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NOREPINEPHRINE LEVELS IN DISCRETE BRAIN NUCLEI IN BORDERLINE HYPERTENSIVE RATS EXPOSED TO COMPOUND STRESSORS

A Dissertation
presented for the
Doctor of Philosophy
Degree
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Guanping Zheng

May, 1998

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DEDICATION

To the memory of my grandmother Li Gui-feng (1906-1976)

李桂鳳

whose love and caring accompanied my childhood

To my father Zheng Hua-chen and my mother Jiang Da-shu

鄭華成　蔣達淑

who give me the best value of life and
their best support for my education

To my wife Jiankun Wang for her love and support, and to my daughter Faye who is my constant source of joy.

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ABSTRACT

The borderline hypertensive rat (BHR), the first generation of an inbred cross between the normotensive Wistar-Kyoto rat (WKY) and the spontaneously hypertensive rat (SHR), appears to be an appropriate model for investigating the role of the environment in producing hypertension.

The central nervous system has been demonstrated to play important role in the regulation of cardiovascular function. Intensive studies using several animal models suggest that pathways that regulate blood pressure involve nuclei in the brain stem and hypothalamus, which may mediate the baroreflex, or influence sympathetic nervous system outflow. One particular neurotransmitter system, the norepinephrine (NE) system, has been suggested to be involved in the regulation of blood pressure. Its cell groups and the connections between them cover several regions in the hypothalamus and medulla oblongata.

Previous studies have demonstrated that the BHR shows chronic blood pressure elevations to both stress and high salt intake. Other studies suggest that interactions between

the brain and kidney play an important role in initiating this hypertension. The central noradrenergic system has been implicated in these effects, especially in the hypothalamus. Because exercise has been found to attenuate stress-induced hypertension in the BHR, the current study sought to examine the impact of compound stressors on central NE levels. Special attention was paid to the effects of exercise paired with other stressors (salt intake or stress).

Male BHR were exposed to either control, salt plus stress, salt plus exercise, or stress plus exercise conditions for either 2 or 6 months, beginning at 2 months of age. Following sacrifice, brain nuclei in the brain stem and hypothalamus were removed using the Palkovits micropunch technique. Punches were analyzed for NE content via high performance liquid chromatography with electrochemical detection.

Compared with the control condition, chronic salt plus stress led to reductions in NE content in selected brain nuclei, especially in the hypothalamus. Compared with salt plus stress, the exercise conditions were associated with elevated NE levels, especially in the early phases of exposure to the treatment. The possible role of exercise

training in preventing a central nervous system trigger from inducing hypertension in the BHR is discussed.

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LIST OF ABBREVIATIONS

SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
CHD	Coronary Heart Disease
SHR	Spontaneously Hypertensive Rat
WKY	Normotensive Wistar-Kyoto Rat
CS	Conditioned Stimulus
UCS	Unconditioned Stimulus
UCR	Unconditioned Responses
CR	Conditioned Response
PAG	Periaqueductal Gray
NTS	Nucleus Tractus Solitarius
IML	Intermediolateral Cell Column
SPNs	SYMPATHETIC PREGANGLIONIC NEURONS
Epi	Epinephrine
NE	Norepinephrine
GABA	Gamma-Aminobutyric Acid
NPY	Neuropeptide Y
PNMT	Phenylethanolamine N-Methyltransferase
RSNA	Renal Sympathetic Nervous Activity
CVLM	Caudal Ventrolateral Medulla
RVLM	Rostral Ventrolateral Medulla
LH	Lateral Hypothalamus
PH	Posterior Hypothalamus

DMH	Dorsomedial Nucleus Of The Hypothalamus
PVH	Paraventricular Nucleus Of Hypothalamus
SO	Supraoptic Nucleus
LC	Locus Coeruleus
Ant	Anterior Hypothalamus
Arc	Arcuate Nucleus
VMH	Ventromedial Hypothalamus

CHAPTER I

INTRODUCTION

According to the National Health and Nutrition Examination survey conducted during 1988-1991 (NHANES, 1993), there are about 50 million adults in the United States with hypertension, defined by the Fifth Joint National Committee (JNC, 1993) as a systolic blood pressure (SBP) of 140 mm Hg or higher and a diastolic blood pressure (DBP) of 90 mm Hg or higher. Looking at the dimension of aging, while only 4% of the youngest (age 18-24) have hypertension, the incidence is increased to 33% in middle age (45-54) and 60% in old age (65-74). Thus, although it has been stated that only about 20% of the US population has hypertension, most Americans will develop it at some point during their life time.

The criteria at or above which hypertension is defined are determined by estimating the risk associated with non-action taken vs. the cost and benefits associated with reducing blood pressure. Like most of the clinical criteria for diagnoses, the definition of hypertension as 140/90 mm Hg or higher is arbitrary. When referring to an individual,

there is no definitive level at which the blood pressure should be considered as normotensive or hypertensive. However, it is true in both populations at large and individuals that higher blood pressure predicts higher risk of cardiovascular disease. MacMahon (1990) performed a meta-analysis including nine studies relating DBP levels to the incidence of stroke and coronary heart disease (CHD), in which 420,000 people were followed up for 6 to 12 years, and 599 fatal strokes and 4,260 deaths from coronary CHD were recorded. They estimated that a DBP that is persistently higher by 5 mm Hg is associated with a 34% boost in stroke risk and at least a 21% increase in CHD. While the arbitrary criteria by which normotension and hypertension are classified provide a practical setting point for clinical practice, other factors should be taken into account when dealing with individual patients, including certain demographic features (e.g., gender, age, and race), pathology of vascular and other target organs induced by high blood pressure, and the presence of other risk factors.

Hypertension can be secondary to a number of causes, such as chronic renal disease, coarctation of the aorta, primary aldosteronism, Cushing's syndrome, etc. However, according to several surveys of hypertensive diagnosis

(Rudnieck et al, 1977; Sinclair et al, 1987), the cause of more than 90% of hypertension cases is not well understood. This large category is referred to as primary or essential hypertension. For hypertension that is secondary to other diseases, high blood pressure is an index of the related disease, and a cause of other physiological failures, such as stroke and renal or heart failure. For essential hypertension, high blood pressure is a risk factor for CHD, as suggested by MacMahon (1990).

Maintenance of appropriate blood pressure adequate to perfuse tissues is a key for survival. Quick responses to environmental stress require rapid allocation of physiological resources to meet a host of potential challenges. Many complex mechanisms have evolved to regulate blood pressure for the survival of the species. Understanding the mechanisms of blood pressure regulation is the key for determining the cause of essential hypertension. Decades of intensive research have accumulated mounting evidence regarding the role of the central and peripheral, nervous, hormonal, cardiovascular and renal systems in blood pressure control. There is no debate that the moment-to-moment stability of blood pressure is achieved by a feedback control system. For long-term regulation of blood pressure,

however, which is the key for understanding essential hypertension, the mechanisms are far less understood.

Two important systems are responsible for long-term blood pressure regulation (Cowley, 1992). One is the nervous system, which detects changes in arterial pressure and provides both rapid stabilization and long-term control of blood pressure, predominantly by adjusting the outflow of sympathetic tone, thus affecting both cardiac performance and peripheral vascular resistance. The second regulatory system is the kidneys, which adjust body fluid volume by pressure diuresis. In general, blood pressure is the outcome of synchronized functions of different systems that work to maintain homeostasis. Thus, it is reasonable to believe that hypertension could be the result of either a malfunction of the regulating systems, or an abnormality of the cardiovascular structure. Ferrario and Averill (1991) argue that it is a primary dysfunction in neural control of arterial pressure that contributes to hypertension. Detailed discussion will be presented in the subsequent sections.

While the mechanisms of essential hypertension are still being debated, research has identified several risk factors thought to contribute to its development.

I. Risk Factors of Essential Hypertension

It has been accepted by most psychologists that the development of personality is determined by the interaction between genetic and environmental factors. Although this cannot be a universal philosophy applicable to every etiological consideration, in many cases, including essential hypertension, the pathological causes involve genetic predisposition and risk factors from the environment.

Genetic Predisposition in Essential Hypertension

Three major approaches have contributed to our understanding of the genetic component of essential hypertension. Epidemiological investigations of familial aggregations represent a conventional approach to understanding genetic contributions to diseases. Pickering (1968) estimated that 33-64% of the variance in blood pressure is a result of hereditary factors, after a detailed review of the evidence from case records of single families, from family histories of a large group of patients, from blood pressure studies in twins and from blood pressure studies in relatives of patients with essential hypertension. Two decades later, Williams et al (1991) reported a similar estimate stating that genetic

contributions to blood pressure variability range between 25% in pedigree studies to 65% in twin studies.

The fundamental goal of family history studies is to partition blood pressure variability into familial and non-familial components, using intraclass correlations as the criterion. This approach provides information on the importance of genetic predisposition in essential hypertension, without clarifying what specific genetic traits contribute to the regulation of blood pressure and how environmental factors influence the development of hypertension.

Finally, animal models provide a necessary tool for studying the interaction between genetic and environmental factors in pathology. Animal inbreeding makes it possible to purify genomes for a phenotype. The spontaneously hypertensive rat (SHR) and the normotensive Wistar-Kyoto rat (WKY) developed by Okamoto et al (1963) are widely used animal models for hypertension research. The blood pressure distributions of SHR and WKY are distinctly separate at a very young age. SHR have higher blood pressure than WKY as early as 24 hours after birth (Smith, et al, 1984), and the difference remains at all ages (Gray, 1984). Another model

which will be discussed later was developed by Lawler and his coworkers (Lawler et al, 1980) to study the interaction between genetic predisposition and environmental factors.

Environmental Stressors in the Development of Hypertension

As summarized by Vasl (1996), there are three approaches toward the concept of stress. The first is the epidemiological approach, where the emphasis is on exposure conditions. In this approach, the identification of the defining properties of a stressor is often finessed by enumerating specific environmental conditions thought to be stressful. The second is the biological approach, which defines stress based on biological responses. In this model, finding defining properties of stress appears to be equivalent to postulating the existence of an integrated biological response pattern in the context of stress, and then selecting some specific aspects of this pattern to be critical characteristics for denoting the presence or absence of stress. The third one is the psychological approach which sees stress as a personal experience. Stress is a negative *experience* associated with threat, harm, or demand. Stress is an aversive state *perceived* by the individual, and the *psychological demand of adjustment* to the stressful situation. Each approach has its emphasis but

none is adequate.

Stress can be defined as an individual's psychic and somatic reactions to demands that approach or exceed the limits of one's coping resources. It is a disturbance of the homeostasis of the body and mind. A stressor, then, can be any event that evokes such a disturbance.

Physiological and Psychological Stress

Based on their impacts on one's physical or mental dynamics, environmental stressors can be classified as physical or psychological in nature. Some physical stressors, such as cold or heat, impose demands on an individual's physiological resources directly, without any intervening cognitive appraisal. Other physical and psychological conditions, such as competitive problem solving or conflict decision making, become stressors via cognitive appraisal and interpretation of the threat. It should be noted that these two classes of stressors are closely related.

In animal models, stress is operationally defined through careful experimental design in which animal behavior, cardiovascular responses and other physiological

parameters are observed, while unrelated variables are kept constant.

Psychological stress and hypertension. One of the earliest attempts to induce hypertension in animals was reported by Rosencrans et al (1967). Normotensive male albino rats were exposed to intensive stressors that lasted four hours each session and consisted of a combination of flashing lights, noise and cage vibration. After eight weeks of exposure for three, five or seven sessions per week, rats developed hypertension and maintained the high blood pressure level through the sixteenth week. Various psychological stress models have been employed to elicit cardiovascular responses and induce chronic hypertension. Table 1 summarizes the most commonly used stressors.

Table 1. Most commonly utilized stressors

Noise
Air jet
Minor electric shock
Restraint
Vibration
Same-species confrontation
Different-species confrontation
Classical conditioning
Aversive
Appetitive
Operant conditioning
Signaled avoidance
Unsignaled avoidance
Conflict paradigm

The criteria for a stimulus being considered a psychological stressor include its ability to evoke emotional discomfort and its minimum physical impact. Social confrontation, approach/avoidance conflict, and unpredictable aversive outcomes are some of life's psychological stressors. In animal research, several models

have been applied to investigate the role of psychological stress in the development of hypertension.

Ecological stressors. Ecological stressors are presented by introducing house aggregation, territorial conflict, and sexual or food competition. Using CBA mice, Henry and coworkers (1967) introduced a sophisticated model that enabled researchers to examine the cardiovascular effects of male-male competition, population density, territorial conflicts and predator threats. They observed elevations of systolic blood pressure after exposure to these stressors, among which territorial conflict produced the highest increase in systolic blood pressure. Similar, but more modest, results were reported by Alexander (1974) using similar protocols.

A later study also by Henry et al (1975) followed the time course of the pathophysiological changes that resulted from a sustained disturbance of the social environment in ten colonies of socially-deprived CBA mice. Sixteen formerly isolated males were placed with 16 normal females in population cages consisting of seven intercommunicating boxes. Six of these socially-disturbed 32-member colonies were studied for periods of interaction ranging from 2 days

to 9 months. The remaining four colonies were terminated a month or more after the males had been returned to individual isolation. The reversibility of the BP returning to control levels depended on the duration of exposure to the stress. After a short period of exposures (1 to 16 days), blood pressure reverted to normal in a few days. Exposure of 6 months or more was associated with sustained increases in heart weight and blood pressure readings. This was accompanied by evidence of aortic arteriosclerosis and myocardial fibrosis. These changes persisted despite prolonged return to isolation.

However, like other types of stress, the effect of social stress on blood pressure varies among strains with different behavior characteristics. Henry and coworkers (1993) reported that Sprague-Dawley rats showed only a moderate BP elevation (10 mm Hg) after 6-months of exposure to ecological stress, while in the Long-Evans strain, which is more aggressive, the blood pressure increase exceeded 20 mm Hg. Another strain, Wistar-Kyoto, hyperactive (WKHA) developed by Hendley et al (1991, 1992), showed no change in blood pressure. Social instability and the associated blood pressure changes were made more severe by periodic mixing of males from different colonies. This had no effect on the

peaceable WKHA rats, some effect on the Sprague-Dawley rats, and a severe effect on the Long-Evans rats. The WKHA rats failed to show blood pressure changes despite stress-induced increases in heart and adrenal weights. The authors suggest that different types of psychosocial stress and different genetics combine to induce a variety of neuroendocrine changes, not all of which necessarily lead to increased blood pressure.

Gelsema et al (1994) reported that 14-16 aggregations of 14-16 BHR, which induced fights, failed to produce high blood pressure. They argue that an aggressive disposition may be a stronger determinant of the hypertensinogenic effects of psychological stress in rats than their predisposition to hypertension, because a similar stress paradigm induced hypertension in aggressive Long-Evans rats, but had no effect on less aggressive BHR and WKHA rats.

