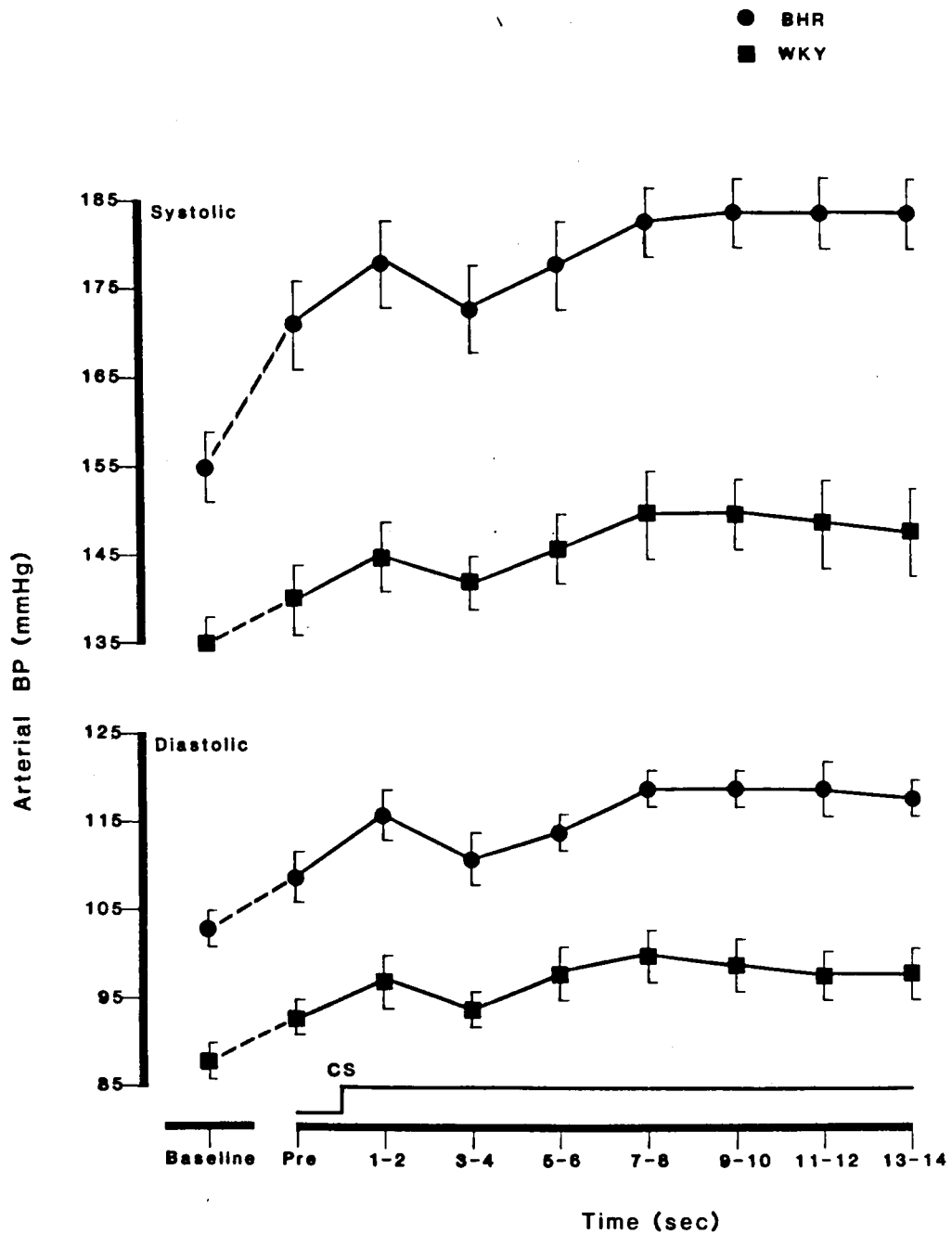


Figure 5. Systolic and diastolic arterial BP responses for the BHR and WKY rats from baseline to the pre-CS and CS periods.

showed a significant increase in systolic and diastolic BP from the pre-CS to the CS period during seconds 1-2 and 5-14. The WKY rats also showed a significant increase in systolic BP from the pre-CS to the CS period during seconds 5-14, and a significant increase in diastolic BP during seconds 1-2 and 5-14. However, the systolic and diastolic BP of the WKY rats was observed to peak on seconds 5-6, since there were no significant differences between this point and seconds 7-14. In contrast, the BHR showed a significant increase in systolic BP on seconds 9-14 compared to seconds 5-6. Diastolic BP also increased significantly for the BHR on seconds 7-12 compared to 5-6.

Figure 6 presents the relative contribution of CI and TPRI to the elevations in mean BP during the pre-CS and CS periods. An analysis of variance for mean BP showed a main effect of strain [$F(1/14) = 29.76$], time [$F(8/112) = 84.21$], and a strain by time interaction [$F(8/112) = 4.70$]. As with systolic and diastolic BP, the interaction for mean BP depicted in Figure 6 illustrates the greater BP reactivity of the BHR to the stress period. The peak change in mean BP from baseline to the stress period was 21 mmHg for the BHR and 14 mmHg for the WKY rats. Subsequent post-hoc tests revealed that both BHR and WKY subjects showed a significant increase in mean BP from baseline to the pre-CS period, and a significant increase in mean BP from pre-CS during seconds 1-2 and 5-14 of the CS period. Mean BP failed to increase significantly beyond seconds 5-6 for WKY rats, but showed a significant increase from this point during seconds 9-12 for the BHR.

There was a main effect of time [$F(8/112) = 4.50$] for CI, and a main effect of strain [$F(1/14) = 5.83$] and time [$F(8/112) = 7.36$] for

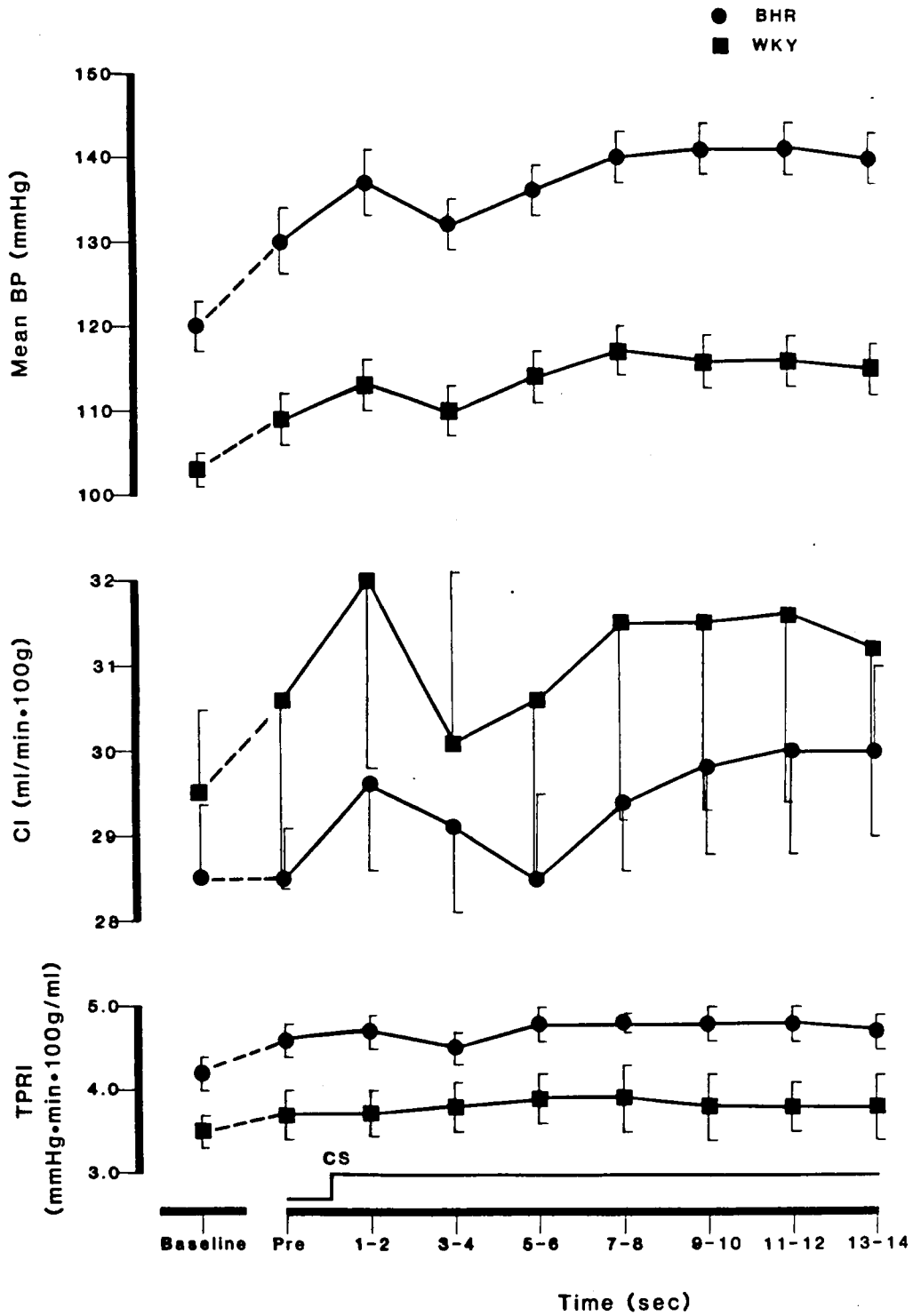


Figure 6. Mean BP, CI, and TPRI responses for the BHR and WKY rats from baseline to the pre-CS and CS periods.

TPRI. Analysis of these simple main effects revealed that the strains showed no significant increase in CI from the baseline to the pre-CS period. There was a significant increase in CI from the baseline during seconds 1-2 and 7-14 of the CS period. However, there was no significant increase in CI from the pre-CS to the CS period. The BHR showed a significantly higher TPRI than WKY rats during baseline, which persisted throughout the stress period. Follow-up analysis of the main effect of time revealed that there was a significant increase in TPRI from baseline beginning at pre-CS and continuing into the CS period. There was no significant increase in TPRI from the pre-CS to the CS period.

Both strains showed a significant increase in HR across time [$F(8/112) = 25.78$] from the baseline to the pre-CS and CS periods (Figure 7). In addition, there was a main effect of time for SW [$F(8/112/112) = 12.81$] and MW [$F(8/112) = 32.78$]. Subsequent follow-up analysis revealed that SW was significantly lower than the baseline during the pre-CS period and during seconds 3-4 of the CS period. All other points during the CS period were not significantly different from the baseline. In addition, SW was significantly greater than the pre-CS period during seconds 1-2 and 7-14 for the strains. Figure 7 shows the increase in MW for BHR and WKY rats from baseline to the pre-CS period. A Tukey (HSD) post-hoc analysis revealed that this change was significant for the strains. There was also a significant increase in MW from pre-CS during seconds 1-2 and 7-14 of the CS period.

Figure 8 shows the dramatic reduction in SI for both BHR and WKY rats from baseline to the stress period (both pre-CS and CS periods).