Classical and operant conditioning. The core idea of conditioning was established by Pavlov through a series animal experiments in the late 1920's. Early research focused on response-independent situations in which animals were constrained and only the passive behavior or physiological variables in response to a stimulus were

recorded. Later, attention turned to the observation of the active performance of animals in response to environmental factors, known as operant conditioning. Several stress paradigms based on classical (response-independent) conditioning, or operant (response-dependent) conditioning have been used in experimental hypertension research in a variety of animal models.

The basic procedures of classical conditioning involve systematically pairing a conditioned stimulus (CS) (e.g., a tone or a light), which elicits no apparent behavioral or physiological responses, with an unconditioned stimulus (UCS) (e.g., electric shock), which evokes profound unconditioned responses (UCR), such as seeking to escape or preparing to fight. After a sufficient number of CS-UCS pairings, the CS acquires the capability to elicit behavioral and physiological responses (conditioned response, CR). Thus, a temporal contingency between the CS and UCS has been learned, such that the previously neutral CS now evokes behavioral and physiological responses in anticipation of the UCS.

Classical conditioning provides a tool for evaluating sympathetic and parasympathetic control of cardiovascular

function by observing changes in blood pressure and heart rate. One example of such research was undertaken by Hatton et al (1981). In both the SHR and WKY, heart rate deceleration and blood pressure elevation were induced by the presentation of a neutral tone. After 30 paired CS (tone) and UCS (shock) trials, SHR showed heart rate deceleration and blood pressure elevation in response to the shock. The WKY showed a smaller pressor response, but a significant increase in heart rate. Bradycardic responses in SHR, but not WKY, were blocked by the parasympathetic muscarinic antagonist methyl atropine. This suggests the presence of enhanced sympathetic activity in the SHR in response to stress.

Acute cardiovascular alterations have been explored in relation to various UCS and CS modalities, such as intensity, temporal pattern and duration. Stress levels could vary depending on the patterns of classical conditioning. Lawler et al (1993a) investigated the relationship between the predictability of signaled shock and the phasic blood pressure of SHR, BHR, and WKY rats. Predictability was manipulated by altering the percentage of trials during which foot shock was associated with a tone, while at the same time keeping the total number of foot

shocks equal in all groups. For the most predictable situation, shock always occurred midway through the tone-on period.. For the group with the least predictability, 75% of the shocks occurred midway through the tone-on period, but 25% of the shocks occurred midway through the tone-off period. In the third group, shocks occurred with equal frequency in the tone-on and tone-off periods. During the first few trials, the most unpredictable foot shock was associated with higher blood pressure, a trend that was significant for the borderline hypertensive rat (BHR) and marginal for the spontaneously hypertensive rat (SHR). It was also noted that, over the 28 foot-shock trials comprising the stress session, habituation of the blood pressure response was obtained for the most unpredictable shock group. Again, this occurred in those rats with a genetic history of hypertension (SHR and BHR).

The classical conditioning procedure provides a valuable means of real time observation of stress-induced nervous activity. For example, Li et al (1997) observed changes in renal sympathetic nerve activity, mean arterial blood pressure and heart rate following the onset of a tone which had previously been paired with shock. They found that the tone-induced pressor response was preceded by a sudden

burst of renal sympathetic nerve activity averaging 177 +/- 22% over baseline in SHR versus 105 +/- 13% for the WKY, suggesting that control of sympathetic and parasympathetic nervous activity during a psychological stressor differs remarkably in hypertensive and normotensive subjects. For the most part, investigations of the cardiovascular concomitants of Pavlov's conditioning procedures have focused on acute phasic changes during the CS-UCS interval.

Operant conditioning refers to procedures in which the presentation of a specific stimulus (e.g., food or shock) is made contingent upon the rate or magnitude of some specified response (operant). Different schedules can be arranged between a stimulus and an operant. In a continuous schedule, the stimulus is presented after each specified behavior (e.g., bar pressing). Ratio-based schedules require animals to respond a certain number of times before a stimulus can be delivered or terminated. In a temporal schedule, a stimulus is presented only after a specified time interval.

Within the operant conditioning framework, the avoidance paradigm has been widely used in various animal models. In this procedure, animals receive an electrical shock at a regular interval unless a response occurs within

that interval. An appropriate response (e.g., lever-press) avoids the shock. For example, a study by Lawler et al (1975) observed the cardiovascular response in dogs during pre-avoidance and avoidance (lever-press) periods. During the pre-avoidance period, an increase in blood pressure accompanied by increased total peripheral resistance was observed, while during avoidance performance, increases in heart rate, cardiac output and blood pressure were seen.

Corley and his colleagues (1975, 1977, 1979) adopted the operant paradigm to study stress-induced myocardial pathology. The procedure of unsignalled shock avoidance was used in which squirrel monkeys in the experimental group learned to press a lever in order to avoid shock. The control group experienced the same shock delivered to the experimental monkeys but did not have any control over the shock delivery. Results showed different pathological patterns between the experimental and the yoked control animals. While fuchsinophilia, necrosis and fibrosis were most evident in the experimental group, bradycardia and ventricular arrest mostly occurred in the yoked group.

A conflict paradigm has been used by Lawler and coworkers (1980a, 1980b, 1981) in studying stress-induced

hypertension in BHR. In the conflict situation, animals were shocked ten times for failing to turn the wheel and once for turning it. Over a period of 12 weeks, BHR subjected to the conflict stress developed hypertension with a systolic blood pressure above 180 mmHg. In another study, twenty-four male WKY and 24 male BHR were randomly assigned to 3 groups of 8 each. Experimental animals were subjected to 2 hr daily of shock-shock conflict. Yoked animals received the same shocks as experimental but had no control over their presentation. Finally, a control group for maturation received no shocks. The experimental and yoked animals were subjected to 14 weeks of conflict and were then monitored an additional 14 weeks in the absence of shock. All animals had their tail cuff blood pressures taken weekly except for 3 times when bloods were obtained for PRA assays. Analysis of blood pressure data revealed that: (1) BHR animals showed more of a blood pressure response to shock than WKY animals; (2) yoked animals showed more of a response to conflict than the experimental group, especially for the BHR group; and (3) BHR shocked animals remained permanently elevated compared to BHR control animals even in the 14 week post-conflict period during which no shocks were given. Although plasma renal activity for BHR animals was significantly higher than for WKY at the beginning of study, the stress-induced

hypertension was associated with either normal or suppressed plasma renal activity values, suggesting that the hypertension in these animals is not a high renin hypertension.

High Salt Intake and Hypertension

The adaptive function of food preference and aversion. In the perspective of evolutionary psychology, both the structure and behavior of organisms are shaped by selective pressures over many generations. The function of those gradual changes allows the individual to solve the environmental problems it faces. One of the major problems that every species and each individual confronts is to find adequate food for survival. Meanwhile, the organism needs to obtain relatively balanced nutrients and to avoid toxins from their food sources. The behavioral organizations for this function are food preference and food aversion, which are the behavioral mechanisms by which one chooses appropriate nutritional elements to meet the growth, development and homeostatic requirements and distinguishes safe from toxic food. The human perceptual system for evaluating foods is so designed that some tastes and smells

are favored while others are avoided. The favorable sweet taste is a cue for carbohydrates, while bitter tastes or pungent odors in plants indicate high levels of toxins, and foul odors in animal foods suggest the presence of toxic bacteria. Some physiological reflexes such as nausea and vomiting function to expel food that could be toxic.

Sodium chloride is an essential nutritive element and sodium is the major cation in every cell in the body. Intracellular sodium content determines fluid osmotic pressure, acid-based balance, and extracellular fluid volume, thereby determining the integrity of the blood circulation and the chemical milieu in which the tissues function. Thus, a salt appetite has survival value by providing a mechanism for sodium balance.

It has been suggested that during years when salt was scarce, hunger for salt could lead humans to obtain it in amounts that just met the requirements for maintaining homeostasis. However, in modern times this hunger will lead to overconsumption of salt. Human bodies have been calibrated over millions of years of evolution for small amounts of salt, but today the high dosage breaks this calibration. The health problem caused by the strong salt

appetite shows the power of selective pressure that is working to re-calibrate the salt regulatory system. A study by Wilson (1986) links the history of salt supplies in ancient West Africa to the blood pressure today. Historical data reveal that in Senegal and Gambia salt production has been extensive since ancient times, whereas in ancient Nigeria the population had to depend on local vegetarian salt or meager imports of the mineral. He found that Nigerians have higher blood pressure than Senegalese and Gambians. This research indicates that what is today a genetic predisposition to a serious health problem resulted from evolutionary pressure to solve a specific adaptive problem. The design feature is no longer adaptive in an altered situation.

Evidence from epidemiological and experimental studies suggests that high salt intake is a major risk factor in the development of hypertension.

High salt intake could lead to blood volume expansion due to the limitation of the kidney's capacity to excrete excessive sodium. This would result in sodium and water retention and blood volume expansion (Guyton et al, 1964). Augmented venous return increases cardiac pre-load,

reflexively enhancing cardiac output and elevating arterial blood pressure. Under normal conditions, this increased cardiac output is accompanied by increased peripheral resistance, and thus blood pressure. By elevating blood pressure and thus accelerating renal sodium and water excretion, blood volume decreases until blood pressure returns to normal. However, sustained, excessive heavy salt load and water retention lead to adaptive peripheral vascular changes, leading to a sustained elevation of peripheral resistance and blood pressure.

On the other hand, high salt may alter renal function in response to environmental challenges. For example, in a study in Lawler's laboratory (Sanders et al, 1988), WKY and BHR were exposed to either a normal (1%) or high (8%) salt (NaCl) diet for 8 weeks before being subjected to tail-shock stress. Compared to BHR on normal diet, BHR fed 8% NaCl showed a significant elevation in systolic blood pressure (186 mmHg) and a decrease in urinary sodium excretion and renal plasma flow during stress. Similar results were reported by Zheng et al (1995).

Renal function is regulated by nervous and hormonal systems at both central and local levels. Thus, excessive

salt intake may affect renal function through nervous and/or hormonal mediators that eventually elevate blood pressure. An increase in renal sympathetic nervous activity (RSNA) could contribute to the antinatriuretic response to stress in BHR fed a high salt diet. Simultaneous recording showed significant increases in RSNA associated with antinatriuresis, while renal denervation abolished the antinatriuretic response (DiBona and Jones, 1995). Typically, acute stress increases arterial blood pressure, heart rate and cardiac output, which results in a reflex response of natriuresis and diuresis to bring blood pressure back to steady-state levels. The antinatriuresis induced by acute stress in high salt BHR could result in an expansion of circulatory volume that may contribute to the development of hypertension.

The failure of reflex control of volume by the kidneys could be the result of a defect in cardiopulmonary baroreceptor reflex control of peripheral sympathetic nerve activity (Ferrari et al, 1984). DiBona and Jones (1995) reported that the stress-induced augmentation in RSNA in BHR fed high salt was so profound that the cardiopulmonary reflex-mediated decrease in RSNA was masked by its influence. The mechanisms by which a stress-induced increase

in RSNA exceeds the volume-expansion-induced decrease in RSNA remain unclear. Evidence from SHR and WKY (Huang and Leenen,. 1995) found that high sodium diet desensitized cardiopulmonary baroreflex control of RSNA in both SHR and WKY, with greater desensitization in SHR. It can be suggested that in high salt BHR there is a desensitization of cardiopulmonary reflex control of RSNA, and thus a stress-induced increase of the central sympathetic outflow to kidneys can be fully unmasked. In Zheng et al's study (1995), the BHR showed decreased RPF when exposed to acute stress, whereas WKY showed similar renal plasma flow responses only after 8 weeks of high salt loading. This result suggests that BHR have higher sympathetic responses to stress, which may contribute to their development of hypertension.

High salt intake may elevate blood pressure through hormonal regulation. For example, natriuretic hormone reduces tubular reabsorption of sodium by inhibiting the Na^+-K^+-ATPase, leading to natriuresis and diuresis. de Wardener and MacGregor (1983a, 1983b) proposed that the plasma's ability to inhibit Na^+-K^+-ATPase causes a reduction in the kidney's capability to excrete sodium, leading to blood volume expansion and blood pressure increase. The

hormone also inhibits Na^+-K^+-ATPase in other types of cells including vascular smooth muscle cells, so that the intracellular sodium concentration rises in these cells. Then, because the vascular smooth muscle cells contain a Na/Ca exchange transport system in their plasma membranes, more calcium than normal will be delivered to these cells. This will cause increased contractility and reactivity, thereby increasing vascular tone and peripheral vascular resistance and, ultimately, blood pressure (Blaustein, 1985).

Exercise in the development of hypertension

It is a popular belief that exercise is beneficial to health. Several studies have observed that low levels of physical activity represent an independent risk factor for CHD or mortality in the general population (Paffenbarger, et al, 1986; Sandvik et al, 1993). Ekelund et al (1988) followed 4276 men, 30 to 69 years of age, for an average of 8.5 years and concluded that a lower level of physical fitness is associated with a higher risk of death from CHD and cardiovascular disease in clinically healthy men, independent of conventional coronary risk factors. A recent

study by Lakka et al (1994) found that higher levels of both leisure-time physical activity and cardiorespiratory fitness had a strong, graded, inverse association with the risk of acute myocardial infarction, supporting the idea that lower levels of physical activity and cardiorespiratory fitness are independent risk factors for CHD.

Exercise has been touted as non-pharmacological treatment for high blood pressure. In a recent meta-analysis involving 166 studies, Linden et al (1994) compared the effectiveness of several pharmacological treatments with various non-pharmacological treatments. The latter included weight reduction, exercise, reduced sodium and alcohol intake, potassium supplements and relaxation treatments. Of these, exercise produced the largest effect size. Veras-Silva et al (1997) reported the effects of low and high intensity exercise training on resting blood pressure, cardiac output, and total peripheral resistance. They found that low-intensity, but not high-intensity, exercise training decreases heart rate and cardiac output and, consequently, attenuates hypertension in SHR.

The nature of exercise differs according to its intensity, duration and mode. Nieman and his associates have

studied the relationship between exercise and immunological response for many years. In a recent review, Nieman (1997) summarizes the findings and reveals that acute exercise bouts of moderate duration (< 60 min) and intensity (< 60% VO2max) are associated with fewer perturbations and less stress to the immune system than are prolonged, high-intensity sessions. Data also suggest that unusually heavy acute or chronic exercise may increase the risk of upper respiratory tract infection, while regular moderate physical activity may reduce symptomatology of upper respiratory tract infection (Nieman et al, 1990).