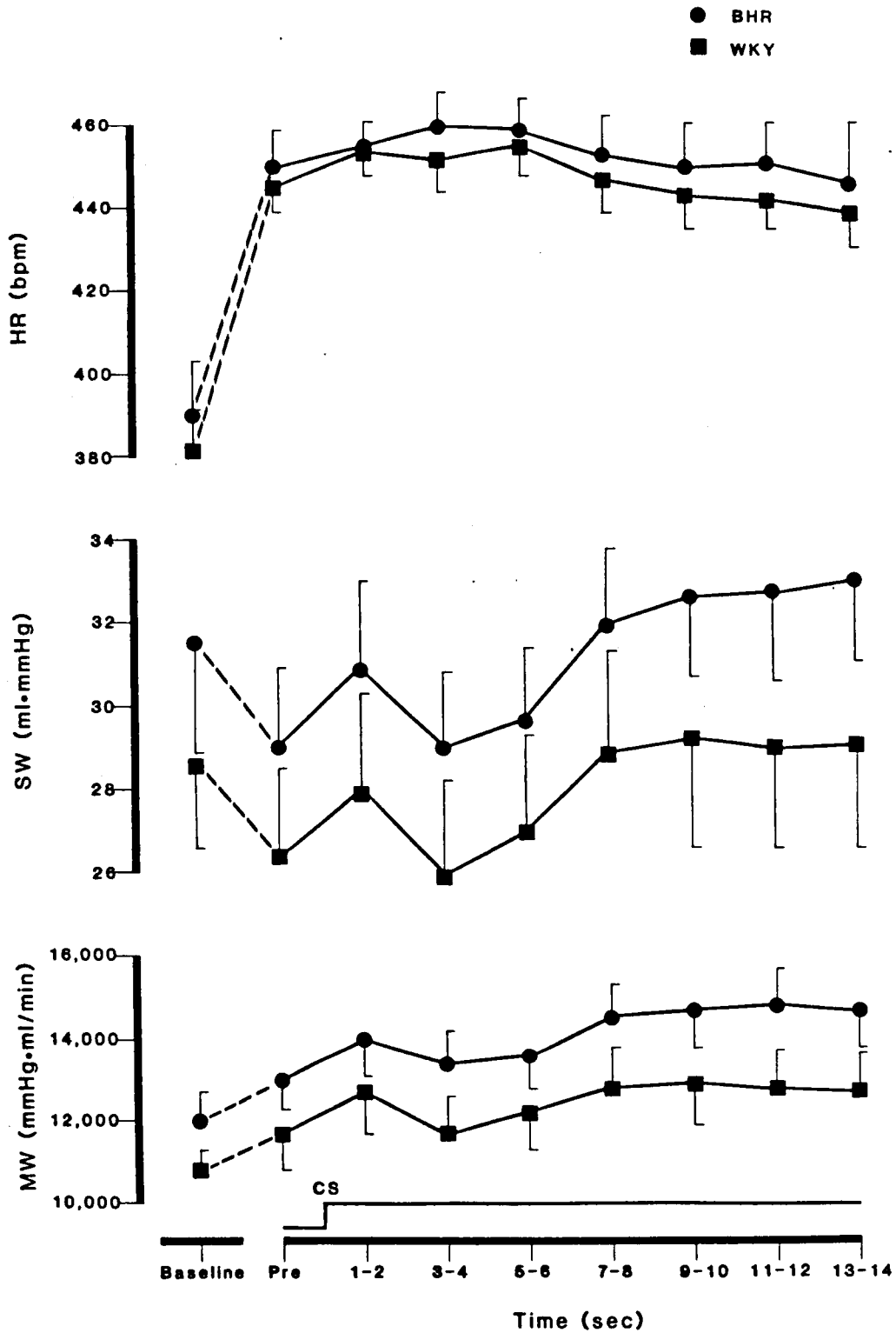


Figure 7. Heart rate (HR), SW, and MW responses for the BHR and WKY rats from baseline to the pre-CS and CS periods.

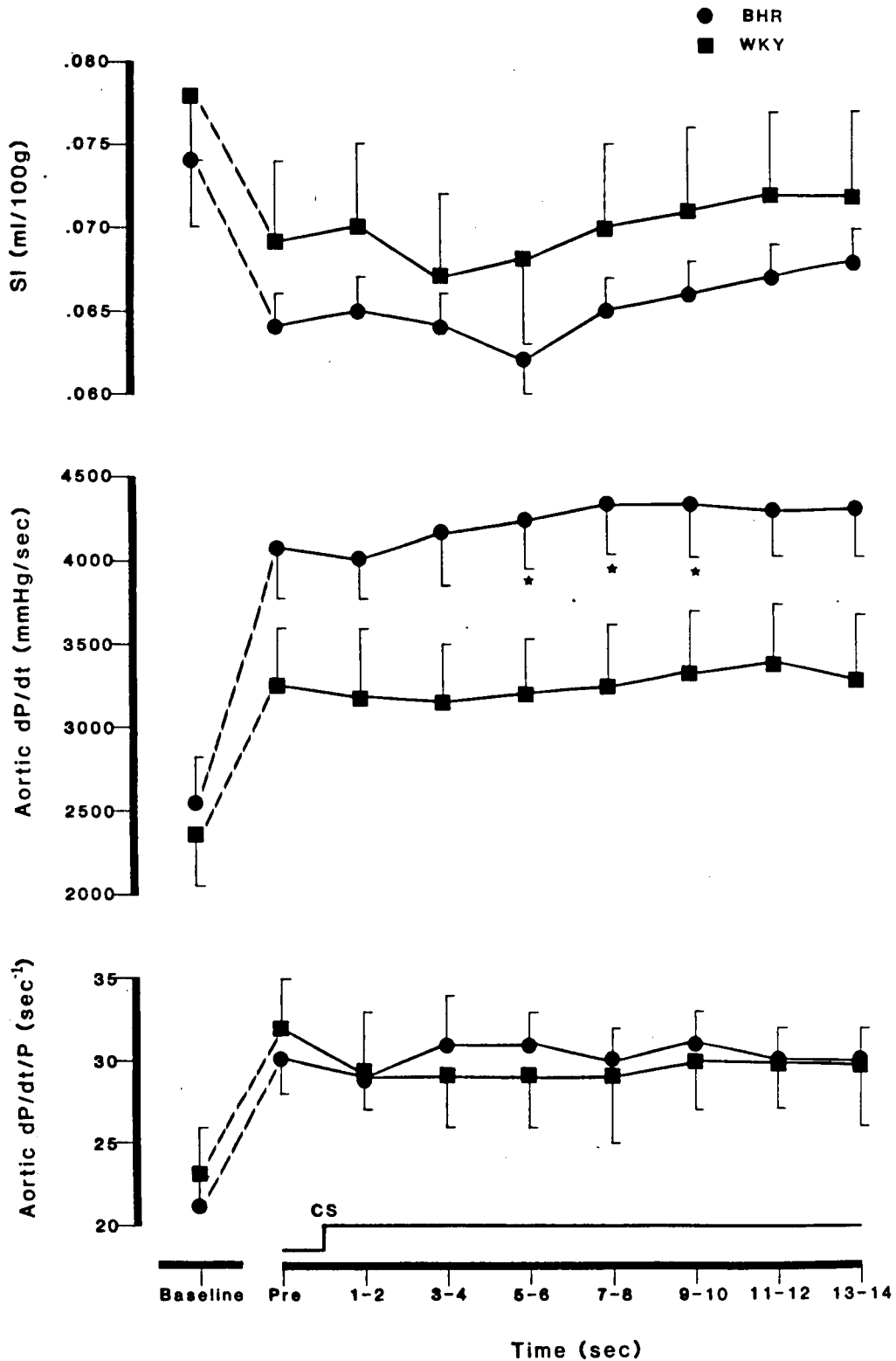


Figure 8. Stroke index (SI), maximum aortic dP/dt, and maximum aortic dP/dt/P responses for the BHR and WKY rats from baseline to the pre-CS and CS periods. Aortic dP/dt values for the BHR differ significantly from WKY rats at * $p < 0.05$.

An analysis of variance revealed a main effect of time [$F(9/112) = 14.64$]. Subsequent post-hoc analysis of this effect for SI showed that the baseline was significantly greater than all time points during the stress period. Figure 8 also shows the changes in maximum aortic dP/dt and $dP/dt/P$ across time for BHR and WKY rats. There was a main effect of time [$F(8/80) = 27.20$] and a strain by time interaction [$F(8/80) = 2.48$] for aortic dP/dt . Post-hoc analysis indicated that aortic dP/dt increased significantly over time, from the baseline to the stress period, for both BHR and WKY rats; however, the BHR showed a significantly greater increase than did the WKY rats. Comparisons between the strains using t -tests showed a significant difference for aortic dP/dt on seconds 5-10 of the CS period (seconds 3-4 and 11-14 were marginally significant, $p < 0.06$). An analysis of variance revealed a significant main effect of time [$F(8/80) = 12.25$] for aortic $dP/dt/P$. The follow-up analysis revealed that aortic $dP/dt/P$ increased significantly from the baseline to the stress period for both strains.

There were no apparent differences in plasma E or NE for BHR and WKY rats during the baseline period (Figure 9). The stress period instigated a significant increase in plasma E for both the BHR, $t(13) = 2.47$, and WKY rats, $t(13) = 5.95$. A significant increase in plasma NE was also seen in response to the stress period, for both BHR, $t(13) = 4.73$, and WKY rats, $t(13) = 4.83$. In addition, the BHR showed a significantly greater increase in plasma NE during this period compared to the WKY rats, $t(12) = 1.79$ (one-tailed).

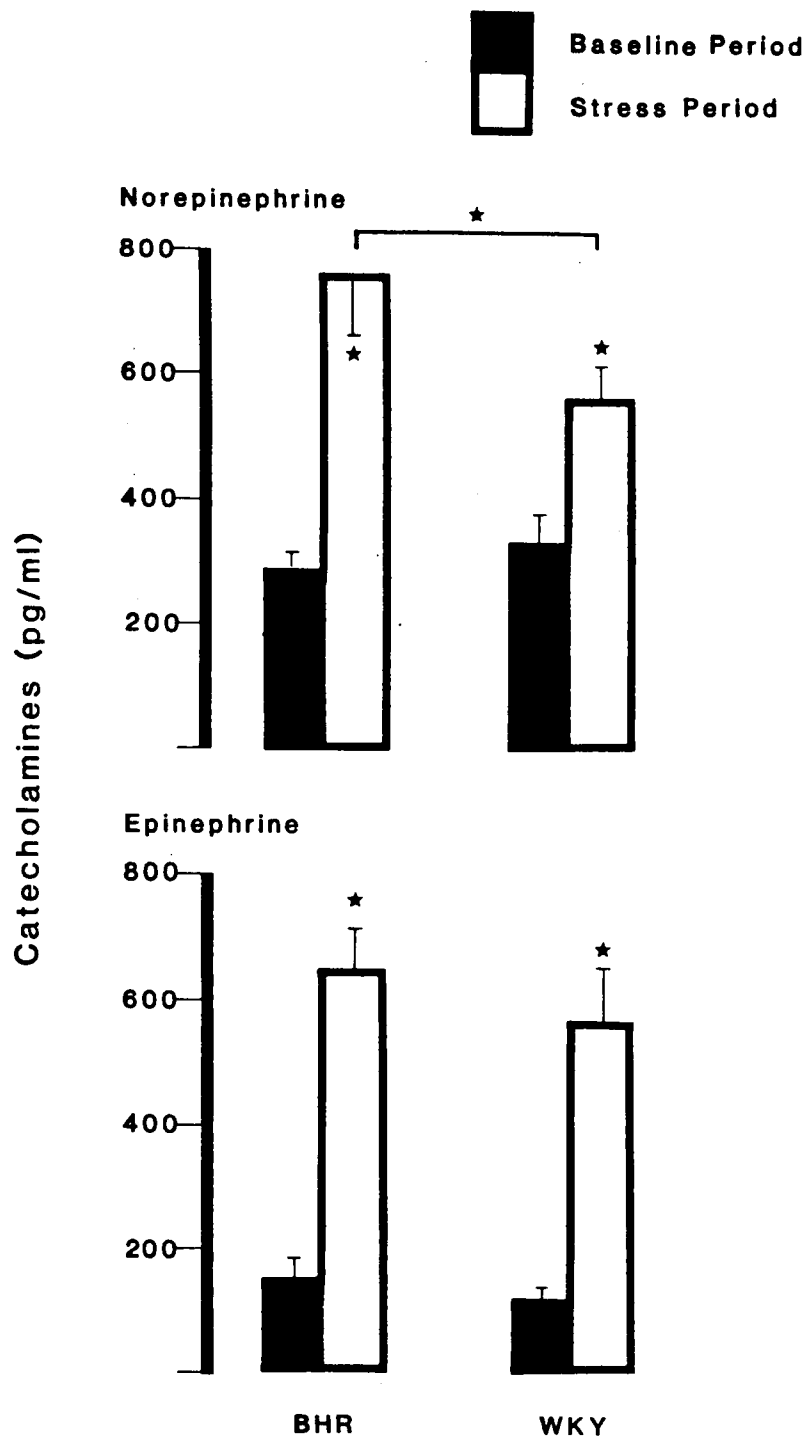


Figure 9. Plasma epinephrine (E) and norepinephrine (NE) levels directly following the baseline period and stress period for BHR and WKY rats. The catecholamine values differ significantly * $p < 0.05$.

The Effects of Clonidine on Cardiovascular and
Sympathetic Response Patterns

Table 5 presents resting preinfusion cardiovascular data for BHR and WKY rats in the saline and clonidine treatment groups. There were no significant differences within the strains for groups receiving saline versus clonidine.

Figure 10 shows a sample physiograph output comparing the effects of i.v.t. saline with clonidine on hemodynamic patterns during the baseline and stress periods in the BHR. The clonidine produced a dramatic reduction in resting HR and in the conditioned HR acceleration. There was a significant decrease in both baseline and stress-induced elevations in arterial BP. In addition, there was a reduction in SV from baseline to the stress period for both the saline and clonidine treatment conditions.

An analysis of variance revealed a significant main effect of strain [$F(1/13) = 5.50$], condition [$F(1/13) = 11.9$], and time [$F(8/104) = 47.85$] for the change in (Δ) systolic BP from the preinfusion baseline across time. Figure 11 shows that an increase in systolic BP throughout the stress period was greater for the BHR than for the WKY rats. In addition, the systolic BP of the clonidine treatment group was significantly lower than the saline treatment group. Follow-up comparisons across time showed that there was a significant increase in systolic BP from postinfusion baseline to the pre-CS period (second 0). There was also a significant increase in systolic BP from second 0 to seconds 1-2 and 5-14 of the CS period.

Table 5
Preinfusion Baseline Cardiovascular Data
for BHR and WKY Rats

Variable	BHR ^a		WKY	
	Saline	Clonidine	Saline	Clonidine
Systolic BP (mmHg)	155 ± 5	155 ± 4	138 ± 2	133 ± 5
Diastolic BP (mmHg)	102 ± 3	104 ± 3	89 ± 3	86 ± 3
Mean BP (mmHg)	120 ± 4	121 ± 3	106 ± 3	101 ± 4
HR (bpm)	405 ± 16	375 ± 16	366 ± 14	396 ± 10
CO (ml/min)	98 ± 7	100 ± 7	106 ± 12	103 ± 5
SV (ml)	.244 ± .022	.270 ± .030	.295 ± .042	.261 ± .018
TPR (mmHg·min/ml)	1.25 ± .11	1.22 ± .07	1.03 ± .12	1.00 ± .08
CI (ml/min·100 g)	28.0 ± 1.4	29.0 ± .7	30.8 ± 1.1	28.2 ± 1.5
SI (ml/100 g)	.070 ± .005	.078 ± .005	.085 ± .005	.072 ± .005
TPRI (mmHg·min·100 g/ml)	4.32 ± .27	4.18 ± .08	3.45 ± .18	3.62 ± .29
SW (ml·mmHg)	29.2 ± 2.7	32.8 ± 4.4	30.8 ± 3.5	26.3 ± 1.6
MW (mmHg·ml/min)	11,720 ± 863	12,092 ± 1,166	11,140 ± 905	10,381 ± 447
Aortic dP/dt (mmHg/sec)	2,658 ± 417 (N = 4)	2,232 ± 341 (N = 4)	2,834 ± 456 (N = 2)	1,804 ± 191 (N = 2)
Aortic dP/dt/P (sec ⁻¹)	21.6 ± 3.5 (N = 4)	18.5 ± 2.7 (N = 4)	28.2 ± 6.1 (N = 2)	19.3 ± 3.6 (N = 2)

^aN = 5 for BHR saline group, all other groups N = 4, unless otherwise specified.

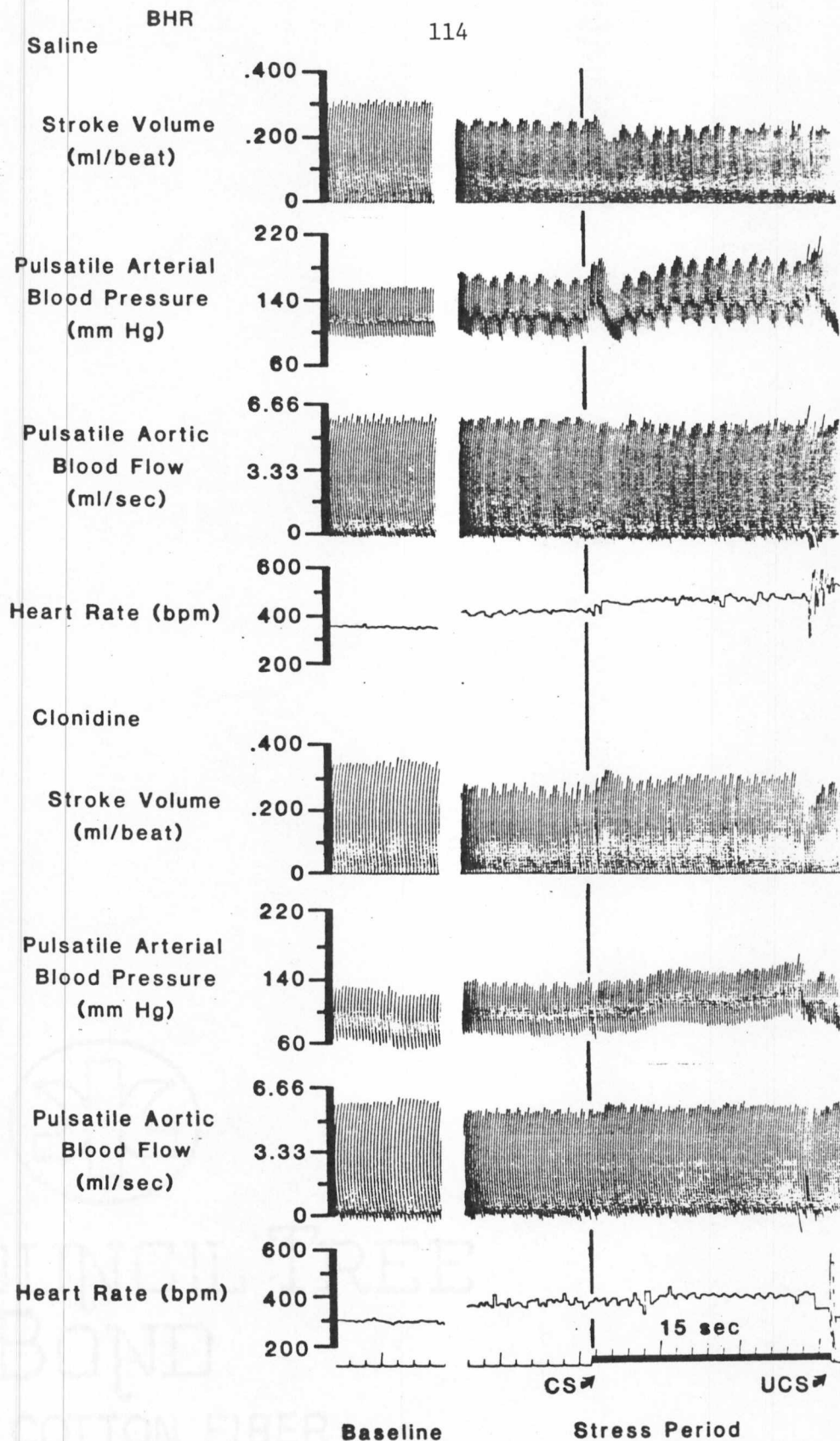


Figure 10. Baseline and conditioned cardiovascular response patterns following either a 4 μ l i.v.t. infusion of normal saline or clonidine (8 μ g) in the BHR.