The role of exercise in the development of environmentally-induced hypertension has been investigated in BHR and other animal models. One such study (Cox et al, 1985) tested the hypothesis that exercise is capable of altering the development of hypertension in this model. Two groups of BHR (8 rats per group) were subjected to 2 hours of daily, predictable, uncontrollable tail shock for 12 weeks. One of these groups was also given 2 hours of daily swim stress (exercise/stress group). A third group served as a maturation control and received neither intervention (n=7). After 12 weeks of stress, direct recording of blood pressure revealed that systolic and diastolic blood

pressures in the shock only group were significantly higher than in both of the other groups. The control group differed from the exercise-trained/shocked group only in diastolic blood pressure. During a short-term stress session, plasma norepinephrine levels in the exercise/stress group were significantly lower than those in the shock only group. Thus, swim-training appears to reduce the severity of stress-induced hypertension in the BHR. An alteration of autonomic function resulting from the exercise training may be related to the resistance to stress-induced hypertension in the exercise/stress group. A later study (McCoy et al, 1993) examined the effect of swim training on renal and cardiovascular responses to stress using the same BHR model. The results suggest that swim training alters the effect that stress exerts on renal and cardiovascular function in BHR, which results in better fluid and electrolyte conservation in trained BHR.

Another study (Melby et al, 1991) sought to determine whether exercise might also exert a similar protective effect on salt-induced hypertension. Two groups of BHR were placed on a high salt diet for 20 weeks; one of the groups also engaged in daily swim training for the entire 20 week period. A third group of BHR served as maturation controls.

At the end of the experimental period, direct measurement of heart rate in conscious subjects revealed a significant resting bradycardia in the trained group on a high salt diet but no significant group differences with respect to blood pressure level. Cardiovascular responses to an episode of acute stress revealed that BHR are capable of elevating pressure in response to a novel stressor after swim training. These observations suggest that exercise may be an environmental intervention capable of increasing cardiovascular responses to acute stressors.

Taken together, these studies suggest a positive role of exercise on cardiovascular and renal function in BHR. In addition to attenuating the development of environmentally-induced hypertension in BHR, exercise allows the organism to produce biological responses to exogenous challenges that may be adaptive in nature.

II. Role of Central Nervous System in Hypertension

The cardiovascular system plays an essential role in the maintenance of homeostasis, and in the appropriate reallocation of resources when dealing with environmental

challenges. The neural system that regulates cardiovascular function consists of negative feedback loops operating at different levels of the central nervous system (CNS), as well as a central command function. Arterial blood pressure, circulating blood volume, cardiac output, and arterial gas tensions are monitored by various peripheral receptors, which project to the CNS, resulting in central modulation of autonomic outflow to the cardiovascular and endocrine systems. Dysfunction in either the CNS or cardiovascular system could contribute to essential hypertension. It has been suggested that neural imbalance at peripheral and/or central levels (Reis, 1981; Ferrario, 1991) and structural adaptation of the cardiovascular system due to prolonged disturbances (Folkow, 1989) may result in a chronic elevation of blood pressure.

Peripheral input and the role of the nucleus tractus solitarius

Regulation of blood pressure is achieved by interactions between peripheral receptor activation and a complex central network composed of several brain regions and neurotransmitter systems. Figure 1 depicts a simplified outline of the neural control of the cardiovascular system.

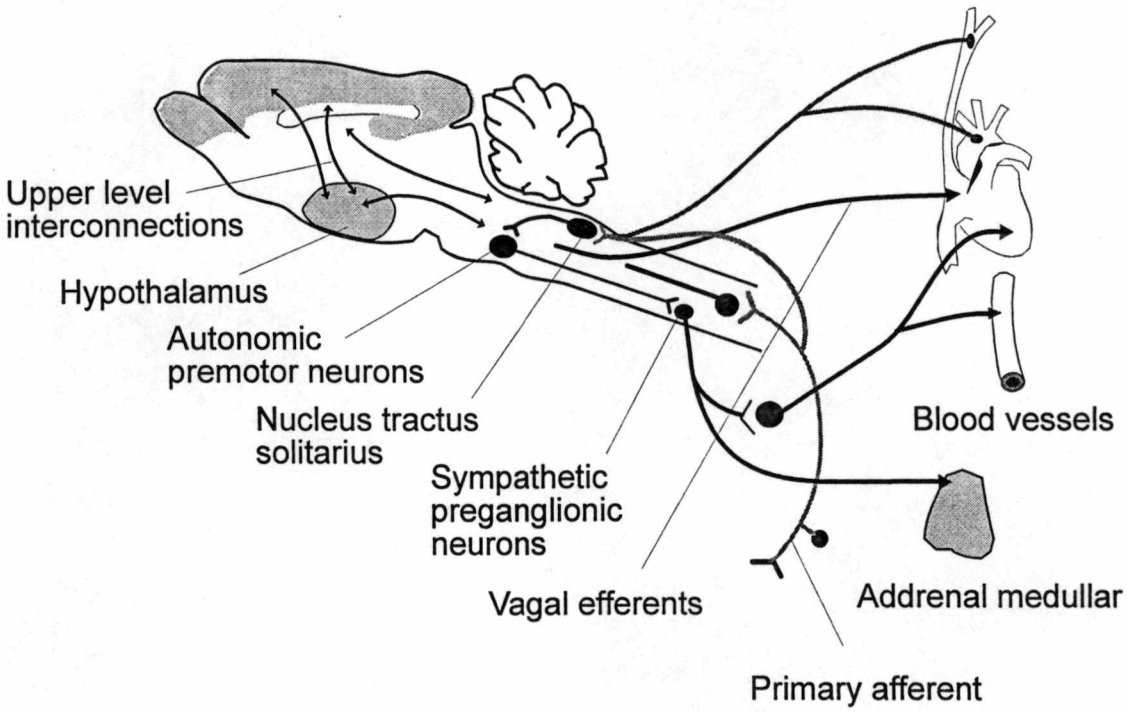

Figure 1. Schematic representation of the neural control of the cardiovascular system.

The initial level of neural regulation of blood pressure is reflex control, which is mediated by both mechanoreceptors and chemoreceptors. Detailed reviews characterizing their functions have been made by Kirchhein (1976), Spyer (1981; 1990) and Smith and Thames (1994). Mechanoreceptors in the aortic arch and carotid sinus, known as arterial baroreceptors, monitor arterial pressure changes by sensing vessel wall distortion. They transmit this information through the sinus nerve, a branch of the glossopharyngeal nerve, to the nucleus tractus solitarius (NTS) of the medulla. Additional baroreceptors, known as cardiac receptors, are located within the heart. Another class of receptors responds to changes in oxygen, carbon dioxide, and acid levels of the blood. These chemoreceptors include one group in the carotid and aortic bodies and others in the ventricles, atria, great veins, pulmonary artery and aorta. Afferent fibers from both classes of receptors are all part of the glossopharyngeal and vagal nerves, and all terminate in the NTS (Spyer, 1981).

Abnormalities of reflex control of blood pressure have been considered as possible factors related to the development of hypertension. It has been demonstrated that receptors exposed to maintained alterations of arterial

pressure are reset, an effect characterized by a parallel shift of the stimulus-response curve of sinoaortic pressure vs. aortic pressure. Early in the 1950's, McCubbin (1958) first described the resetting of the carotid and aortic receptors. By using electrophysiological recording of carotid and aortic nerve activity in renal hypertensive dogs, these studies revealed that the set point of the baroreceptors rose progressively as hypertension developed. Evidence of resetting has also been reported in other species, including rabbits (Dorward et al, 1982), rats (Mifflin and Kunze, 1982; Moreira et al, 1989) and humans (Ludbrook et al, 1976). One possible mechanism for the resetting may be adaptation of the receptors after prolonged activation (Munch et al, 1983; Heesch et al, 1985). Another possibility is a change in the elastic characteristics of vessel walls (Saum et al, 1976), which would result in an elevated threshold for the activation of the baroreceptors.

The NTS plays a key role in the regulation of the baroreflex in the CNS. It receives afferent input from both baro- and chemoreceptors, which constantly provide mechanical and chemical information from the cardiovascular and respiratory systems. Other afferent inputs to the NTS arise from nociceptors in the skin and chemoreceptors in

both skeletal muscle and the kidney.

The NTS has broad connections with other areas of the CNS. Wheat germ agglutinin-horseradish peroxidase (WGA-HRP) injections into the periaqueductal gray (PAG) of the cat revealed that this region has an extensive set of afferent and efferent connections with NTS (Bandler and Tork, 1987). Using a virus as a retrograde tracer, Gieroba et al. (1991) marked broad areas of the CNS which appear to connect with the NTS. In this study, herpes simplex virus type 1 was injected into the aortic nerve. Virus-positive cells were found ipsilaterally within the NTS, area postrema, caudal and rostral ventrolateral medulla, the spinal trigeminal complex, raphe nucleus, A5 area, locus coeruleus, parabrachial nucleus, PAG, ventrolateral hypothalamic area, paraventricular nucleus, amygdala, bed nucleus of the stria terminalis, and insular cortex, all of which may have reciprocal projections to the NTS. For example, reciprocal projections have been found from NTS to the ventrolateral medulla (Ross et al, 1985; Dampney, 1987; Otake et al, 1993), the parabrachial nucleus (Spacer, 1982; Herbert, et al., 1990), lower brain stem regions and spinal cord (Loewy and McKellar, 1978), forebrain areas such as the hypothalamus and amygdala (Ricardo and Koh, 1978), and the

olfactory tubercle (Guevara-Aguilar et al, 1987).

The NTS functions to modulate central signal transmissions from upper levels of the brain. One well-documented phenomenon is the elicitation of the defense reaction, including fight/flight behavior and associated cardiovascular changes, in response to stimulation of the perifornical region of the hypothalamus. One result of this stimulation is the inhibition of the baroreflex (Coote et al, 1979; Jordan, 1990). Mifflin et al (1988) reported an in vivo investigation of the effects of stimulation within the hypothalamic defense area (HDA) on the activity of neurons in the NTS. HDA stimulation exerted marked influences on NTS neurons. Approximately two-thirds of the NTS units receiving a carotid sinus nerve input were inhibited by HDA stimulation. All units shown to receive an excitatory input from carotid sinus baroreceptors were inhibited by HDA stimulation, whereas a much smaller percentage of baroreceptive units were inhibited from hypothalamic stimulation outside the defense area. These results clearly show that HDA excitation inhibits the NTS excitation normally evoked by peripheral receptors.

Intracellular recordings from the same study revealed

that HDA stimulation evoked a long-lasting hyperpolarization of membrane potential that resulted from postsynaptic inhibition (rather than dysfacilitation). The HDA-evoked inhibitory post-synaptic potential (IPSP) 'shunted' the evoked excitatory post-synaptic potential (EPSP) when the stimulus was timed to occur during the initial peak hyperpolarizing phase of the HDA-evoked IPSP. HDA stimulation disinhibited cells receiving an inhibitory input from the carotid sinus baroreceptor. These results explain, at the intracellular level, the mechanism for the central suppression of the baroreceptor reflex that forms an integral part of the defense response.

Central regulation of blood pressure is achieved by modification of autonomic outflow to the cardiovascular system and by activation of the endocrine system. Sympathetic preganglionic neurons (SPNs), which are located in the intermediolateral cell column of the spinal cord (IML), the adjacent white matter of the lateral funicularis, the intercalated cell group between the IML and the central canal, and a cell group lying dorsolateral to the central canal (Coote, 1988; Cabot, 1990), are the final nodes that receive information from multiple levels of CNS processing and execute the sympathetic command to the target organs. As

Coote (1988) described, the sympathetic preganglionic nuclei are controlled by descending pathways arising from various nuclei in the brain stem, hypothalamus and other upper levels of the brain. The importance of the sympathetic contribution to the etiology of primary hypertension has been well recognized. The first generation of antihypertensive agents, such as propranolol (Eckberg et al, 1976; Takeda and Bunag, 1980) and clonidine (Kobinger, 1983), lower blood pressure by binding on the alpha-2-adrenoreceptor to reduce sympathetic drive. The second generation of antihypertensives, such as rilmenidine and moxonidine, reduce blood pressure by acting on the newly found imidazoline receptor also to reduce sympathetic tone (Schafer, et al, 1995). The action of these drugs suggests a role for the sympathetic nervous system in the development and maintanance of hypertension.

Direct Projections from the Brain to Sympathetic Preganglionic Nuclei: Role of the Rostral Ventrolateral Medulla

By injecting tracers into the IML cell column and then mapping the brain areas that are labeled, researchers are able to identify the nuclei from which direct projections

reach the IML cell column (Amendt et al , 1979; Loewy, 1981a; Loewy and McKeller, 1981; Helke et al, 1986; Miura et al 1983; Sasek and Helke, 1989). Tracers injected in the IML at levels of the thoracic or lumbar spinal cord can be mapped in the medulla, pons, and several forebrain areas.

As mentioned above, another advanced technique which uses a virus as a labeling tracer provides a powerful tool for studying the pattern of anatomical organization of the CNS (Strack et al, 1989). The virus (herpes or pseudorabies varieties) crosses the synapse and is transported back to the cell bodies of the neuron that project to the injection sites. In a study from Loewy's group (Spencer et al, 1990), pseudorabies virus was injected into various sympathetic ganglia and the adrenal gland in rats. This produced immunohistochemically-detectable retrograde viral infections of ipsilateral SPNs and transneuron infections of the specific sets of second order neurons in the spinal cord and brain that innervate the infected SPNs. Combined with other functional studies utilizing stimulation and ablation, researchers have identified several central neuron groups that are important in the central regulation of blood pressure.

It is well documented that the rostral ventrolateral medulla (RVLM) plays an important role in the tonic and phasic regulation of blood pressure. The RVLM corresponds to the C1 adrenaline-containing neuron group, which was first described by Hokfelt et al (1973). In fact, cells in this region also contain other types of neurotransmitters, such as gamma-aminobutyric acid (GABA), glutamate, enkephalin, and neuropeptide Y (NPY) (Chalmers and Pilowsky, 1991). Early in the 1970s, Feldberg and Guertzenstein (1972) produced significant reductions in arterial pressure by bilateral application of either pentobarbital sodium or nicotine to the ventral surface of the medulla (Feldberg, and Guertzenstein, 1976), or by destruction of the ventral surface of the medulla (Guertzenstein and Silver, 1974). Subsequent research from Ross et al (1984) and McAllen (1986a; 1986b) using electrical or chemical stimulation elicited pressor responses.

By utilizing anterograde tracers, researchers have mapped the projections from the C1 region to the IML and specified its termination in sympathetic preganglionic nuclei (Dampney et al., 1987; Ross et al., 1984). It is believed that the C1 area contains a majority of epinephrine cell bodies (Dahlstrom and Fuxe, 1964; Hokfelt et al, 1974; Palkovits,

1978). However, the specific neurotransmitter which is responsible for the pressor effect has not been clearly defined (Chalmers et al, 1992). Evidence indicates that neurons containing the enzyme phenylethanolamine N-methyltransferase (PNMT) are responsible for the pressor effect (Dampney and Moon, 1980; Dampney et al, 1985). Others have demonstrated that administration of epinephrine in the area containing sympathetic preganglionic neurons can attenuate the discharge of these neurons (Guyenet and Cabot, 1981; Guyenet et al, 1982), and neither alpha nor beta-adrenoreceptor blockade has an effect on pressor responses elicited by stimulation of the RVLM (Mills et al, 1988). Connor and Drew (1987) reported that inhibition of PNMT did not affect pressor responses elicited by rostral ventrolateral stimulation. Other putative neurotransmitters, such as glutamate (Sun et al, 1988; Guyenet et al, 1989), NPY and substance P have also been implicated. It is possible that neurons in the C1 area are functionally heterogeneous and that their ultimate effect on sympathetic outflow depends on the properties of the SPNs and/or the final information processing in these neurons.