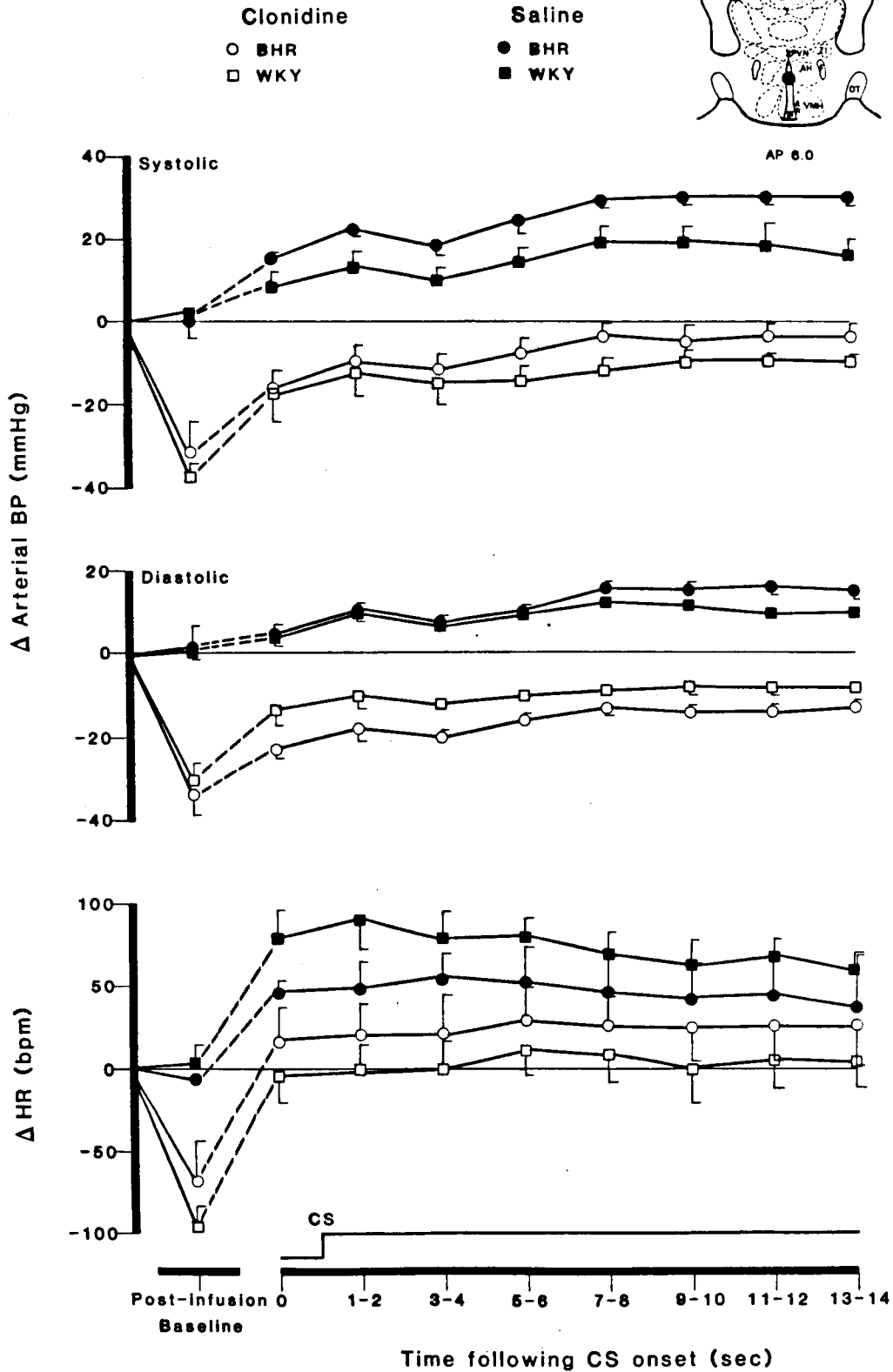


Figure 11. Change in (Δ) systolic and diastolic arterial BP, and HR from preinfusion to postinfusion baseline, pre-CS (second 0), and CS periods for BHR and WKY rats given either i.v.t. saline or clonidine (8 μ g).

For the Δ diastolic BP, there was a main effect of condition [$F(1/13) = 258.27$], time [$F(8/104) = 40.97$], strain by condition interaction [$F(1/13) = 7.63$], and a time by condition interaction [$F(8/104) = 4.18$]. Figure 11 shows that the interaction between strain and condition was the result of a greater reduction in diastolic BP for the clonidine treated BHR than for the WKY rats when they were compared to their saline treated counterparts. Tukey post-hoc tests revealed that the saline treatment group showed no significant change in diastolic BP from the preinfusion to the postinfusion baseline and from the postinfusion baseline to the pre-CS period (second 0). There was a significant increase in diastolic BP for the saline treatment group from the postinfusion baseline to the CS period during seconds 1-14. There was also a significant increase in diastolic BP from second 0 to seconds 1-2 and 5-14 of the CS period. In addition, there was a progressive increase in diastolic BP from second 3-4 which reached statistical significance during seconds 7-12.

In contrast, the clonidine treatment group showed a significant reduction in diastolic BP from preinfusion to the postinfusion baseline, which persisted throughout the stress period. A significant increase in diastolic BP for this group was observed from the postinfusion baseline to second 0 of the pre-CS period. A significant increase in diastolic BP was also observed during seconds 7-14 of the CS period compared to second 0. However, there was no significant increase in diastolic BP from seconds 3-4 throughout the remainder of the CS period. A comparison of the Δ diastolic BP for the saline and clonidine treatment groups

using t-tests revealed significant differences at all time points (from the postinfusion baseline throughout the stress period).

There was a main effect of condition [$F(1/13) = 8.67$], time [$F(8/104) = 42.16$], and a time by condition interaction [$F(8/104) = 3.08$] for the Δ HR (Figure 11). Follow-up analysis revealed that the saline treatment group showed no significant change in HR from the preinfusion to the postinfusion baseline. There was a significant increase in HR compared to the pre- and postinfusion baseline, beginning on second 0 and continuing throughout the stress period. In contrast, the clonidine treatment group showed a significant reduction in HR from the preinfusion to the postinfusion baseline. There was also a significant increase in HR from the postinfusion baseline to second 0, which persisted throughout the stress period. Both groups showed no significant change in HR from the second 0 to the CS period.

Comparison of saline and clonidine treatment groups across time using t-tests revealed that the saline group had significantly higher HR differences than the clonidine group during the postinfusion baseline, second 0, and seconds 1-8 of the CS period. The HR differences during seconds 9-14 of the CS period were not significantly different between the groups. As can be seen in Figure 11, this effect was due to a progressive drop in HR for the saline group across time.

Figure 12 shows the depressor effect of i.v.t. clonidine and the relative contributions of CI and TPRI to changes in mean BP during the postinfusion baseline and stress periods. An analysis of variance indicated there was a main effect of condition [$F(1/13) = 222.23$] and

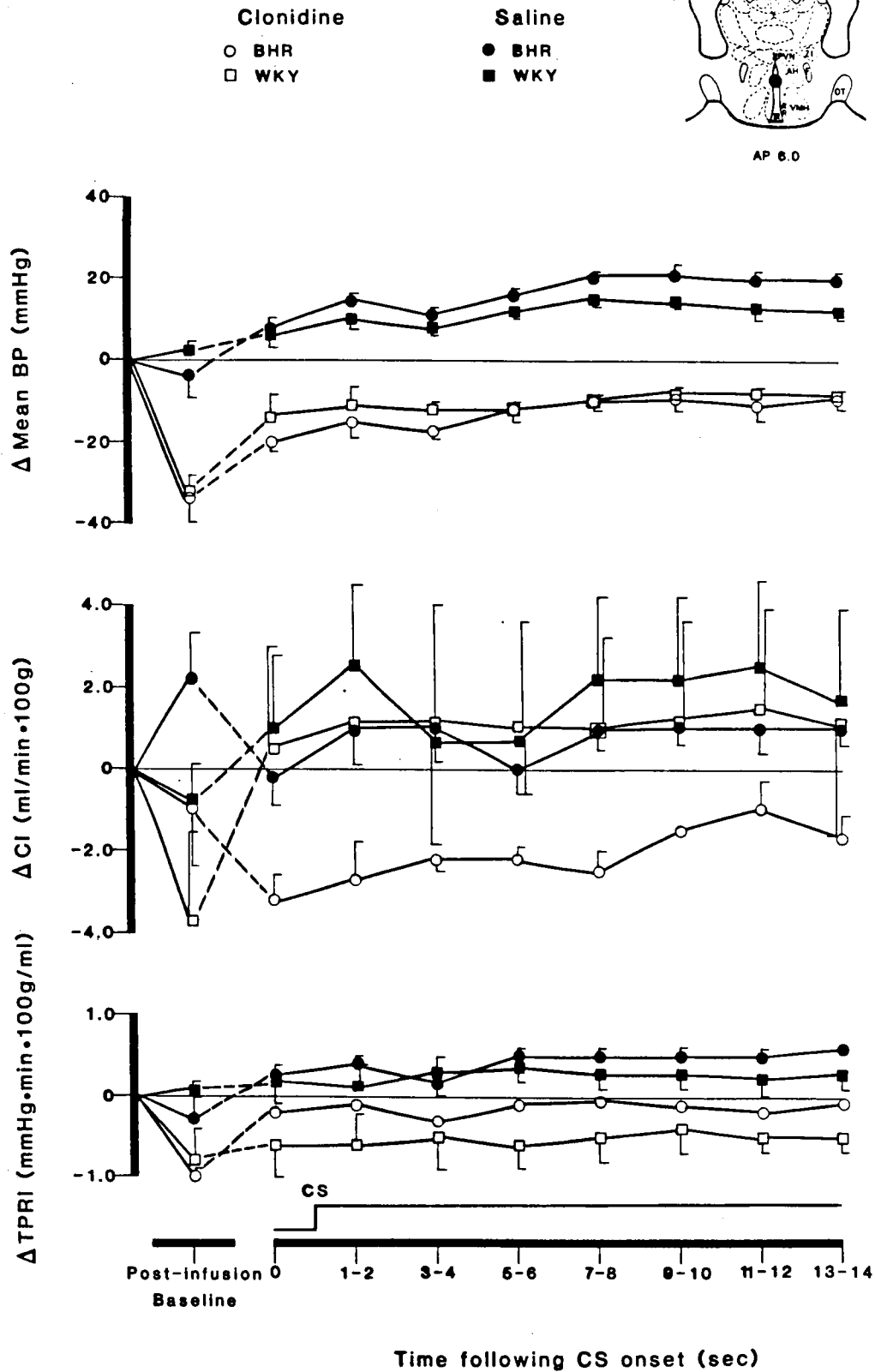


Figure 12. Change in (Δ) mean BP, CI, and TPRI from preinfusion to postinfusion baseline, pre-CS (second 0), and CS periods for BHR and WKY rats given either i.v.t. saline or clonidine (8 μ g).

time [$F(8/104) = 46.81$] for the Δ mean BP. The mean BP of the clonidine treatment group was significantly lower than the saline treatment group. Post-hoc tests revealed that there was a significant increase in mean BP from the postinfusion baseline to second 0, and from second 0 to seconds 1-2 and 5-14 of the CS period.

For the Δ CI from the preinfusion baseline, there was a main effect of time [$F(8/104) = 3.11$] and a time by strain interaction [$F(8/104) = 5.89$]. Tukey post-hoc tests showed that there was a significant reduction in CI from the postinfusion baseline to second 0 for the BHR. In addition, there was no significant change in CI from the postinfusion period to the CS period. In contrast, the WKY showed a significant increase in CI from the postinfusion baseline to seconds 0-14 of the stress period. Additional comparisons using t -tests revealed that there were no significant differences in the Δ CI between the strains across time. Figure 12 graphically depicts these changes in CI for BHR and WKY rats.

An analysis of variance on the Δ TPRI showed a main effect of condition [$F(1/13) = 14.03$], time [$F(8/104) = 13.24$], and a time by strain interaction [$F(8/104) = 4.85$]. The TPRI of the clonidine treatment group was significantly lower than the saline treatment group. Follow-up comparisons revealed that the time by strain interaction was the result of divergent changes in TPRI from the postinfusion baseline to the stress period (Figure 12). The BHR showed a significant increase in TPRI from the postinfusion baseline to the stress period, while the WKY rats showed no significant change across this time period. Comparisons between the strains across time using t -tests revealed no significant differences in the Δ TPRI.

A main effect of time [$F(8/104) = 31.34$], time by strain interaction [$F(8/104) = 5.07$], and time by condition interaction were seen for the Δ SI from the preinfusion baseline across time (Figure 13). Post-hoc tests revealed that both BHR and WKY rats showed a significant drop in SI from the postinfusion period to second 0; however, this change was significantly greater for the BHR. There was no significant change in SI from the pre-CS (second 0) to the CS period for either strain. In addition, comparisons between the strains revealed that there were no significant differences in the Δ SI across time.

Additional post-hoc tests showed that there was a significant increase in SI from the preinfusion to the postinfusion baseline for the clonidine treatment group. The saline control groups showed no significant Δ SI across this time period. There was a significant reduction in SI from the postinfusion baseline to second 0 of the pre-CS period for both treatment groups. The clonidine treatment group showed no significant Δ SI from second 0 to the CS period. However, the saline control group showed a significant increase from second 0 to the CS period during seconds 13-14. Comparisons between the two treatment groups revealed a significant difference in the Δ SI during the postinfusion baseline. The clonidine group had a significantly larger SI than the saline group during this time period. All other comparisons were nonsignificant.

An analysis of variance for the Δ SW revealed a main effect of condition [$F(1/13) = 8.55$], time [$F(8/104) = 14.37$], time by strain interaction [$F(8/104) = 3.84$], time by condition interaction [$F(8/104) =$

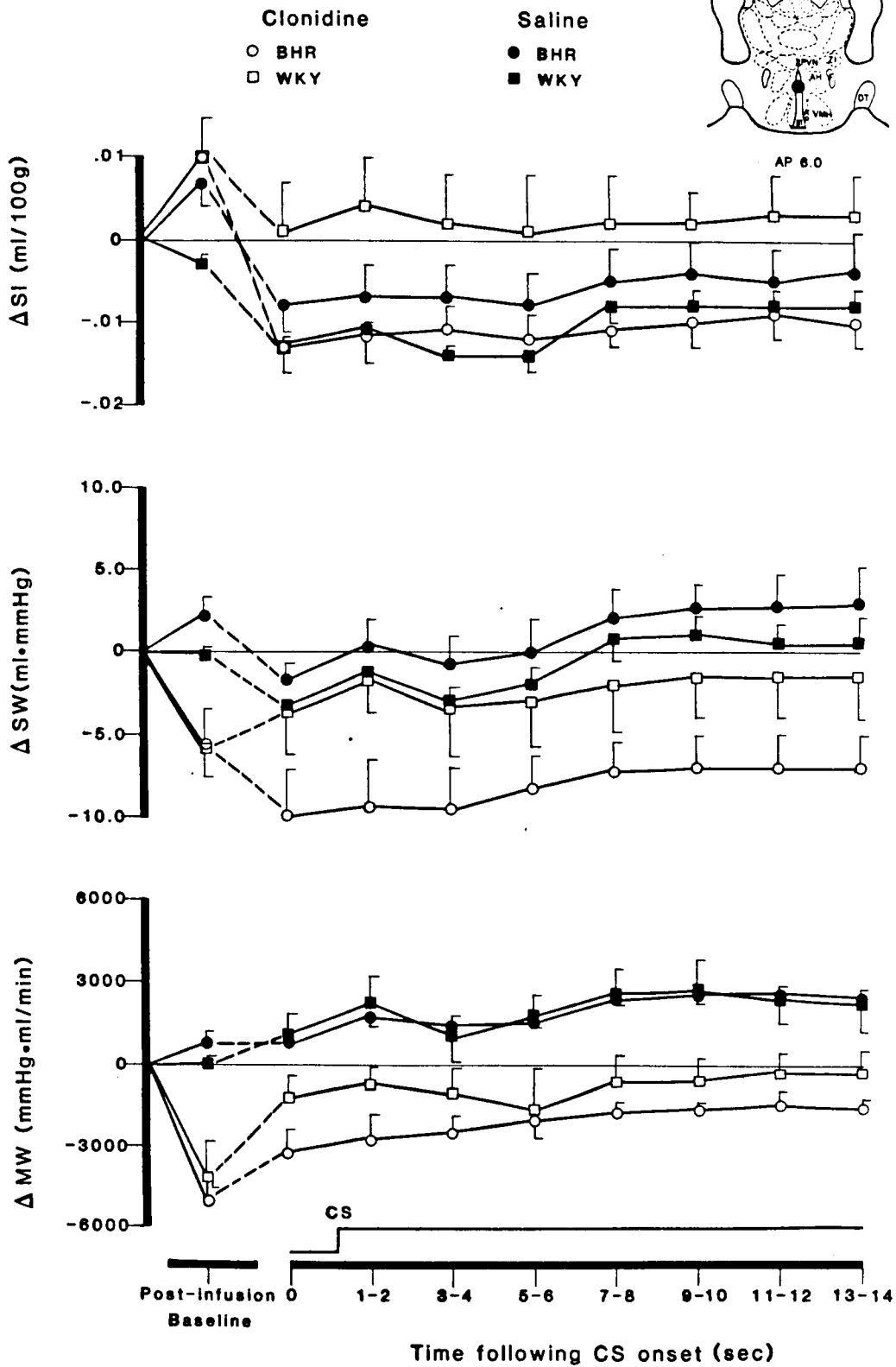


Figure 13. Change in (Δ) SI, SW, and MW from preinfusion to postinfusion baseline, pre-CS (second 0), and CS periods for BHR and WKY rats given either i.v.t. saline or clonidine (8 μ g).

2.17], and a triple interaction of time by strain by condition ($F(8/104) = 2.48$). Figure 13 depicts this latter effect. Follow-up analysis comparing the Δ SW from the postinfusion period across time, between the strains receiving either clonidine or saline, revealed the source of the interaction. No significant difference between the saline treated BHR and WKY was found across all time points; however, the clonidine treated BHR and WKY differed dramatically across time. The BHR showed a decrease in SW following the postinfusion period, while the WKY showed an increase in SW following the postinfusion period. These points were significantly different from each other during seconds 0, 1-4, and 7-14 of the stress period.

For the Δ MW, there was a main effect of condition [$F(9/13) = 29.73$] and time [$F(8/104) = 17.91$]. Figure 13 shows that the clonidine treated group had a lower MW than the saline treated group. Post-hoc comparisons across time revealed a significant increase in MW from the postinfusion baseline to second 0 and a significant increase from second 0 to seconds 7-14 of the CS period.

Figure 14 shows the changes in maximum aortic dP/dt and $dP/dt/P$ from the preinfusion baseline across the stress period. An analysis of variance revealed a main effect of time [$F(8/64) = 37.70$] for aortic dP/dt and [$F(8/64) = 20.41$] $dP/dt/P$. There was a significant increase in both of these variables from the postinfusion baseline to the stress period.

Figure 15 shows the plasma catecholamine responses to the postinfusion baseline and stress period for BHR and WKY rats treated

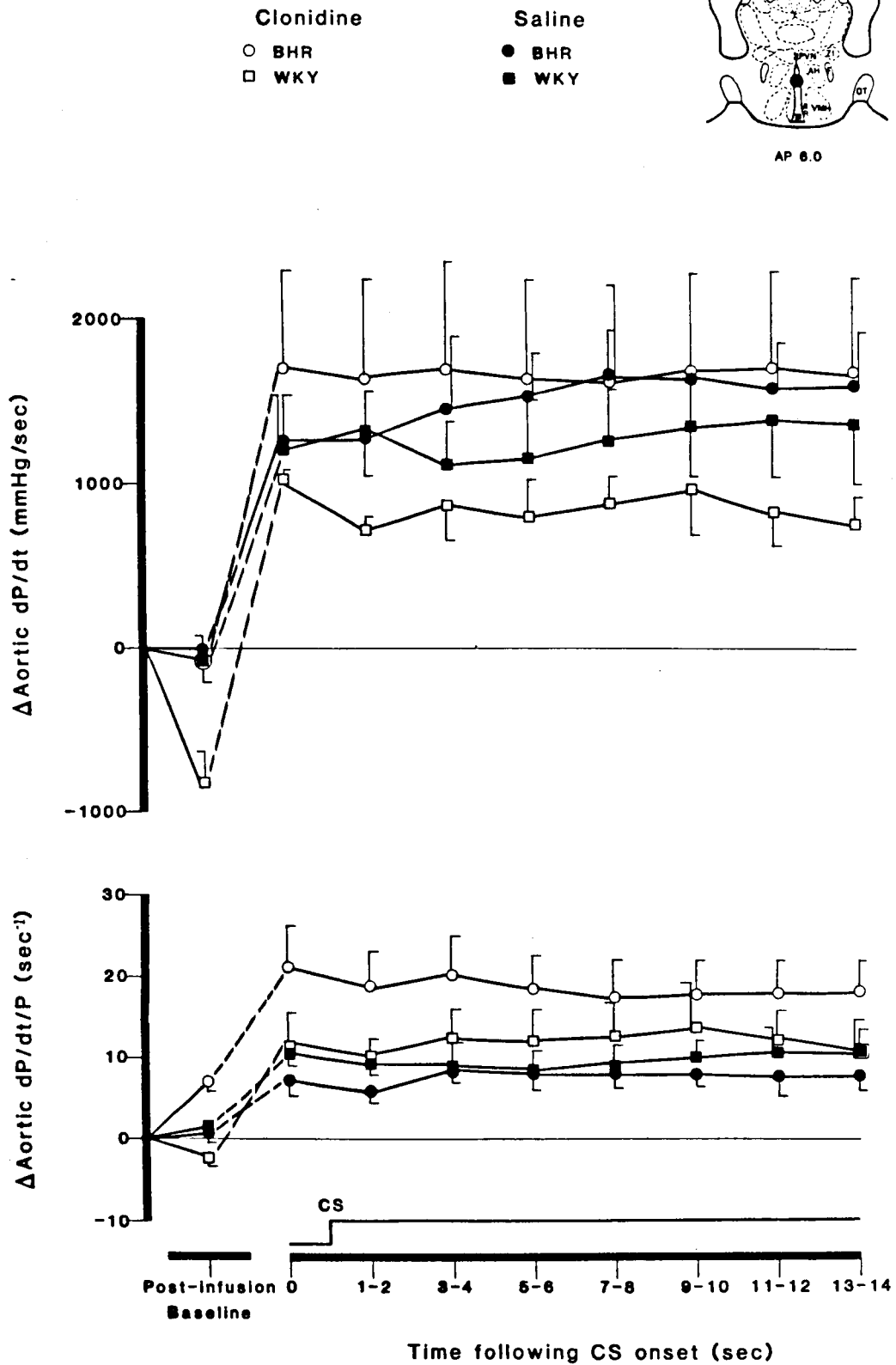


Figure 14. Change in (Δ) maximum aortic dP/dt and maximum aortic dP/dt/P from preinfusion to postinfusion baseline, pre-CS (second 0), and CS periods for BHR and WKY rats given either saline or clonidine (8 μ g).