The RVLM receives input from the NTS, caudal ventrolateral medulla (CVLM), midbrain PAG, and lateral and

paraventricular nuclei of the hypothalamus (Dampney et al, 1982; 1987; Granata et al, 1985; Carrive et al, 1988; Somogyi et al, 1989). Other lesser inputs arise from the central amygdaloid nucleus and the medullary raphe nuclei (Takayama et al, 1990; Carrive et al, 1988). However, the evidence that these inputs involve cardiovascular control is incomplete. Studies utilizing anterograde transport of tracers can not provide precise information that these projections synapse with cardiovascular neurons, since there may be other functional RVLM inputs, such as those serving respiration and pain modulation. It can be assumed that these pathways form a complex network that integrates circulatory and behavioral responses.

The Inhibitory Input from Caudal Ventrolateral Medulla to RVLM

Another important region in the regulation of blood pressure is the CVLM located between the nucleus ambiguus and lateral reticular nucleus. Stimulation of this area elicits a decrease in blood pressure and heart rate (Feldberg, 1976; Willette et al, 1987; Drolet et al, 1993). Inhibition of this area results in an increase in blood pressure (Blessing and Reis, 1983; Drolet et al, 1993), suggesting that these cells are tonically active. Some

observations suggest that A1 noradrenergic cells, which were first described by Dahlstrom and Fuxe (1964), may be responsible for the depressor response (Granata et al, 1985; Willette, 1987). However, recent studies indicate that this may not be the case. For instance, the sympathoinhibitory pathway originating in the CVLM includes an inhibitory GABAergic synapse with RVLM neurons (Willette et al, 1984; Blessing, 1988), and there may be reciprocal control from the RVLM to the CVLM, because the CVLM depressor region can be antidromically activated from the RVLM (Agarwal and Calaresu, 1991; Cieroba et al, 1992).

Anatomical studies have demonstrated the connection between CVLM and RVLM by using phaseolus leucoagglutinin and herpes simplex virus as anterograde tracers to map the structure (Li et al, 1992). Another study by Reis's group (Jeske et al, 1995) used the double labeling method to trace the CVLM-RVLM-IML arc. They found double labeling in the RVLM after administration of the anterograde tracer biocytin into the barosensory sympathoinhibitory region of the CVLM and the retrograde tracer wheat germ-agglutinate apo-horseradish conjugated to gold particles into the IML, suggesting a direct synapse from CVLM to RVLM.

There is evidence to indicate that the CVLM may mediate baroreflex regulation of blood pressure. For instance, injection of D-2-amino-5-phosphonovaleric acid, an N-methyl-D-aspartate receptor antagonist (Gordon, 1987), or the glutamate antagonist kynurenic acid (Guyenet, et al, 1987) into this area blocked the baroreflex response. Lesion of the rostral area of the CVLM abolished the baroreceptor reflex and eliminated the synchronization of sympathetic nerve activity to the cardiac cycle (Cravo et al, 1991). As previously discussed, anterograde and retrograde tracing studies demonstrate direct connections between the NTS and CVLM (Ross et al, 1985; Sun and Guyenet, 1986).

Another study by Paton et al (1991) suggests that the ventrolateral medulla may provide a source of tonic excitatory drive to rhythmically firing neurons in the cardiovascular nucleus tractus solitarius of the rat. Calaresu and associates (Agarwal et al, 1989) reported that neurons in RVLM are inhibited by chemical stimulation of CVLM. Further observations (Agarwal et al, 1990) indicate that inhibition of the RVLM by baroreceptor activation is relayed through the CVLM. They observed that electrical stimulation of the NTS silenced the activity of neurons in the RVLM, and application of kynurenic acid into the CVLM

blocked the inhibition in the RVLM. It was suggested that projections from CVLM may not reach the SPNs in the IML. Rather, its depressor effect may be achieved by modulating RVLM pressor activity (Blessing and Reis, 1982; Blessing 1988, Blessing and Li 1989; Dembowsky et al, 1989).

Thus, compelling evidence suggests that the CVLM sends inhibitory projections to the RVLM to modulate the pressor effect of neurons in this structure. In addition, the CVLM is involved in baroreceptor function, since it receives input from the NTS (See Figure 2).

Nuclei in the Hypothalamus

The hypothalamus is an important structure that integrates certain behaviors with appropriate autonomic responses. The most often-used techniques in early years were lesions or electrical stimulation of different areas of the hypothalamus to elicit behavioral and physiological responses. Electrical stimulation of the lateral hypothalamus (LH) (Wasman and Flynn, 1962) evokes an attack response that is considered a predatory behavior. The region dorsal to the anterior and posterior hypothalamus may be responsible for flight behavior (Romaniuk, 1965).

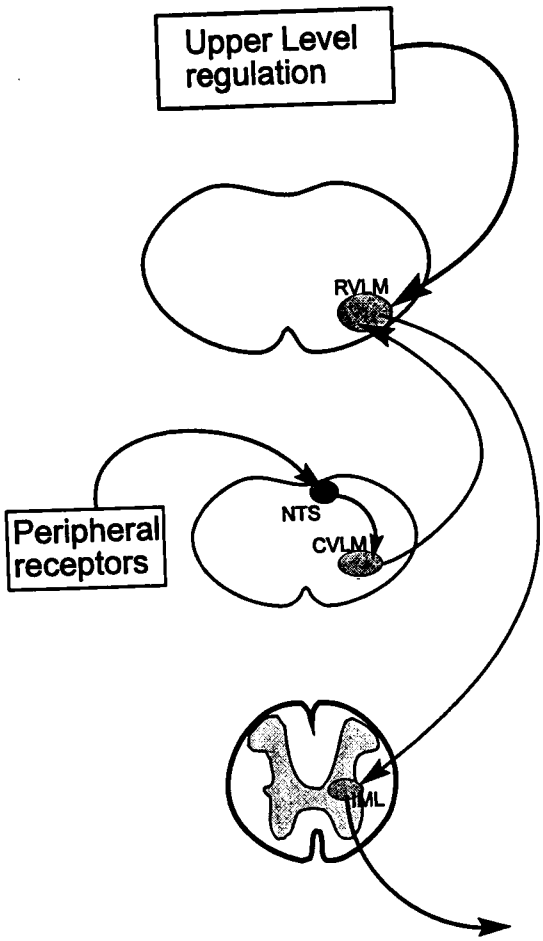

Figure 2. Diagrammatic representation of the medullary pathways regulating sympathetic outflow.

RVLM, rostral ventrolateral medulla
CVLM, caudal ventraolateral medulla
IML, intermediolateral cell column

The importance of the hypothalamus in the regulation of cardiovascular activity has been the focus of research for several decades. By stimulating the LH and the perifornical region of rats, Folkow and Rubenstein (1966) observed sympathetically-mediated responses, including an increase in blood pressure, cardiac output, heart rate, stroke volume and vasoconstriction in some regions. Later observations by Spyer (1989) revealed a similar cardiovascular pattern in response to stimulation of the perifornical area of the hypothalamus in the cat. Stimulation of the posterior hypothalamus (PH) also leads to pressor responses and increases in locomotor activity (Morpurgo, 1968), while electrical lesion of this area (Bunag and Eferakeya, 1976) reduces arterial blood pressure. Zanchetti (1976) reported that stimulation of the hypothalamus resulted in the release of renin (from the kidney), which is related to enhanced sympathetic activity.

However, despite these decades of study, the precise effects of electrical and chemical stimulation of the hypothalamus on cardiovascular function remain controversial. For example, Spencer et al (1989) demonstrated that microinjections of the excitatory amino

acid L-glutamate into the tuberal region of the lateral hypothalamic area caused a fall in blood pressure and heart rate in pentobarbital-anesthetized rats. In research from Calaresu and associates (Gelsema et al, 1989), injecting the excitatory amino acid DL-homocystate into 417 histologically-verified sites in the hypothalamus of 46 urethane-anesthetized, paralyzed and artificially ventilated rats, produced depressor responses in 271 sites, pressor responses in 77 sites and no response in 69 sites. Analysis of the anatomical distribution of responsive sites showed that depressor responses predominated in all hypothalamic subdivisions except in the paraventricular nucleus, where mainly pressor effects and tachycardia were elicited. The authors concluded that excitation of cell bodies in most hypothalamic regions elicits neurally mediated changes in arterial pressure and heart rate and that the traditional functional division of the hypothalamus into a rostral depressor and a caudal pressor area is probably based on the combined excitation of fibers of passage and cell bodies. However, some other observations are not consistent with this conclusion. For example, DiMicco and associates (Wible et al., 1988; Lisa et al., 1989; De Novellis et al, 1995) suggested that several regions of the hypothalamus, including the posterior and dorsomedial nuclei, contain a

sympathoexcitatory system that is modulated by changes in GABA-ergic tone. In one of these studies, Wible et al. (1988) observed that GABA antagonists microinjected bilaterally into the posterior hypothalamus in conscious rats rapidly increased heart rate, blood pressure, and sympathetic nerve activity. Microinjection of the GABA agonist muscimol into this same region decreased heart rate, blood pressure, and sympathetic nerve activity in conscious rats. In another study, Lisa et al (1989) found that injection of muscimol blocked the increase in blood pressure and heart rate normally evoked by stress in conscious rats. Finally, in a recent study, De Novellis et al (1995) reported that microinjection of a GABA antagonist into the dorsomedial nucleus of the hypothalamus (DMH) evoked a profound elevation in blood pressure and heart rate, while injection of the same substance into the paraventricular nucleus of hypothalamus (PVH) caused a much smaller response. They suggested that the DMH is the true site responsible for the observed cardiovascular responses, while reactions elicited by injecting into the PVH are caused by diffusion into the DMH.

Dampney (1994) provided an explanation for these conflicting results. He stated that sympathoexcitatory

neurons are scattered rather diffusely within the hypothalamus. Thus, microinjections of excitatory amino acids may excite not only sympathoexcitatory neurons, but also interneurons that inhibit them. Another possibility could be that there are multiple neurochemical codes within the hypothalamus whose net effect on cardiovascular responses may depend on the characteristics of the stimulus directly applied to this region. Furthermore, since the final gate of sympathetic outflow is governed by a complex network in the central nervous system, the synchronization of multiple processing in different cell groups could be an essential factor in controlling overall cardiovascular responses. Without the consideration of this synchronization, the function of an individual nucleus cannot be fully assessed.

In any case, the hypothalamus is an important site in the regulation of autonomic and neuroendocrine processing, and in coupling the behavioral response (fight and flight) to the acute physiological adaptation. There are two functional sets of cells in the PVH and supraoptic nucleus (SO) relevant to these processes. One of them executes both direct autonomic command to the cardiovascular system and indirect command via endocrine control of the adrenal gland.

Research from Loewy's group (Jansen et al, 1995) demonstrated reciprocal connections between the PVH and both the IML (via RVLM, and perhaps CVLM) and the adrenal gland. In this study, two forms of the Bartha strain of pseudorabies virus were used as transneuron tracers. One virus was injected into the stellate ganglion, the major sympathetic ganglion that innervates the heart, and the other was injected in the ipsilateral adrenal of anesthetized rats. The brain areas infected by both viruses included the RVLM, CVLM, caudal raphe nuclei, PVH, and the perifornical region, indicating a role for those areas in the regulation of both cardiovascular and endocrine function.

Another set of neurons in the PVH are magnocellular cells which synthesize the peptide vasopressin. Additional vasopressin cells are localized in the supraoptic nucleus of the hypothalamus. Vasopressin influences water retention in the kidney and the constriction of the peripheral vasculature. Vasopressin release is controlled through a reflex loop which includes a tonic inhibitory influence of low-pressure receptors located in the atria and high-pressure receptors (baroreceptors) from the carotid and aortic arch. The ascending noradrenergic (or norepinephrine,

NE) projections from A1 and C1 cell groups reach the hypothalamus after integrated multiple inputs from upper and lower levels. Functional studies have revealed the regulation of vasopressin release by the central noradrenergic system. For example, Bachelard et al. (1992) reported that microinjection of NE into the paraventricular nuclei of Long-Evans rats produced dose-dependent increases in blood pressure. However, there was no effect of injection on the Brattleboro rat, which is a genetically vasopressin-deficient strain, suggesting that NE in the paraventricular area along with the vasopressin system may contribute to the elevation of blood pressure.

Destruction of the projections from A1 and C1 cells by the microinjection of 6-hydroxydopamine, a neurotoxin relatively specific for noradrenergic and dopaminergic containing fibers, abolished the liberation of vasopressin which normally accompanies hemorrhage (Lightman et al., 1984). In another report (Banks and Harris, 1984), chemical destruction of noradrenergic neurons in the locus coeruleus (LC) eliminated the increased vasopressin release that usually follows baroreceptor stimulation (Harris, 1979). However, these results do not necessarily mean that there is a direct tonic noradrenergic excitation from the medulla to

the PVH and SO.

A later study by Cunningham et al (1993) found that injection of norepinephrine into the diagonal band of Broca induced a reduction in the activity of vasopressin cells in the SO region. These results suggest that the baroreceptor-sensitivity of vasopressin neurons is mediated by a noradrenergic mechanism in the diagonal band of Broca. In a study by Voisin et al (1994), GABA levels in the SO of rats were measured by microdialysis after either manipulation of blood volume or intervention of the neuron activities in the diagonal band of Broca area. They reported that in rats, removal of 1.5-2 ml of blood resulted in a 30-60 mmHg fall in blood pressure but a non-significant decline in GABA outflow. Replacement of blood resulted in an abrupt 50 mmHg increase in blood pressure and a significant 22% increase in GABA outflow. Repeated intravenous injections of the alpha-adrenoceptor agonist, metaraminol, similarly evoked approximately 50 mmHg increments in blood pressure and a 26% increase in GABA outflow. Electrical stimulation of the diagonal band of Broca for 10 min produced a two-fold increase in GABA outflow from the SO. These data support the hypothesis that GABA inputs to supraoptic neurons are part of a baroreceptor reflex,

relaying through the diagonal band of Broca, and suggest the physiological importance of GABA inputs to the supraoptic nuclei and indicate that GABA may be activated by noradrenergic neurons to influence the activity of magnocellular neurons.

However, the site where the GABAergic neurons mediate baroreceptor reflex control of vasopressin secretion may not necessarily be located in the diagonal band of Broca. Nissen (1995) observed that lesion of the lateral hypothalamus greatly decreased the baroreceptor- and diagonal band of Broca-evoked inhibitions in electrical activity of vasopressin neurons in the SO, suggesting that the GABAergic interneurons which mediate a baroreceptor-induced inhibition of SO vasopressin-secreting neurons may be located in an area of the lateral hypothalamus close to the target neurons. Finally, these vasopressin cells may also be influenced by the CVLM and/or RVLM via their direct links to the PVH and SO, because anatomical studies suggested projections from CVLM and RVLM to the PVH and SO (Jansen et al, 1995).

Thus, the hypothalamus is an important area that couples the behavioral response following environmental challenges

with the physiological adaptations necessary to sustain the behavior. It also plays an important role in the integration of neural and hormonal control of the cardiovascular system to maintain homeostasis.

Role of NE in the Regulation of Blood Pressure

The existence of multiple neurotransmitters within a given nucleus has recently been regarded as a general principle of the nervous system (Holets, 1990). Although the CNS is a multiple chemically-coded neuron network, and although results from research on individual neurotransmitters may not be able to provide a definitive understanding of the complexity of neural interactions, each neurotransmitter or neuromodulator system has its characteristics that are relatively independent. This justifies studies of individual neurotransmitters. In addition, we cannot learn how these systems interact until we understand the anatomical, chemical and functional characteristics of individual neurotransmitters. We now examine one of these neurotransmitter systems: NE.

The central distribution of NE cell groups and the connections between them cover several regions in the hypothalamus and medulla. The evidence is summarized in a

recent review by Holets (1990). The ensuing review discussion is based on this review. Following Dahlstrom and Fuxe's (1964) original designations of NE cells, Hokfelt et al (1974) proposed the nomenclature for the NE cell groups which is widely accepted today. NE cell bodies have been identified in the CVLM (A1 group), NTS (A2 group), rostral medullary reticular formation (A5 group), LC (A4, A6 group), regions ventral to the LC and within the reticular nucleus (A5), and an area dorsal to A5, within or near the lateral lemniscus (A7).

Although the noradrenergic system serves a large area of the brain, it nevertheless involves a relatively small number of neurons. The projections of the noradrenergic cell groups have been described using fluorescence histochemical, immunohistochemical and autoradiographic techniques (see reviews by Ungerstedt, 1971; Lindvall and Bjorklund, 1983; Moore and Card, 1984). Cells in the LC region (A6) give rise to the dorsal noradrenergic projection bundle, the major noradrenergic projection system, which innervates virtually the entire central nervous system, including the brain stem, cerebellum, hypothalamus, amygdala, hippocampus, septum, fornix, cingulum, external capsule, and lateral and dorsal neocortex, as well as olfactory nuclei. Many of the axons of

these A6 neurons initially bifurcate with a T-shaped pattern to send one projection to higher brain locations and another to the lower brain stem. The second noradrenergic pathway, arising from A1, A2, A5 and A7 regions, constitutes the central tegmental bundle, and travels rostrally to merge with the ventral bundle. The merged central tegmental bundle and ventral bundles ascend and merge with the dorsal bundle originating from LC (A6 cells) to form the median forebrain bundle. These pathways terminate in a variety of brain regions (See Figure 3)

As discussed above, the central noradrenergic neuron system may control blood pressure by mediating vasopressin release via the GABAergic system. Another role that the noradrenergic system plays is to influence the outflow of sympathetic responses. Folkow (1959) suggested that an increase in the release of NE in the anterior hypothalamus resulted in a decrease of blood pressure due to sympathoinhibition. Dejong et al's research (1977) supported the sympathoinhibitory function for the central NE system. They reported that microinjection of NE into the NTS elicited a rapid decrease in blood pressure and heart rate which is the main effect of sympathetic inhibition. When the

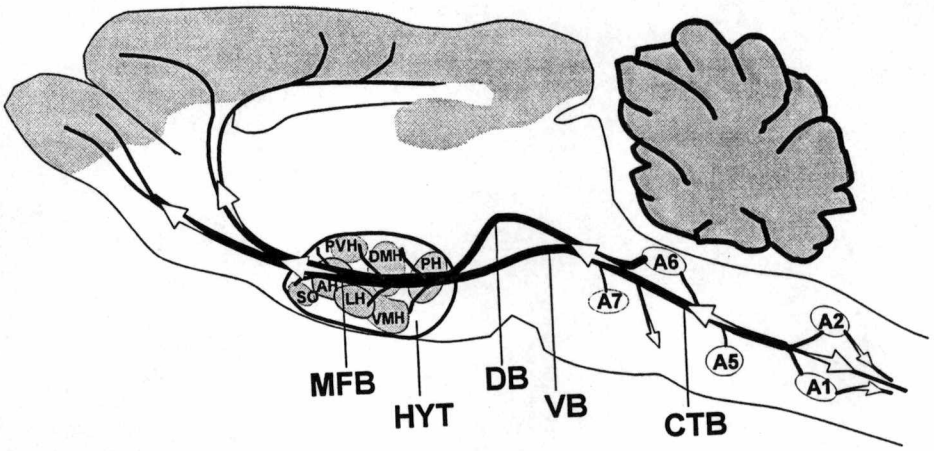

Figure 3. Schematic illustration of the noradrenergic pathways regulating sympathetic outflow

CTB, central tegamental bundle
VB, ventral bundle
DB, dorsal bundle
MFB, median forebrain bundle
HYT, hypothalamus

(Modified from Ungerstedt, 1971)

alpha-adrenoreceptor antagonist phentolamine was injected into this area prior to NE injection, the effect of NE was diminished. Other research supports this view as well. For example, Benarroch et al. (1983) observed that blood pressure and heart rate were reduced after injection of the NE agonist clonidine into the posterior hypothalamic nucleus, while increased blood pressure and heart rate were observed after depletion of NE from this area by microinjection of 6-hydroxydopamine. Sved and Felsten (1987) reported that unilateral microinjection of L-glutamate into LC of anesthetized rats elicited decreases in blood pressure and heart rate. Microinjections into the LC of DL-homocysteic acid and carbachol, agents which (like L-Glutamate) stimulate neurons within the LC, also produced depressor and bradycardic responses. Vagal blockade with methyl atropine eliminated the bradycardia, but had no effect on the decrease in arterial pressure elicited by stimulation of neurons within the LC, indicating a decrease in total peripheral resistance (an indication of a fall in sympathetic drive).

Destruction of the noradrenergic neurons of the LC by local administration of 6-hydroxydopamine eliminated the cardiovascular responses elicited by injection of

L-Glutamate or carbachol into the LC (Sved and Felsten, 1987). Another study by Miyawaki et al (1991) directly assessed sympathetic tone by measuring renal nerve activity. Unilateral microinjection of L-glutamate into the LC elicited dose-dependent decreases in arterial pressure and heart rate. Renal sympathetic nerve activity showed transient but strong inhibition with this stimulation.

The coexistence of neurotransmitters and neuromodulaters within one anatomical or functional structure makes it more complicated to explain the specific role of one single neurochemical element. The way that the NE system modulates sympathetic outflow could be direct, by affecting the sympathetic preganglionic neurons in the IML or the central nuclei in the hypothalamus and other brain regions, or indirect, by affecting actions of other neurotransmitters and neuromodulaters.

Thus, the central NE network comprises a major neurotransmitter system which works either to affect sympathetic activity in response to an acute challenge, or to affect vasopressin release in a slower fashion. The development of hypertension may thus be due in part to dysfunction of the NE system.

In summary, the central nervous system, consisting of complex neural networks, plays an essential role in the regulation of cardiovascular function. Although inconsistent results have been obtained, due to limitations in technique and experimental design, and more information is needed to clarify the mechanisms of central cardiovascular regulation, advances in research techniques have made it possible to identify specific functions subsumed by specific structures. Research reviewed above allows us to make several conclusions about central mechanisms of cardiovascular control.

1. The peripheral afferent input from the cardiovascular and respiratory systems ascends to the CNS with the first synapse in the NTS, where information is integrated and relayed to higher levels for additional neural processing.

2. The activity of preganglionic sympathetic and vagal neurons controlling the heart and blood vessels is modulated by synaptic input from structures throughout the CNS.

3. The RVLM is the origin of neurons that project to preganglionic sympathetic neurons in the IML of the spinal cord. The RVLM also integrates inputs from a wide range of peripheral and central sources.

4. The NTS, CVLM, RVLM and ILM work together as functional systems mediating cardiovascular reflexes. While the NTS acts as a transient relay station with limited integration, the RVLM is the principal integration site receiving input from peripheral receptors and central nuclei at different levels. The CVLM exerts an inhibitory effect by providing negative feedback for the excitatory influence of the RVLM neurons. The ILM cell column is the final pathway for descending sympathetic preganglionic neurons.

5. The hypothalamus is involved in coupling the behavioral responses following environmental challenges to cardiovascular adjustment, and integrating neural and endocrine responses to disruptions in homeostasis.

III. The BHR as A Model for the Interaction between Genetic and Environmental Factors

The genetic constancy of the inbred hypertensive and normotensive animal strains, such as SHR and WKY, makes these models particularly important in the study of both environmental sources of variance in blood pressure and the etiology of hypertension. Because genetic variance is excluded as a cause of blood pressure differences between

groups of the same strain as well as within groups, the measure of environmental impact is much more reliable. However, limitations exist in using SHR and WKY to understand the influence of environmental variables. First, SHR will inevitably become hypertensive, even without environmental manipulations. Second, cardiovascular reactivity may be capped due to higher baseline levels; this could mask the impact of environmental factors. Third, the nature of spontaneous hypertension in the SHR is not the same as that for human hypertension, in which the abnormality of blood pressure is the result of an interaction between genetic predisposition and environmental influences. Finally, the observed physiological differences between strains may not be related to hypertension, per se, but to "accidental" genetic differences that were passed on to succeeding generations when the models were first developed.

An excellent model for understanding the roles of genetics and environment in the development of hypertension is the BHR established by Lawler and coworkers (Lawler et al, 1980). The BHR is the first generation offspring of the female SHR and the male WKY. Under normal conditions, BHR has a resting systolic blood pressure between 140-160 mmHg

at the age of 4 months, and does not show any further age-related increase in blood pressure. However, the BHR is very susceptible to environmental stressors. It becomes hypertensive following chronic exposure to either psychological stress (Lawler et al, 1980a, 1980b, 1981, 1984; Sanders et al, 1989; Mansi and Drolet, 1997), or high salt diet treatments (Lawler et al 1987, 1993a, 1993b, 1993c; Sanders et al, 1988). Over a decade of investigation in this laboratory and some others, it has been demonstrated that the BHR is a suitable model for studying the interactions between genetic and environmental factors in the development of hypertension.

IV. Rationale Statement

The interaction among high salt diet, psychological stress and genetic predisposition is an important determinant of hypertension in the human population. The BHR has been proven to be an important animal model for human essential hypertension. Recent attention has turned to the mechanisms of environmentally-induced hypertension in this model. As is suspected by some for human hypertension (Abbound, 1984; Oparil et al, 1995), the CNS may play a

critical role in the development of hypertension in the BHR.

Intensive studies using several animal models suggest that pathways that regulate blood pressure involve nuclei in the brain stem and hypothalamus, which may mediate the baroreflex or influence sympathetic nervous system outflow. Also, it is well documented that the hypothalamus is involved in the defense reaction, which involves the allocation of physiological resources that represents the major function of the cardiovascular system.

Folkow and Rubenstein (1966) observed increased blood pressure, cardiac output, heart rate and stroke volume resulting from the stimulation of the hypothalamus. Zanchetti (1976) reported that stimulation of the hypothalamus resulted in the release of renin (from the kidney), which is related to enhanced sympathetic activity. Smith et al (1980) suggested that the hypothalamus participates in the cardiovascular response associated with emotion. Baboons showed elevated blood pressure, heart rate and cardiac output when subjected to conditioned emotional stress. Bilateral ablation of certain regions of the hypothalamus eliminates this cardiovascular response pattern. Broad connections are found between the

hypothalamus and other brain areas. It is thought that the hypothalamus is essential to the normal operation of the baroreflex, since lesions in the hypothalamus ventral and caudal to the anterior commissure diminish the response to baroreceptor stimulation (Spyer, 1981). Stimulation of the hypothalamic defense area inhibits the activity of the neurons in the nucleus tractus solitarius (NTS), which receive input from the arterial baroreceptors (Ciriello et al, 1983, Mifflin et al 1988).

The lower portion of the medulla oblongata is a vital area of blood pressure regulation. It is suggested that neural elements in the rostral ventrolateral medulla (RVLM) are essential for the neurogenic maintenance of arterial pressure (Ross et al, 1983; 1984). Microinjection of excitatory amino acids such as L-glutamate, N-methyl-D-aspartic acid and D, L-homocystic acid into the RVMM, which is medial to the RVLM, increases arterial pressure (Brody et al, 1989; Minson et al, 1987; Yardley et al, 1989). Conversely, microinjections of the inhibitory amino acids gamma amino butyric acid or glycine into this area elicit reductions in arterial pressure (Yardley, 1989).

Electrical stimulation and lesions can reveal brain

structures responsible for a specific function. However, detailed information is necessary to trace the pathways that comprise the circuits for regulating blood pressure. One important aspect is to investigate the neurotransmitters and neuromodulators involved in these circuits. Investigators have found that the central noradrenergic system plays a significant role in the regulation of blood pressure and the adaptation to environmental stimuli.

The distribution of NE cell groups and the connections between them cover several regions in the hypothalamus and medulla oblongata. The evidence is summarized by Holets (1990). Several aspects of research have shown that noradrenergic projections from A1, A2 and locus coeruleus areas of the brain stem to the anterior hypothalamic nucleus play a role in the regulation of blood pressure. For example, Benarroch et al (1983) reported that depletion of NE stored in the anterior hypothalamus of Wistar rats increased blood pressure and heart rate, whereas administration of the NE agonist clonidine into these areas elicited a reduction of blood pressure (Yang et al, 1989), indicating that NE has a inhibitory effect in these areas. Bachelard et al (1992) reported that microinjection of NE into the paraventricular nuclei of Long-Evans rats produced

dose-dependent increases in blood pressure. However, there was no effect of the injection on the Brattleboro rat, which is a genetically vasopressin-deficient strain, suggesting that vasopressin mediated the pressor response to NE in this nucleus. Results from microdialysis research by Woo et al (1993) showed an elevation of NE content in the paraventricular nuclei of the spontaneously hypertensive rats (SHR) compared to that of normotensive Wistar-Kyoto rats (WKY). These results suggest a role for CNS levels of NE in blood pressure control.