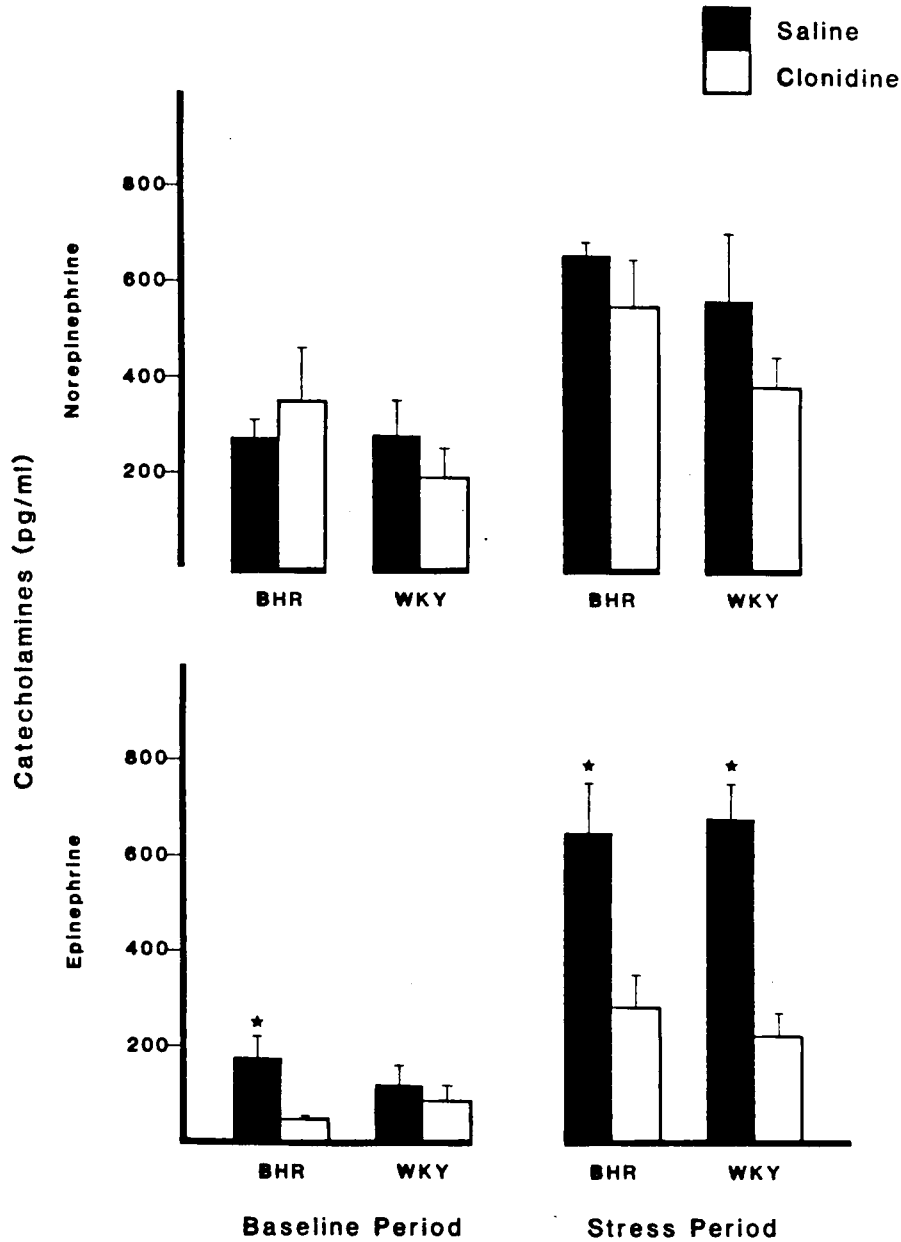


Figure 15. Plasma epinephrine (E) and norepinephrine (NE) levels directly following the postinfusion baseline and stress period for BHR and WKY rats receiving i.v.t. saline or clonidine (8 μ g). Epinephrine values differ significantly between saline control and clonidine treatment groups at $p < 0.05$.

with either i.v.t. saline or clonidine. Comparisons were made between saline and clonidine treated animals within each strain. It can be seen that there were no significant differences in plasma NE between the saline and clonidine treatment groups during either the baseline or stress periods. However, there was a significant reduction in plasma E for clonidine treated BHR during the postinfusion baseline, $t(6) = 2.35$ (one-tailed). There was also a significant reduction in plasma E during the stress period for both BHR, $t(6) = 2.98$, and WKY rats, $t(5) = 4.91$.

CHAPTER IV

DISCUSSION

The hemodynamic and sympathetic-adrenal medullary response patterns of chronically instrumented BHR and WKY rats were compared during baseline (rest) and aversive classical conditioning periods. In addition, intracerebroventricular infusion of clonidine was employed to assess the role of central α_2 -adrenoreceptors in the mediation of these cardiovascular and sympathetic responses during the two behavioral states, for both BHR and WKY rats.

The baseline hemodynamic data obtained in the first study indicate that the mild hypertension seen in 18-28 week old BHR is the result of an elevated TPRI (or TPR) with a normal CI (or CO) compared to the WKY control rats. The other cardiovascular variables were not significantly different between the strains at rest. Also, the BHR and WKY rats did not differ from each other with regard to basal plasma E and NE levels. Plasma E concentrations have been shown to reflect adrenal medullary secretion and activity, while plasma NE levels have been related to average sympathetic outflow, i.e., spillover from the synaptic cleft into the circulation and adrenal medullary secretion (Goldstein, McCarty, Polinsky, & Kopin, 1983). Consequently, there does not appear to be an elevation in resting sympathetic tone in the BHR as reflected by plasma catecholamine levels.

This observation coupled with the elevated TPRI seen in the BHR compared to WKY rats suggests that: (1) an increased vascular

sensitivity to sympathetic stimulation either directly or from circulating catecholamines is responsible for the elevated vascular resistance; and/or (2) structural resetting of the resistance vessels results in an increased wall:lumen ratio and altered vascular design which increases the vasoconstrictor response for a given level of sympathetic stimulation.

Previous investigations have shown that the BHR develops a spontaneous borderline hypertension, which remains stable unless the animal is exposed to psychological stress (Lawler et al., 1980; Lawler, Barker, Hubbard & Schaub, 1980). Chronic exposure to 12 weeks of shock-shock conflict has been shown to instigate pathological hypertension in this animal (Lawler et al., 1981). Consequently, the susceptibility of the BHR to stress-induced hypertension has been well established; however, the mechanisms responsible for the transition from the borderline hypertensive condition to a pathological hypertension remain to be established. One possible mechanism initiating this effect might involve a pathological interaction between the sympathetic nervous system, vascular smooth muscle, and myocardium which does not occur if the animal is left undisturbed, but can be triggered by repeated exposure to psychological stress.

The acute stress response of the BHR to the aversive classical conditioning schedule was similar to that of the WKY, but greater in magnitude for several cardiovascular variables. A biphasic pressor response to the CS was noted for both strains. The initial rise in arterial BP is thought to represent a conditioned OR, which is then

followed by the CR (the unconditioned response, UCR, was not examined in this study). Systolic, diastolic, and mean arterial BP increased significantly more in the BHR than in the WKY rats in response to the stress session. However, the most dramatic increase in systolic BP for the BHR was seen from the baseline to the pre-CS period, manifested as an increase in systolic BP of 16 mmHg compared to 5 mmHg for the WKY rats. In addition, the peak pressor response during the CS period was 29 mmHg greater than the baseline for BHR and only 15 mmHg greater than the baseline for the WKY subjects. Thus, it appears that the BHR not only have a higher resting arterial BP, but also show a greater pressor responsiveness to psychological stress compared to WKY rats.

The initial increase in mean arterial BP from the baseline to the pre-CS period was the result of an increase in TPRI for both BHR and WKY subjects. However, the TPRI of the BHR was significantly greater than the WKY both at rest and during the stress period. The conditioned OR and CR pressor responses were the result of an increased CI compared to the baseline period, which was superimposed on the elevated TPRI. The main analysis of variance indicated that the CI response during stress was not different between the two strains. Thus, the increased mean arterial BP of the BHR in response to the stress period was the result of a multiplicative interaction between an elevated TPRI and normal CI compared to the WKY subjects. These observations suggest that the mechanisms utilized for elevating mean arterial BP in both strains were the same (an increase in both TPRI and CI compared to the baseline period); however, the contribution of TPRI to this response pattern for the BHR was significantly greater than the WKY subjects.

Both strains showed a significant increase in HR in response to the stress period, which did not change from the pre-CS to the CS period and was not significantly different between the strains. The lack of a significant change in CI, given this dramatic increase in HR, can be ascribed to the significant drop in SI seen for both strains across this time period (see Figure 8, p. 109). This reduction in SI was associated with an increased maximum aortic dP/dt and $dP/dt/P$ from the baseline to the pre-CS period. Since increases in aortic dP/dt and $dP/dt/P$ have been correlated with an augmented inotropic state (Grossman, Brooks, Meister, Sherman & Dexter, 1971; Lawler et al., 1975), it must be concluded that the drop in SI was due to a reduction in venous return to the heart coupled with a shorter diastolic filling period. The increase in arterial vascular resistance seen in both strains could lead to a direct reduction in central venous pressure and subsequent drop in preload. This response pattern would act in conjunction with the elevated afterload to favor a reduced SI (even with an increase in sympathetic drive to the heart). The progressive increase in CI during the latter seconds of the CS period was due to an increase in SI while HR showed only a slight reduction across this time period.

Stroke work (SW) dropped significantly for both strains from the baseline to the pre-CS period. This effect was due to a significant reduction in SV (reported as SI on Figure 8, p. 109). However, there was a subsequent recovery and increase in SW over the pre-CS period during seconds 7-14 of the CS period. This response was due to an increase in SV. The initial increase in MW from the baseline to the

pre-CS period was the result of the significant tachycardia seen during this time period. The increase in MW during the CS period was the result of changes in SW. The peak in MW during the OR was due to an increase in mean BP, while the secondary peak during the CS was due to an augmented SV. This latter increase in SI (or SV) which contributed to the secondary changes in these cardiovascular variables may have been due to an interaction between a slight reduction in HR and modest increase in dP/dt during this time period (seconds 7-14). The drop in HR would augment ventricular filling by increasing the length of the diastolic period and the increase in the inotropic state would favor a larger ejection fraction from the left ventricle.

It appears that the BHR also showed a greater sympathetic response to the aversive classical conditioning paradigm than did the WKY rats, as indicated by higher plasma NE levels during this period. This response may reflect an adaptive autonomic pattern by the BHR to maintain myocardial performance in the face of a reduced preload and elevated afterload. Previous studies have shown that hypertensive animals, such as the SHR, require a greater preload to maintain a normal CI (Hallbäck et al., 1975; Hallbäck et al., 1979). The presence of this condition in the BHR is supported by the observation of a greater increase in maximum aortic dP/dt for the BHR during the stress period as compared to the WKY subjects. A rank order correlation (ρ) between the maximum change in aortic dP/dt and NE from the baseline to the stress period for BHR and WKY rats revealed a significant positive correlation, $r = +.624$, $t(8) = 2.26$ (one-tailed). This suggests that

the greater increase in NE for the BHR was acting to enhance contractility and thereby improve SI. However, the increase in sympathetic tone would also elevate peripheral vascular resistance, which would further compromise the preload.

Thus, it appears that the adult BHR has retained several characteristics from its SHR progenitor. The most salient of these is a high resistance, normal CI hypertension. This observation suggests that an inherited vascular sensitivity to vasopressor substances such as AII, E, or NE might contribute to the development of stress-induced hypertension in the BHR. The BHR also shows a greater sympathetic response and pressor reactivity to psychological stress than does the WKY, a characteristic pattern well documented for the SHR (Judy, Watanabe, Henry, Besch, Murphy & Hockel, 1976; Lundin & Thorén, 1982; McCarty & Kopin, 1978). Depressed noradrenergic activity in specific regions of the CNS, which regulate sympathetic drive in response to environmental perturbations, has been shown to modulate this autonomic pattern in the SHR (Wijnen et al., 1980), and, consequently, may also play an important modulatory role in the BHR. The role of central α_2 -adrenoreceptors and noradrenergic activity on cardiovascular and sympathetic response patterns at rest and during stress were examined in conscious BHR and WKY rats using clonidine.

Intracerebroventricular infusion of clonidine (8 μ g) in conscious BHR and WKY rats produced a significant reduction in baseline systolic, diastolic, and mean BP. The drop in mean arterial BP was the result of a significant reduction in TPRI for both strains. There was no

significant change in CI for the strains following clonidine administration during the postinfusion baseline period. Clonidine also produced a significant decrease in resting HR for both strains. The combination of a decreased TPRI and HR resulted in a significant increase in resting SI for the clonidine treatment group. There were also concomitant decreases in SW and MW for the clonidine treatment group during the postinfusion period. No significant differences were observed between the saline treatment group and the clonidine treatment group with regard to resting aortic dP/dt and dP/dt/P.

Baseline plasma catecholamine levels for the WKY rats were unaffected by clonidine when compared to the saline treatment group. In contrast, the BHR showed a significant decrease in resting E following i.v.t. clonidine compared to the saline control group. There was no significant change in resting NE for the BHR between saline and clonidine treatment groups.

These observations suggest that intracerebroventricular clonidine alters basal cardiovascular function in the BHR and WKY in a similar manner. The selective reduction of resting plasma E for the BHR may reflect a greater availability or sensitivity of central α_2 -adreno-receptors for clonidine. A slight increase in plasma NE at rest for the BHR may act as compensatory response to offset the decrease in plasma E following clonidine treatment.

Intracerebroventricular clonidine initiated a rapid depressor effect for both strains (within 5 minutes of the infusion) which persisted throughout the classical aversive conditioning paradigm. It

was found that clonidine-treated animals were capable of generating a conditioned increase in systolic BP from the postinfusion baseline which was not significantly different in magnitude from the saline-treated animals. A similar effect was also seen for mean arterial BP. However, diastolic BP was selectively reduced to a greater degree in the BHR than in the WKY rats. In addition, clonidine-treated animals showed a greater increase in diastolic BP from the postinfusion baseline to second 0 of the pre-CS period when compared to the saline-treated subjects. This response for the clonidine treatment group was then followed by an attenuated diastolic BP CR which was evident during seconds 3-14. Thus, diastolic BP reactivity was left intact as reflected by the change from the postinfusion baseline to second 0 of the pre-CS period. However, the more controlled pressor response to the CS was reduced for the clonidine treatment group compared to the saline saline treatment group. This rather selective effect of clonidine on diastolic BP is probably related to the mode of administration (i.v.t.) and the locus of action within the CNS.

Postmortem examination of the brain tissue following a 4 μ l i.v.t. infusion of Evans Blue revealed that the dye was localized almost exclusively in the ventral portion of the third ventricle and hypothalamus. Small amounts of dye were seen at the entrance to the aqueduct of Sylvius in only a few animals, and no dye was seen in the fourth ventricle. This technique for mapping the diffusion of centrally applied drugs has been shown to be quite reliable (Tadepalli et al., 1978). Consequently, the cardiovascular effects of clonidine reported

in this study most likely reflect an action of the drug on the hypothalamus, rather than structures in the medulla such as the NTS.

The selective reduction of plasma E, but not NE, in response to the stress session for clonidine-treated BHR and WKY rats also supports the idea of a specific site of action within the hypothalamus. Previous studies have demonstrated that i.v.t. clonidine can depress adrenal nerve activity in anesthetized rats and significantly reduce the efflux of E from the adrenal vein (Togashi, 1983). Lesion studies have also shown that damage to the anterior hypothalamus can instigate a fulminating hypertension which is the result of a hypersecretion of catecholamines from the adrenal medulla (Nathan & Reis, 1975; Suarez et al., 1981). Thus, it appears that central noradrenergic activity in the anterior hypothalamus regulates adrenal medullary secretion of E selectively via α_2 -adrenoreceptors.

Clonidine treatment reduced resting HR, but did not attenuate the increase in HR during the stress session. This demonstrates that HR reactivity to the onset of the stress session was left intact regardless of the clonidine treatment. In addition, maximum aortic dP/dt and dP/dt/P were not affected by the clonidine treatment and showed a significant increase from the postinfusion baseline to the stress period. It is hypothesized that the initial drop in HR was mediated by an increase in resting vagal tone stimulated by the action of clonidine on the anterior hypothalamus. The evidence for this assertion is indirect, based on studies which have demonstrated that stimulation of the anterior hypothalamus elicits a vagally mediated bradycardia (Hilton

& Spyer, 1971; Hilton & Spyer, 1980). Also, the observation that basal plasma NE was unaffected by the clonidine also supports this hypothesis. However, the increase in HR, aortic dP/dt and dP/dt/P from the post-infusion baseline to the stress period was probably the result of an increase in sympathetic activity for both the clonidine and saline treatment groups. The increase in plasma NE from the postinfusion baseline to the stress period supports this contention.

It was found that the BHR had a higher TPRI than WKY rats regardless of treatment condition. The TPRI for the clonidine treatment group was lower than the saline control group throughout the stress period. Cardiac index remained unchanged between the two treatment groups. Consequently, the lower mean BP of the clonidine treatment group throughout the stress period was due to the reduction in TPRI.

Stroke index decreased from the postinfusion baseline to the stress period for all groups and then remained relatively stable. Although it was not tested, there is an apparent difference in SI between the BHR and WKY clonidine treatment groups. The WKY rats infused with clonidine maintained SI during the stress period at a significantly higher level than the BHR subjects infused with clonidine. This difference becomes important when discussing the significant difference in SW between these two groups of subjects. The BHR clonidine group showed a significant reduction in SW compared to the WKY clonidine treatment group from the postinfusion baseline throughout the stress period. The BHR and WKY saline treated subjects did not differ in SW across this time. Since mean arterial BP increased significantly during this time for the

clonidine and saline-treated subjects, the reduction in SW for the clonidine-treated BHR must be due to a drop in SV (reported as SI in Figure 13, p. 122). This reduction in SV (SI) for the BHR must be attributed to a reduction in preload since both aortic dP/dt and $dP/dt/P$ increased significantly during the stress session. This observation again emphasizes the importance of an elevated preload for the BHR to maintain a normal SI. The increased HR during this period also decreased the diastolic filling period which would further compromise SI. The MW for the clonidine-treated subjects was significantly lower throughout the stress period compared to the saline-treated subjects.

In summary, it appears that the mild hypertension in the BHR is the result of an elevated TPRI with a normal CI. Psychological stress instigates a greater increase in arterial BP in the BHR compared to normotensive WKY rats. This response pattern appears to result from an elevated sympathetic drive in the BHR compared to the WKY rats. Both strains show an increase in TPRI and CI in response to the stress; however the TPRI of the BHR is significantly higher which results in a greater change in mean arterial BP. Intracerebroventricular infusion of clonidine significantly reduces the arterial BP of the treated subjects compared to the saline treated group. The reduction in mean arterial BP is due to a drop in TPRI with a slight, but nonsignificant, reduction in CI. The intracerebroventricular clonidine also caused a selective reduction in E for BHR and WKY rats in response to psychological stress.

Thus, the results of this study suggest that a combination of electromagnetic flowometry, in conscious normotensive and hypertensive

rats, in conjunction with neuropharmacological and behavioral techniques may provide valuable information regarding the role of various neurotransmitters in normal and abnormal cardiovascular control.

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APPENDIXES

APPENDIX A

LIST OF ABBREVIATIONS

Cardiovascular Variables

BP:	Blood pressure (mmHg) Systolic Diastolic Mean = Diastolic + $1/3$ (Systolic-Diastolic)
CI:	Cardiac index (ml/min $\times$ 100 g)
CO:	Cardiac output (ml/min) = SV $\times$ HR
dP/dt:	Maximum rate of change in pressure (P) with time (t), (mmHg/sec)
dP/dt/P:	Maximum rate of change in pressure (P) with time (t) divided by the instantaneous pressure (P), (sec^{-1})
HR:	Heart rate (beats per minute) or (bpm)
MW:	Minute work (mmHg $\times$ ml/min) = SW $\times$ HR
SI:	Stroke index (ml/100 g)
SV:	Stroke volume (ml) or (ml/beat)
SW:	Stroke work (ml $\times$ mmHg) = SV $\times$ mean BP
TPR:	Total peripheral resistance (mmHg $\times$ min/ml) = mean BP/CO
TPRI:	Total peripheral resistance index (mmHg $\times$ min $\times$ 100 g/ml)

Neural Structures and Terminology

CNS: Central nervous system

Spinal Cord:

DLF: Dorsolateral Funiculus

Medulla:

d-MOC:	Dorsal Nucleus Medulla Oblongata Centralis
GRN:	Gigantocellular Reticular Nucleus
ION:	Inferior Olivary Nucleus
LRN:	Lateral Reticular Nucleus
NA:	Nucleus Ambiguus
NC:	Nucleus Commissuralis
NPH:	Parahypoglossal Nucleus
NTS:	Nucleus and Tractus Solitarii
PRN:	Paramedian Reticular Nucleus
PvRN:	Parvocellular Reticular Nucleus
RF:	Reticular Formation
RN:	Raphé Nuclei
RNM:	Nucleus Raphé Magnus

Cerebellum:

FN:	Fastigial Nucleus
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Pontine, Midbrain Region:

LC:	Locus Coeruleus
NPB:	Nucleus Parabrachialis

Forebrain:

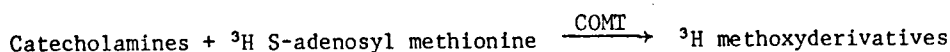
AH:	Anterior Hypothalamus
AN:	Arcuate Nucleus
AH/PO:	Anterior Hypothalamic/Preoptic Area

AV3V:	Anteroventral Third Ventricle
F:	Fornix
H:	Field H of Forel
LH:	Lateral Hypothalamus
LS:	Lateral Septum
MBN:	Midline Bed Nucleus
HC:	Hippocampal Commissure
MFB:	Medial Forebrain Bundle
NM:	Nucleus Medianus
MS:	Medial Septum
NIST:	Nucleus Interstitialis Striae Terminalis
OVLT:	Organum Vasculosum of the Lamina Terminalis
PAH:	Paraventricular Hypothalamic Nucleus
PH:	Posterior Hypothalamus
PMH:	Posteromedial Hypothalamic Nucleus
PON:	Preoptic Nucleus
PVN:	Periventricular Hypothalamic Nucleus
SON:	Supraoptic Nucleus
SM:	Stria Medullaris
VMH:	Ventromedial Hypothalamic Nucleus
ZI:	Zona Incerta

APPENDIX B

PROCEDURE FOR CATECHOLAMINE ASSAY

I.