Several aspects of the research concerning the central mechanisms of stress-induced hypertension in BHR involve surgical or chemical lesions, electrical stimulation and *in vivo* or *in situ* neurotransmitter analysis. A recent review (Lawler and Edgemon, 1998) of studies utilizing the BHR summarizes the efforts of this approach. Since the brain NE system participates in the regulation of blood pressure in most animal models, it is worth investigating the role of the NE system in the development of stress-induced hypertension in BHR. An early approach examined the NE contents in discrete brain areas, including nuclei in the brain stem and hypothalamus. Mitchell and Lawler (1989) reported that, compared to the age-matched groups, the NE

levels in the A2 and A1 cell groups of the brain stem were elevated after three days of stress, while NE content in some hypothalamic nuclei were reduced. After four weeks of stress, there were no significant differences between groups. After either 10 or 16 weeks, NE levels in some nuclei of the brain stem and hypothalamus were higher in the stressed groups. The results indicate that different durations of conflict stress affect NE levels in various brain regions. In everyday life, human beings confront multiple stressors, including work pressure, high salt diet, exercise, etc. It is possible that single stressors and multiple or compound stressors have different impacts on the organism. An experimental design that mimics the human situation would be informative.

The current study was designed to characterize the role of noradrenergic pathways in the CNS in the development of hypertension induced by compound stressors, consisting of mild electric shock plus daily high salt diet, daily swim plus shock and swim plus salt. Thus, we would predict that stress plus salt would elicit greater NE changes in the CNS than those previously reported for stress alone (Mitchell and Lawler, 1989). Also, since exercise has been shown to prevent stress-induced hypertension in the BHR (Cox et al,

1985), it would be predicted to counteract the central changes evoked by stress or high salt alone. Thus, in the present study, we will examine the norepinephrine levels of several nuclei in the brain stem and hypothalamus using the micropunch technique and HPLC-EC assay. The nuclei include: the A2 cell group coinciding with the nucleus tractus solitarius, which is a depressor center in the baroreflex pathway; the A1 cell group located in the caudal ventral medulla, which also is a depressor center of the reflex; the C1 cell group in the rostral ventrolateral medulla, which is a pressor center; the locus coeruleus (LC), which is the major NE cell group in the CNS; and several nuclei in the hypothalamus, which have wide connections with A1, A2, C1, and LC cell groups, including the anterior (Ant), posterior (PH), arcuate (Arc), dorsomedial (DMH), lateral (LH), ventromedial (VMH), paraventricular (PVH) and supraoptic nuclei.

The following is a list of the questions to be answered through this study:

1. Are there changes in NE content in certain brain nuclei of the BHR as a result of maturation?
2. Are there any differences among BHR groups subjected to high salt/shock, high salt/swim, shock/swim in terms of

NE content in certain brain areas at a particular period?

3. Are there any differences of NE alteration of NE content due to the duration of stresses?

CHAPTER 2

METHODS

Animals

Male BHR were used in this study. Inbred parent strains of female SHR and male WKY were obtained from Taconic Farms (Germantown, NY). Before breeding, indirect measurements of systolic blood pressure using tail plethysmography were obtained in order to insure that SHR and WKY were indeed hypertensive and normotensive, respectively. Two female SHR were group-housed for two weeks prior to mating in order to synchronize estrous. One male was then introduced for a two-week period. Following the breeding period, each SHR female was singly housed. Male BHR offspring were weaned at 21 days. Beginning at 8 weeks of age, all male BHR were handled daily for 5 minutes to adapt them to human contact.

Apparatus

Home cages

All subjects were doubly housed in pairs in standard plastic laboratory cages measuring 21.6 x 41.9 x 14.6 cm and were maintained on a 12 hour light/dark cycle. Laboratory rat chow and tap water were available ad libitum throughout

the remainder of study. Laboratory chow contained either normal (1% NaCl), or high salt (8% NaCl, ICN Nutritional Biochemicals), depending on group assignment.

Stress Cages and Computer Control

The stress of mild cutaneous electric shock was conducted in a plexiglass box measuring about 7.0 x 28.0 x 9.0 cm inside. Each animals was confined in the box with its tail constrained on the back side of the box. Animals were prevented from jumping and turning by this constraint.

Three plexiglass boxes were placed in each of six sound-attenuated chambers measuring 0.3 x 0.8 x 0.25 m. Each chamber contained a light and a ventilation fan.

Control for lights and shocks delivered to the animals during the stress session was managed using a 6502-based microcomputer (MOS Technology, Inc.; Norristown, PA). The hardware interface provided individual control of current delivered to each rat. Each shock system placed the rat in series with a 1.0 Mohm resistor. An additional potentiometer (0-5 Mohm) allowed control of the 1600 V AC shock at the desired current (0.2-0.4 mA). Software for the control of tone and shock delivery was written in machine code by James

E. Lawler.

Swimming Tank

Plastic tanks, 0.6 m in diameter and 1.1 m in depth were used for the swimming session. The tank was filled to 0.7 m with 36°C water.

Cryostat

A Pearse cold microtome cryostat, Type HR/M (Slee Manufacture, London, England) was utilized for brain sectioning and micro-sampling. The cryostat is capable of maintaining a sub-zero temperature necessary for brain slicing and for preventing the catecholamines in the brain tissue from oxidating.

High performance Liquid Chromatography

Assay for catecholamines was performed using high performance liquid chromatography with electrochemical detection. The system consisted of a Phenomenex column (5mm, ODS20, reverse-phase, 80 x 6.2 mm), a Bioanalytical Systems LC-4B amperometric detector, interfaced with a Waters autoinjector and pump, and a Hewlett Packard integrator. The HPLC was maintained at ambient temperature of 25°C.

Procedures

General Description

BHR were randomly assigned to groups at 2 months of age. There were four treatments: control, salt plus stress, salt plus swim, and stress plus swim. The control group consisted of age-matched rats subjected to neither shock nor swim, and maintained on a normal salt diet. Animals were studied for either 2 or 6 months (i.e., until either 4 or 8 months of age). Cell size of animals successfully completing the protocol varied between 10-12 rats. Group assignment is summarized in Table 2.

Table 2. Group assignment

Group	treatment	Duration (months)	Age at End (months)
1	salt + swim	2	4
2	salt + swim	6	8
3	swim + shock	2	4
4	swim + shock	6	8
5	salt + shock	2	4
6	salt + shock	6	8
7	control	2	4
8	control	6	8

Animals subjected to stress were exposed to five, 90 minutes sessions per week, after a one-week training period. Stress sessions utilized a signalled shock avoidance schedule. A response to the onset of a light, consisting of a one-quarter turn of a low frictin wheel, could both avoid and escape a mild (0.2-0.4 mA shock, 0.2 second in duration) tail shock. Each response reset a clock. When a 10 second period elapsed without a response, a light was turned on. This signalled that shock would commence in 5 seconds. If no response was make in this interval, up to 10 consecutive shocks were administered, once per second. After the shock began, a response terminated the shock and the light, and the light was reset. Control of the presentation of light and shock was programmed using a microcomputer as previously described.

Animals subjected to exercise were exposed to five, 90 minutes sessions per week, after a one-week training period. Exercise sessions consisted of forced swimming in isothermic (36°C) water in groups of three.

Animals on a high salt diet received 8% sodium chloride ad libitum.

After 2 or 6 months of study, rats were sacrificed by decapitation. Brains were quickly removed and placed on dry ice, and transferred to an ultracold (-85 °C) freezer. The Palkovits micropunch technique was used to obtain the brain nuclei of interest.

Microsection and Micropunch Techniques

About 8 hours before the series sectioning, brains were removed from the freezer and placed in the cryostat at -9 °C. Brains were mounted on the platform with Tissue-Tek (Miles, Inc., IN). Series sections were performed at -9 °C. Sections alternated between 150 um (thin) and 300 um (thick) in thickness. Slices were mounted on cold glass slides. The thin sections were allowed to thaw slightly to reveal major anatomical landmarks which were easily located without using dye by utilizing a dissection microscope (Olympus, Tokyo, Japan) at a magnification of 10x. A thick slice was obtained for micropunch after a thin slice revealed that the correct nucleus was visible. The brainstem and hypothalamic nuclei studied were A2, A1 cell groups, C1 cell group, the locus coeruleus (LC), and several hypothalamic nuclei, including the anterior (Ant), posterior (PH), arcuate (Arc), dorsomedial (DMH), lateral (LH), ventromedial (VMH), paraventricular (PVH) and supraoptic (SO) nuclei.

The stereotaxic coordinate map for the rat brain by Paxinos and Watson (1982) was utilized to assist in identification of the nuclei. Plate 75 of the map was used for reference to identify A1 and A2; plate 69 for C1; plate 59 for LC; plate 32 for Post and Arc; plate 31 for DMH; LH and VMH; plate 25 for Ant and PVH; and plate 23 for SO. When the nuclei of interest were identified, bilateral micropunches were performed using a 0.5 mm ID needle to obtain the nuclei. The dissected bilateral nuclei were injected into a cold vial containing 100 ul perchloric acid (0.01 M) and 3,4-dihydroxybenzylamine hydrobromide (DHBA, 375 pg/ml). The DHBA served as an internal standard for HPLC assay.

Norepinephrine Assay

The NE content of each nucleus was detected by the HPLC-EC system as described in the previous section. Samples were sonicated in an ice-water bath for 30 seconds using a Branson Cell Disrupter Sonicater (Danbury, CT. Model 200). Homogenized samples then were centrifuged at 10,000 rpm for 10 minutes. Supernatant (75 ul) was injected onto a 5mm, ODS (20), reverse-phase HPLC column (Phenomenex, 80 x 6.2 mm). The NE was detected by the electrochemical detector (BAS,

LC-4B). The applied potential was set at 700 mv. The flow rate of the mobile phase was fixed at 1.0 ml/min.

The mobile phase was optimized by running test samples with different mobile phases which varied in types of and concentrations of components. Final mobile phase was chosen based on its ability to maximize separation of NE, Epi, DHBA and dopamine within a time window of 15 minutes. The 1000 ml mobile phase for the assay consisted of 40 ml of n-propanol, 5 ml of 10% sodium octane sulfate, 3 ml of 10% Na2EDTA, 100 ml of 1.0 N NH4H2PO4 and distilled water. The pH was brought to 4.0 by addition of 50% H3PO4. The solution was filtered using nylon 45 membrane filters (Magna, NY), sparged with helium gas and then subjected to a vacuum to remove gases.

Calibration of the HPLC system was performed each day before assays started. Additional calibrations were carried out after any system adjustment. The calibration standard consisted of 0.01 M perchloric acid, DHBA, NE, E, Dopamine, all with a concentration of 375 pg/ml. Figure 4 shows a typical calibration and an assay result.

RUN # 1478 FEB/03/94 13:26:54
ID 653-654

ISTD RT	HEIGHT	TYPE	CAL#	AMOUNT
0.06	1000	D BB		0.000
0.19	1113	BB		0.000
0.67	582	D BB		0.000
0.85	513	BB		0.000
1.10	398	D BB		0.000
1.50	109135	D BB		0.000
1.83	114900	→ BB		0.000
2.03	201962	→ BB		0.000
2.82	3829	BB		0.000
4.21	3735	PB	1R	366.920
4.78	3180	PB	2R	376.220
8.31	2709	BB	3R	378.710
9.18	2171	BB	4&	375.000

TOTAL HGHT= 445360
ISTD AMT= 3.7500E+02
MUL FACTOR= 1.0000E+00

RUN # 1483 FEB/03/94 14:36:40
ID 653-654

ISTD RT	HEIGHT	TYPE	CAL#	AMOUNT
0.06	1005	D BB		0.000
0.19	936	BB		0.000
0.67	273	PB		0.000
1.51	117475	D VB		0.000
1.82	129965	BB		0.000
2.03	191212	BB		0.000
2.88	2090	PB		0.000
2.94	518	BB		0.000
3.17	217	BB		0.000
3.95	293	PB		0.000
4.20	2632	BB	1R	196.540
5.56	321	PB		0.000
9.17	2205	VB	4&	375.000

TOTAL HGHT= 448480
ISTD AMT= 3.7500E+02
MUL FACTOR= 1.0000E+00

Figure 4. A typical HPLC calibration (left) and an NE assay result (right)

Protein Assays

NE data were expressed as pg NE per ug protein for each pair of nuclei. Protein assays were performed for each bilateral pair of punches. The protein was allowed to air dry. To each tube, 50 ul of 1N NaOH was added and then 950 ul of 0.1M sodium phosphate buffer was added. The tubes were sonified for six seconds using the Branson Cell Disrupter Sonifier at a setting of 4. From each tube, duplicates of 400 ul each were taken. To this solution, 400 ul more of sodium phosphate buffer was added. Duplicate assays used a Bio-Rad protein assay kit (Bio-Rad, Chemical Div., Richmond, CA). Bio-Rad dye reagent (0.2 ml) was added to each tube. Following vortexing, samples were allowed to sit for five minutes before absorbance readings at 595 nm were made using a Beckman DU-8 spectrophotometer (Beckman Instruments, Inc., Irvine, CA). Protein content was determined using a standard curve.

Statistics

Data were analyzed using Crunch statistical software. Planned comparisons were made between the groups. The Kruskal-Wallis nonparametric statistic was used to test differences between groups due to small and unequal cell size (average cell size 7.2, range of 4-10, yielding a 70%

success rate for sample processing). A probability level of $p<0.05$ ($H>3.8415$) was chosen as significant. Marginal trends with $0.05<p<0.1$ ($3.8415>H>2.7055$) were also noted.

CHAPTER 3

RESULTS

Maturation effects were observed in the LC and Ant nuclei. NE levels were lower in the older rats ($H=8.077$, $p<0.001$ for LC; $H=4.48$, $p<0.05$) (Figure 5). Duration effects of the stressors in NE levels were found in several nuclei. After 6 months of swim plus stress, NE content in C1, LC, DMH, LH, PVH and Ant nuclei was significantly reduced compared to 2 months of treatment ($H=5.3570$, $p<0.05$ for C1; $H=5.415$, $p<0.05$ for LC; $H=5.897$, $p<0.05$ for DMH; $H=5.357$, $p<0.05$ for PVH ; $H=9.267$, $p<0.01$ for Ant) (Figure 6). With swim-salt, NE levels were lower in A1, C1, LC, and Ant nuclei in the 6-month group compared to the 2-month group (Figure 7). For the stress and high salt diet group, NE contents in C1, DMH and Ant nuclei were decreased in the 6-month group compared to the 2-month group (Figure 8).

Two months of swim-stress treatment revealed no significant change in NE levels in any nucleus compared to the non-intervention group. However a marginal NE elevation in PVH was noted ($H=2.676$, $p<0.1019$) (Figure 9). Similar results were obtained in the two months of swim-salt group which showed a NE increase in the VMH area ($H=3.431$,

p<0.0640) (Figure 10). Two month of exposure to stress-salt had a different pattern of NE alteration in which NE content in the VMH nucleus deceased compared to the no-intervention group (H=5.556, p<0.0184) (Figure 11).

Marginal increases in NE in the LC and SO nuclei were found in the swim plus salt group (H=3.153, p<0.0758 for LC; H=3.488, p<0.0618 for SO) (Figure 12). After six months of treatment, swim plus stress significantly reduced NE content in VMH and LH compared to the non-intervention groups (H=5.672, p<0.0172 and H=8.333, p<0.0039, respectively) (Figure 13). A significant NE decrease in LH (H=4.821, p<0.028) and a marginal reduction in DMH (H=3.449, p<0.0633) were detected in the stress plus high salt group (Figure 14).

The final four figures depict how the compound stressors involving exercise (swim+salt, swim+stress) compared with the stress+salt condition. Figure 15 depicts the NE content in brain nuclei for swim+salt vs salt+stress in rats studied for two months. NE content in the latter group appears lower in several nuclei, especially the A2 area and several hypothalamic nuclei (PH, Arc, DMH, VMH, PVH, and SO). These trends were significant in VMH and SO,

and marginal in PVH.

Figure 16 depicts the NE content in brain nuclei for these same groups studied for six months. Once again, several nuclei (LA, DMH, LH, VMH, Ant and SO) in the salt+stress group appear to be lower that the swim+salt group, while one (A1) appears to be higher. However, only two nuclei (Ant and SO) were marginally different.

Figure 17 depicts the NE content in brain nuclei from the swim+stress and salt+stress groups studied for two months. Inspection of the means suggests several differences, especially in the A2 region and in the VMH and PVH. However, only one comparison (PVH)reached statistical significance, while another nucleus (VMH) showed a marginal difference between groups.

Finally, Figure 18 depicts the NE content in brain nuclei in the same groups studied for six months. Inspection suggests two possible differences, in the Arc and Ant nuclei. However, no comparison between groups reached even marginal significance for any nucleus studied.

Table 2 NE Contents in Discrete Nuclei

	2 Months of Treatment				6 Months of Treatment			
Nuclei	Control	SW-SLT	SW-STR	SLT-STR	Control	SW-SLT	SW-STR	SLT-STR
A2	44.73(18.76)	49.58(7.76)	25.36(5.12)	35.10(5.88)	26.59(5.19)	22.10(8.13)	17.12(9.43)	19.47(7.05)
A1	21.71(2.67)	24.97(3.86)	22.34(6.40)	26.07(3.75)	21.75(6.62)	14.23(3.09)	16.19(2.17)	21.97(4.98)
C1	21.39(4.64)	25.52(5.08)	19.40(2.53)	19.63(2.50)	17.51(5.06)	13.80(2.84)	10.98(2.14)	11.10(1.91)
LC	38.11(10.73)	40.05(7.91)	25.15(6.40)	32.01(11.47)	6.89(0.97)	12.23(2.31)	7.58(3.02)	6.43(1.36)
PH	34.94(9.56)	46.87(13.01)	27.14(3.64)	30.31(8.66)	30.80(9.91)	23.35(5.86)	27.82(10.08)	33.76(19.61)
Arc	50.00(10.64)	54.01(10.05)	42.42(6.25)	34.13(6.14)	41.92(10.57)	43.07(13.30)	30.28(4.14)	50.73(14.99)
DMH	58.87(7.31)	72.71(14.53)	51.55(8.37)	51.83(10.07)	50.52(10.43)	40.50(9.23)	25.59(4.18)	28.17(3.07)
LH	50.28(9.88)	41.03(4.30)	40.24(5.91)	36.07(8.11)	32.28(5.71)	29.15(10.61)	15.31(2.62)	16.72(3.45)
VMH	53.79(6.34)	77.40(13.69)	48.32(6.78)	33.91(5.49)	50.35(15.00)	50.03(11.59)	36.15(7.44)	33.07(8.17)
PVH	57.74(7.47)	76.22(10.84)	83.25(11.92)	46.45(8.01)	59.82(7.96)	46.49(15.18)	45.04(9.29)	40.86(11.10)
Ant	53.41(5.73)	50.49(6.55)	65.69(6.29)	53.51(9.09)	35.17(6.50)	32.87(5.30)	28.88(5.66)	19.85(3.20)
SO	42.18(8.24)	50.94(6.82)	36.94(6.41)	30.70(5.20)	27.27(2.57)	36.01(3.63)	29.34(4.13)	26.62(2.64)

Data are expressed as *pg* NE per *ug* protein. Mean (S.E.).

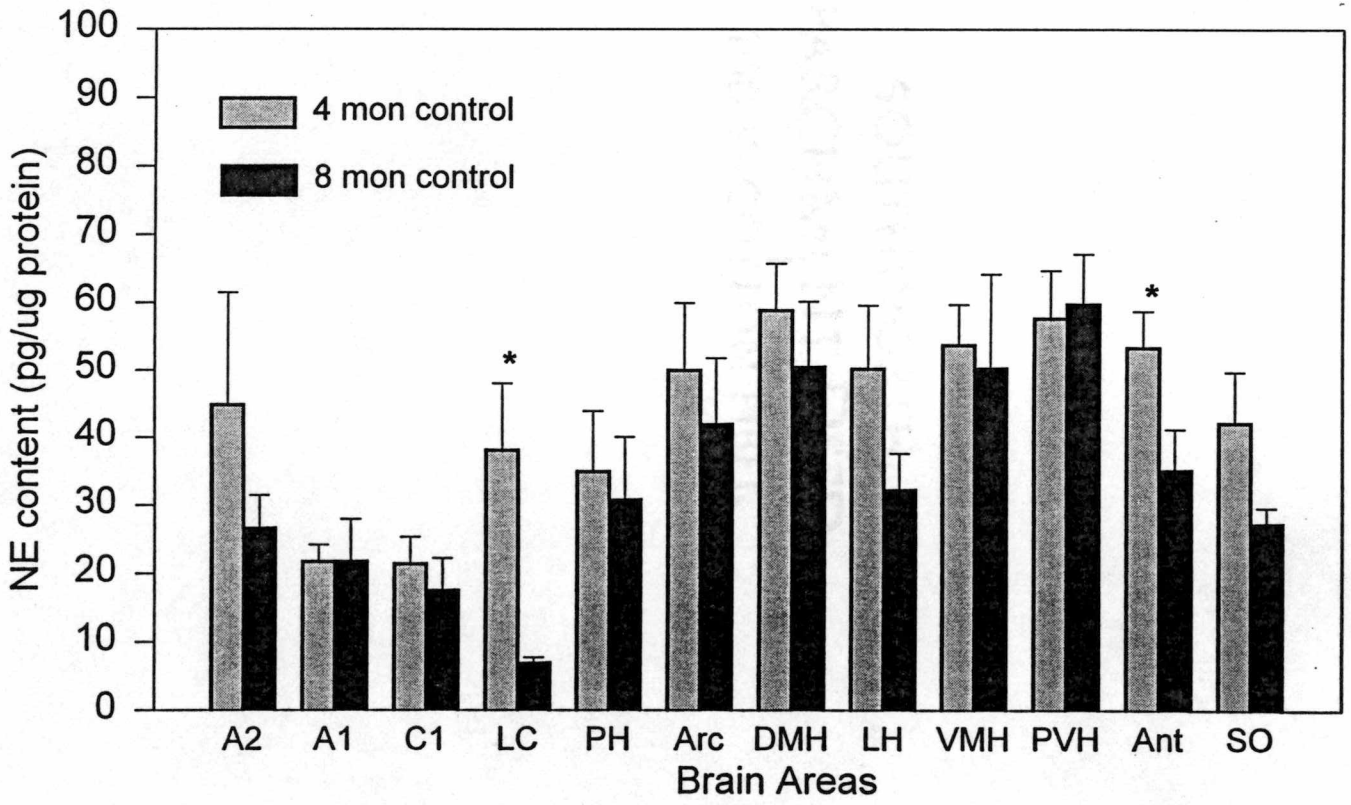

Figure 5. NE levels in the brain nuclei of nonintervention (control) groups of BHR after 2 or 6 months of treatment (*$p<0.05$).

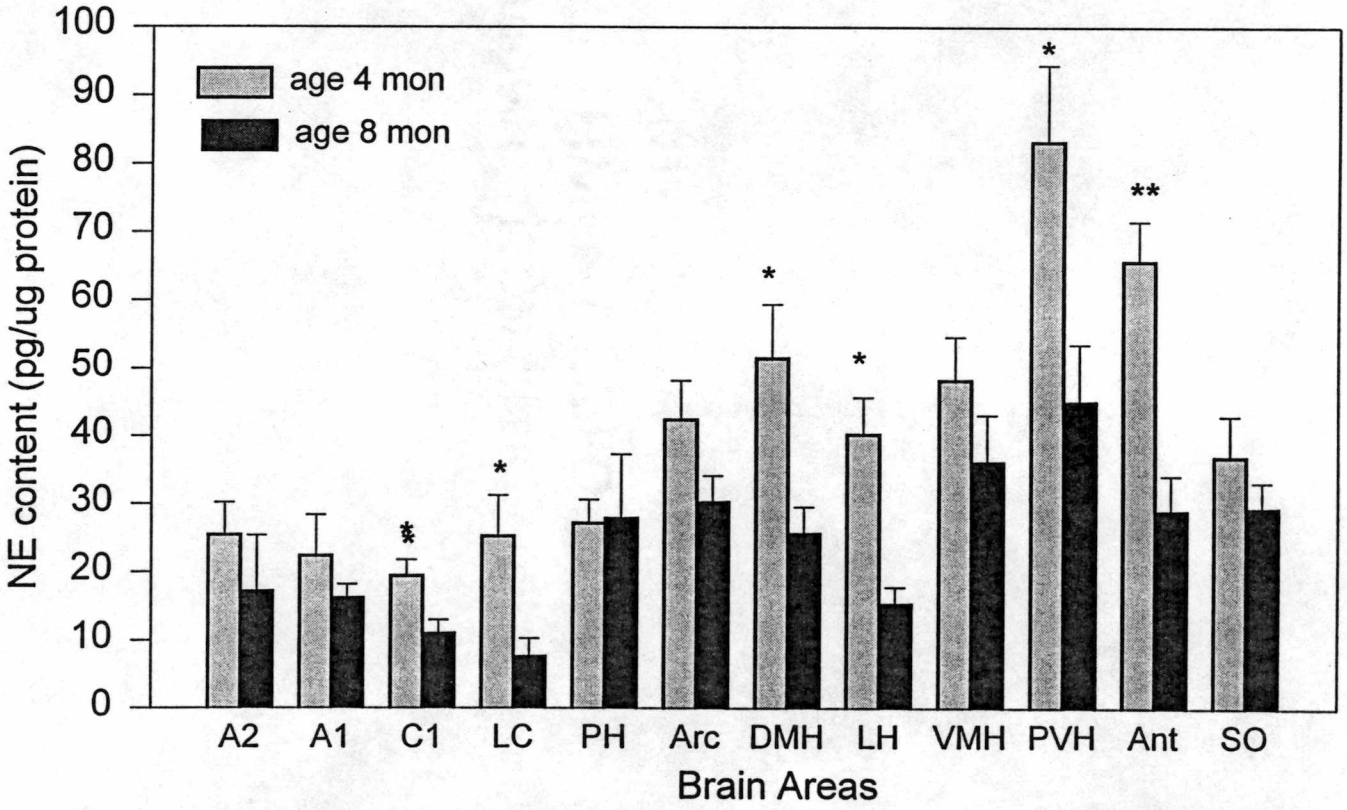

Figure 6. NE levels in the brain nuclei of BHR after 2 or 6 months (age 4 or 8 mon) of swim+stress (SW-STR) treatment (*p<0.05; **p<0.01).

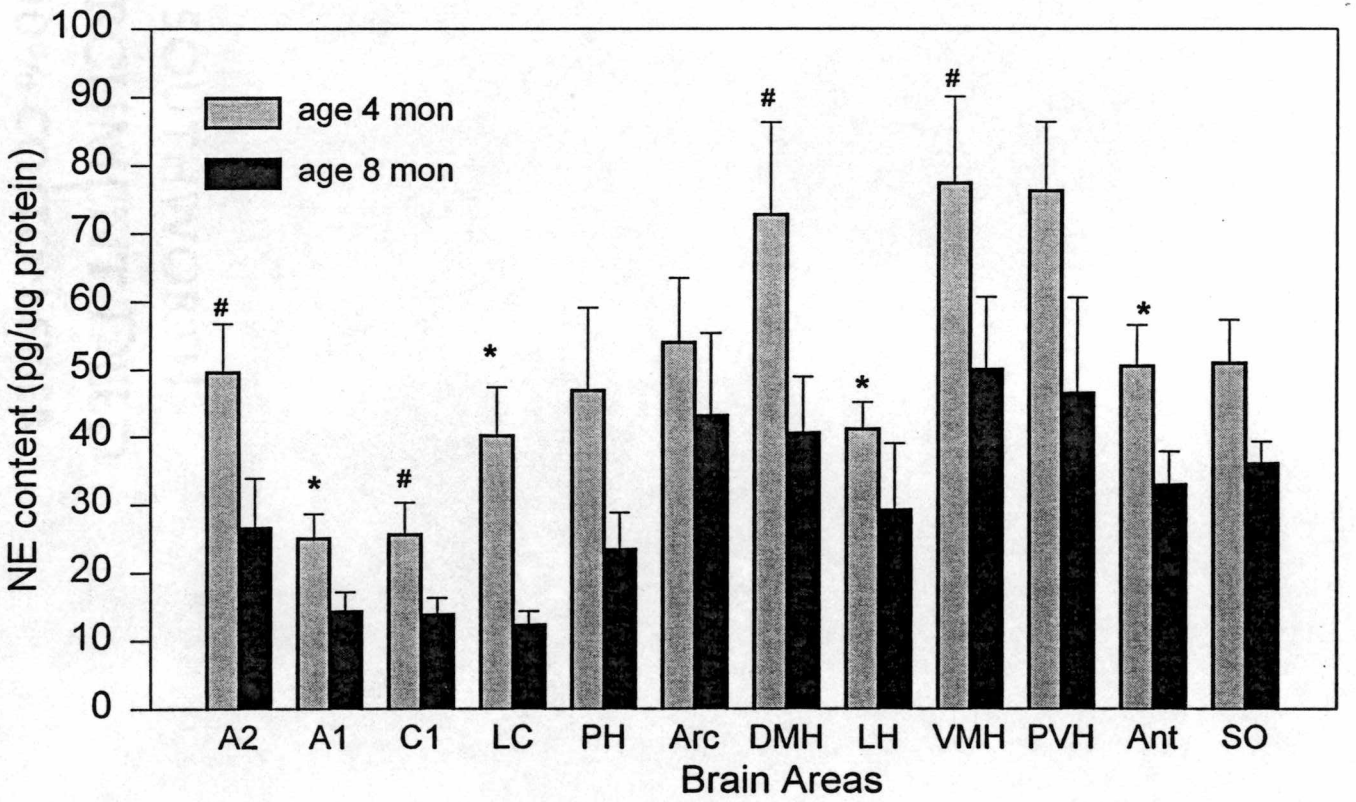

Figure 7. NE levels in the brain nuclei of BHR after 2 or 6 months (age 4 or 8 mon) of swim-salt (SW-SLT) treatment (*p<0.05; **p<0.01; #p<0.1).