Epinephrine and norepinephrine are converted to ${}^3\text{H}$ metanephrine and ${}^3\text{H}$ normetanephrine, respectively, during incubation with catechol-O-methyl-transferase (COMT) and ${}^3\text{H}$ S-adenosyl methionine (${}^3\text{H}$ -SAM). The corresponding methanephrines are extracted into acetic acid and washed with a series of toluene isoamyl washes. The metanephrine and normetanephrine are separated by thin layer chromatography (TLC). They are eluted from the TLC silica gel and oxidized to vanillin with sodium periodate. The vanillins corresponding to epinephrine and norepinephrine are extracted into a toluene-liquifluor solvent and counted in a scintillation counter. The output is proportional to the levels of catecholamines.

II. Reagents

1. THAM--Fisher T395
2. EGTA $\cdot$ Na₂--Sigma E3251
3. MgCl₂ $\cdot$ 6H₂O--Fisher M33
4. Glutathione--Sigma G4251
5. Boric Acid--Matheson Coleman & Bell (MCB) BX865,CB225
6. EDTA $\cdot$ Na₂--Mallinckrodt 4931
7. Metanephrine HCl--Sigma M8625
8. Normetanephrine HCl--Sigma 7127
9. Toluene--Fisher T-324
10. Isoamyl Alcohol--Fisher A-393
11. Glaceol Acetic Acid--Fisher A-38
12. Sodium Periodate--Fisher S-398
13. Glycerin--Fisher G33
14. Ammonium Hydroxide--Fisher A669
15. HCl--Fisher A144
16. Epinephrine--Fisher SO-F-148
17. Norepinephrine (100 $\mu\text{g}/\text{ml}$) BIORAD
18. ${}^3\text{H}$ SAM--New England Nuclear (5-15 μCi)
19. Ethanol--Chem Stores
20. Methanol--Chem Stores
21. Silica Gel Plates (10 $\times$ 20 cm 25 μ) ANALTECH
22. Benzene--Fisher B-245
23. Tert Amyl Alcohol--Fisher A-730
24. Methyl Amine (40%)--Fisher M223
25. Liquifluor--New England Nuclear NEF903
26. H₂SO₄ (pH 2):ethanol (9:1)

III. Daily Working Solutions

- A. Assay Buffer: Tris
Add 6.05 gm of THAM, 1.9 gm EGTA- Na_2 , and 3.05 gm $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ to approximately 20 ml of water.* Adjust pH to 8.3 with concentrated HCl or NaOH. The initial pH is close to this value. Bring volume to 25.0 ml with water.
- B. Glutathione Solution: .1 Molar
Add 30.7 mg of reduced glutathione to a small test tube and add 1 ml of water. Vortex lightly and keep on ice.
- C. Stopping Solution: 1.0 m Boric Acid + .2 m EDTA- Na_2
1. Add: 1.55 gm boric acid + .93 gm EDTA- $2\text{H}_2\text{O} \cdot \text{Na}$ to 20 ml H_2O .
2. Adjust pH to 11.1.
3. Bring to 25 ml with water.
- This solution can be prepared in advance and aliquots can be stored frozen.
- D. Metanephrine & Normetanephrine: .2 $\mu\text{moles}/50 \mu\text{l}$ of borate buffer, which is .934 mg/ml of Met and .88 mg/ml of NorMet. For every 5 ml of the stopping solution add 4.67 mg of metanephrine and 4.4 mg of normetanephrine. Prepare prior to use.
- E. Toluene-isoamyl alcohol (3:2)
Combine 300 ml of toluene and 200 ml of isoamyl alcohol.
- F. Acetic Acid: .1 N
Bring 2.95 ml of glacial acetic acid up to 500 ml with water.
- G. Sodium Periodate Solution: 4% w/v NaIO_4
1 gm of NaIO_4 up to 25 ml with water. Prepare fresh.
- H. Ammonium Hydroxide Solution: .05 M NH_4OH
1 ml of NH_4OH up to 300 ml with water.
- I. HCl: 1.0 N
1. 8.3 ml of concentrated HCl up to 100 ml.
2. .01 HCl: 1.0 ml of 1.0 N HCl to 100 ml.
3. .001 HCl: 1.0 ml of 1.0 N HCl to 1000 ml.
- J. Epinephrine & Norepinephrine Standards: 100 pg/10 μl
1. 200 μl of Fisher Epinephrine (50 $\mu\text{g}/\text{ml}$) and 100 μl of BioRad Norepinephrine (100 $\mu\text{g}/\text{ml}$) are brought to 50 ml with .01 N HCl in a volumetric.

*It is understood that distilled water is always used.

2. Bring 5 ml of the above (1) to 100 ml with .001 N HCl in a volumetric.
3. 10 μ l of (2) will give 100 pg of E & NE.

K. Enzyme: COMT

Prepared according to the procedure of Axelrod and Tomchick (1958).

L. H₂SO₄:Ethanol Solution: (9:1)

Prepare a H₂SO₄ solution of pH = 2. Simply add concentrated H₂SO₄ to water (ca 250 ml) until pH = 2. This will require only a drop or two. Use 9 ml of this for every 1 ml of Ethanol (95%).

M. Toluene--Liquifluor (1000:50 v/v)

Add 50 ml of Liquifluor to 1000 ml of toluene.

IV. Reaction

- A. Turn on water bath and set to 37°C.
- B. Prepare two sets of test tubes (13 × 100 mm). Each sample should be done in duplicate. The number of internal standards will depend on the type of experiment but a duplicate should be considered a minimum. Label tubes with a magic marker (not water based).
- C. Remove enzyme, samples, buffer, and standard plasma from freezer. Thaw and keep on ice. Thaw ³H SAM only before use.
- D. Prepare the enzyme cocktail in a round bottom 13 × 100 mm tube. Add in the following order in amounts sufficient for the assay plus 3 (see below): Buffer solution, glutathione and ³H SAM and H₂SO₄:Ethanol (9:1); vortex lightly; and add COMT enzyme.

Assay	×1	×20	×50	×100
Buffer	5 μ l	100 μ l	250 μ l	500 μ l
Glutathione	1 μ l	20 μ l	50 μ l	100 μ l
³ H SAM	5 μ l	100 μ l	250 μ l	500 μ l
H ₂ SO ₄ -Ethanol (9:1) [†]	5 μ l	100 μ l	250 μ l	500 μ l
Enzyme	24 μ l	480 μ l	1.2 μ l	2.4 μ l

[†] This solution is used to dilute the isotope. It is the solvent vehicle for the ³H SAM. If higher counts are required for the assay, this should be omitted and the quantity of ³H SAM indicated on the chart should be doubled (i.e., 10 μ l/tube).

E. Incubation mixture

The first set of tubes will receive the following:

1. Blanks

- a. 50 μ l of sample vehicle (i.e., water that is mixed with EGTA-Glutathione in the same concentration as that of blood samples).
- b. 40 μ l of enzyme cocktail (#4).
- c. 10 μ l of .001 N HCl.

2. Standards (internal)

- a. 50 μ l of plasma.
- b. 40 μ l of enzyme cocktail.
- c. 10 μ l of 100 pg standard.

3. Plasma samples

- a. 50 μ l of sample plasma.
- b. 40 μ l of enzyme cocktail.
- c. 10 μ l of .001 N HCl.

4. The plasma samples and blanks can be pipetted with the fixed volume 50 μ l positive displacement pipette. The tip does not have to be replaced after each sample.

5. The enzyme cocktail, .001 N HCl and standards can be pipetted with the Eppendorf repeater pipette. Please note correct setting and adaptor size for volume desired.

F. Incubation

75 minutes at 37°C in oscillating water bath.

G. Stopping the Reaction

Place reaction tubes in ice bath and add 50 μ l of the Borate stopping solution that contains the metanephrine and normetanephrine carriers (see section III-C and III-D).

Vortexing is not necessary. Gently shake tube rack.

V. Extraction

The second set of tubes will be used here. They will each receive 100 μ l of .1 N acetic acid (see section III-F).

- A. Add 2 ml of toluene-isoamyl alcohol (3:2) to incubation tubes. Vortex well and centrifuge.[†]

B. Quick freeze:

Prepare an ice bath of methanol and dry ice. Methanol level should only be deep enough to cover the bottom of test tubes (i.e., ca $\frac{1}{2}$ -1 inch).

[†]The centrifuging is done on setting 4 (centrifuge #1) and #7 (centrifuge #2) for ca. 1-2 min. Not critical.

1. Submerge tube to level of aqueous phase.
 2. Blot outside of tube. Decant the organic phase into its corresponding tube containing the .1 N acetic acid (see section V above).
 3. Rhythm: decant one tube while the next tube is in ice bath.
- C. Vortex and centrifuge the acetic acid, organic solution (see section V-B-2).
- D. Quick freeze and aspirate off organic phase without touching suction pipette to pellet.
- E. Add 1 ml of toluene-isoamyl alcohol to thawed aqueous phase.
- F. Vortex and centrifuge.
- G. Quick freeze, aspiration, and discard organic phase as in D above. The assay may be stopped at this point. Cap tubes and keep at -20°C .

VI. Thin Layer Chromatography

- A. Add 100-150 μl of ethanol to the thawed acetic acid from section V-G above (for the number of tubes in one spotting run, i.e., 14).
- B. Slowly pull acetic acid-ethanol solution into syringe. Do not pull excess air into syringe. Should have small bubble on the end of plunger.
- C. Turn on dryer and spotter.
- D. Place dryer approximately 3 cm from plates and set temperature control between 200-300.
- E. Position syringes so that tips touch TLC plates. Set plunger speed to slowest possible speed.
- F. Prepare 44 ml of developing solution (6:2:3) in a 50 ml graduate.
 1. 24 ml tertiary amyl alcohol.
 2. 8 ml benzene.
 3. 12 ml methylamine.
 4. mix gently (solution should be clear).
- G. Put developing solution in developing tank immediately before use. Carefully place plates in tank. Develop until solvent runs the entire plate (ca. 40-50 minutes).

- H. Dry plates and visualize spots under UV. Utilizing a scapel, carefully outline the spots. (Hint: best separation is obtained while plates are still somewhat damp.)
- I. Scrape spots into acid washed scintillation vials (use a scapel). The metanephrines are the top zone and normetanephrines are the bottom zone.

VII. Oxidation

- A. Add 1 ml of .05 M NH_4OH to vials. Vortex vigorously.
- B. Line up vials and add 50 μl of 4% periodate solution at time intervals. Vortex vigorously.
- C. Stop reaction by adding 50 μl of 10% glycerol solution, five minutes after step B. Maintain order and timing of step B.
- D. Add 1 ml of .1 M acetic acid to each vial and vortex.
- E. Add 10 ml of toluene-liquifluous (1000:50 v/v) to each scintillation vial. Shake by hand vigorously (for about 10 sec).
- F. Count each vial for 2-10 minutes.

VITA

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In the Fall of that year he moved to Knoxville and entered The University of Tennessee, where he completed the requirements for the degree of Doctor of Philosophy with a major in physiological psychology in 1983.

The author enjoys quiet evenings at home, good movies, all major sports, and devotion to his wife and career.

In 1980 he married Jeanne Marie Strobach, of Saratoga, California. They presently reside in San Antonio, Texas, where John was accepted as a postdoctoral fellow in the Department of Pharmacology at the University of Texas Health Science Center.