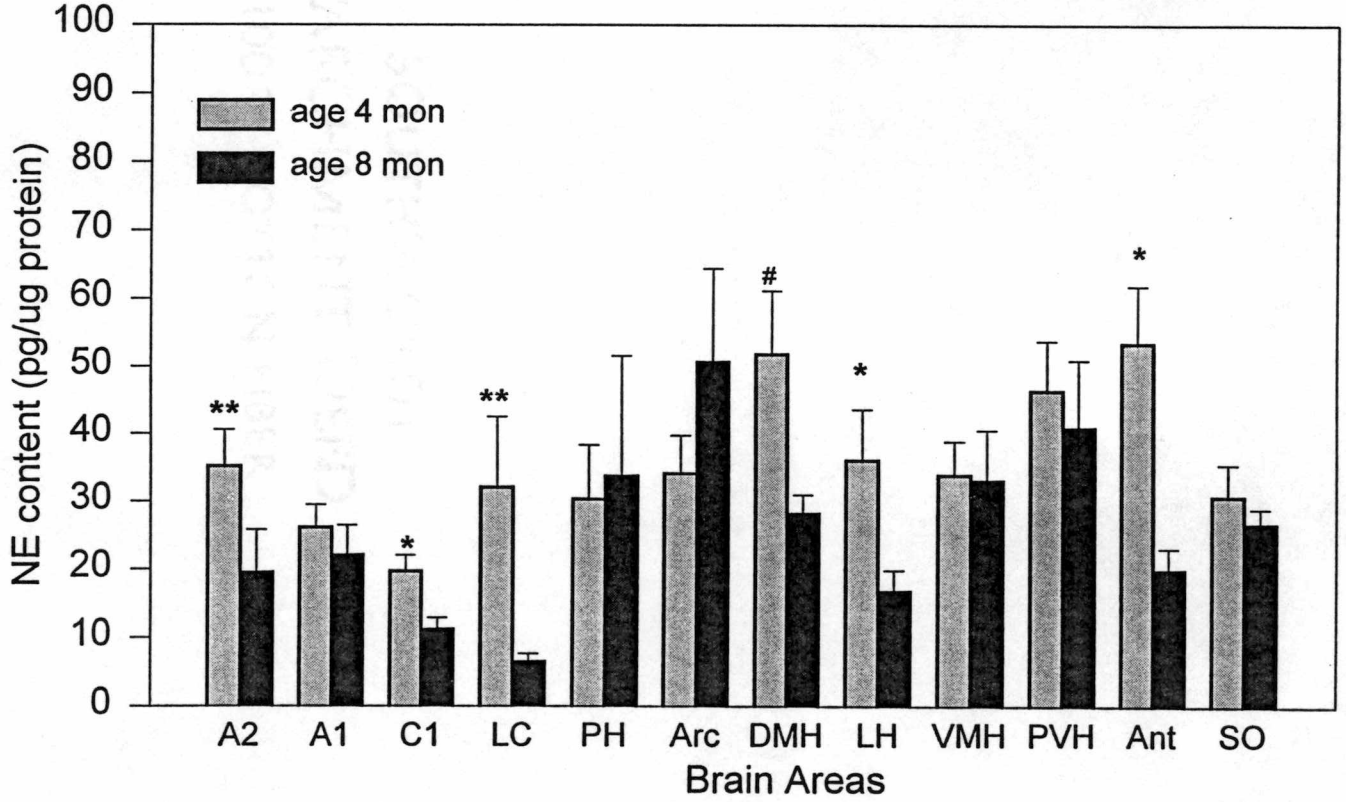

Figure 8. NE levels in the brain nuclei of BHR after 2 or 6 months of slt-stress (SLT-STR) treatment (*p<0.05; **p<0.01; #p<0.1).

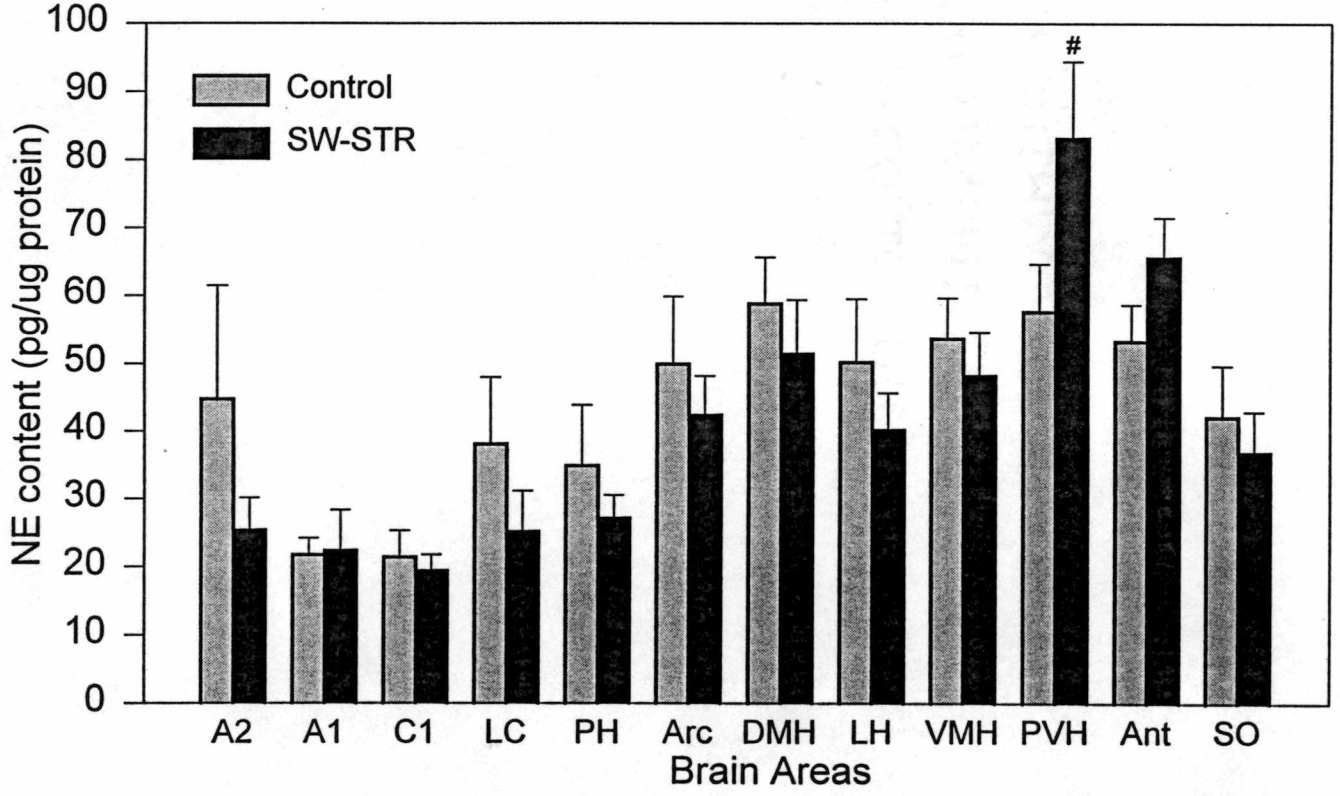

Figure 9. NE levels in the brain nuclei of BHR after 2 months of swim-stress (SW-STR) treatment vs. nonintervention (#p<0.1).

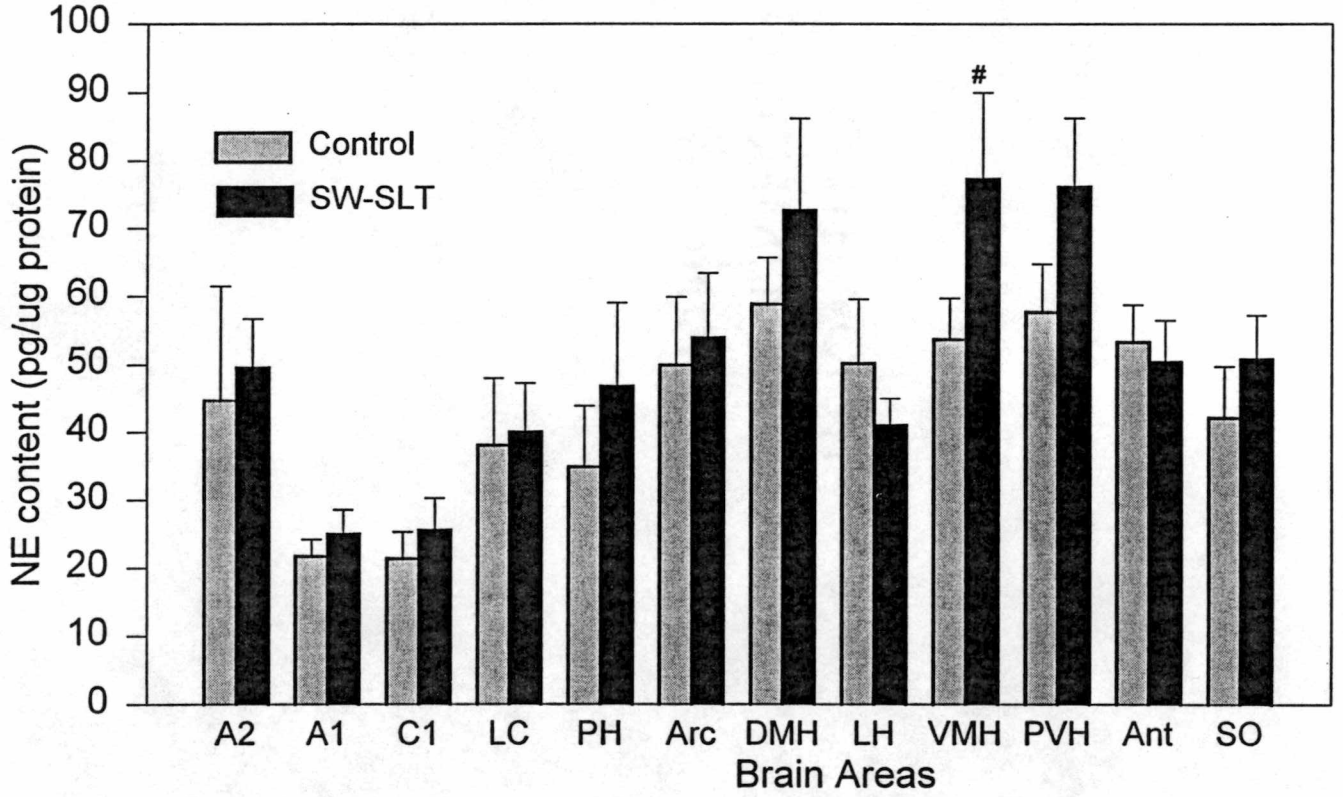

Figure 10. NE levels in the brain nuclei of BHR after 2 months of swim+salt (SW-SLT) treatment vs. nonintervention (*p<0.05, #p<0.1).

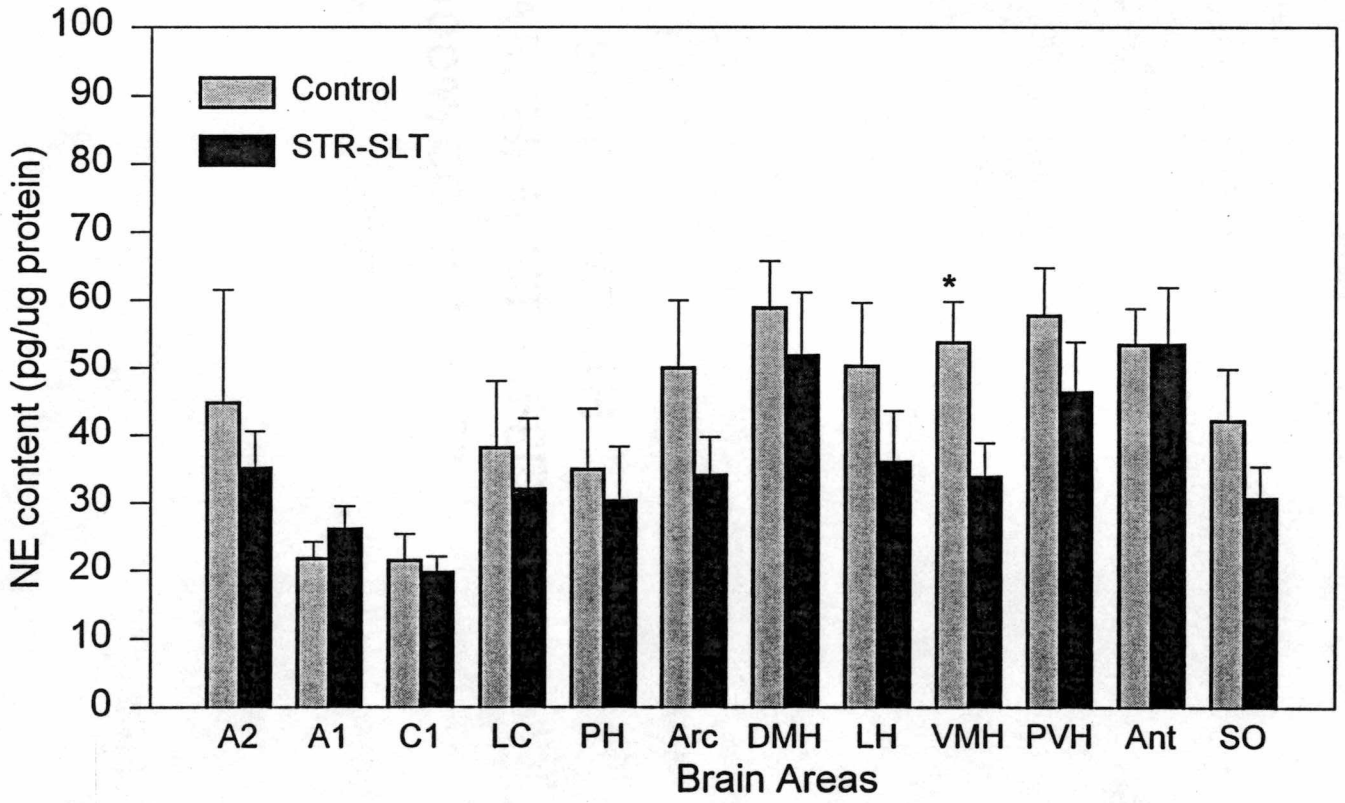

Figure 11. NE levels in the brain nuclei of BHR after 2 months of stress+salt (STR-SLT) treatment vs. nonintervention (*p<0.05).

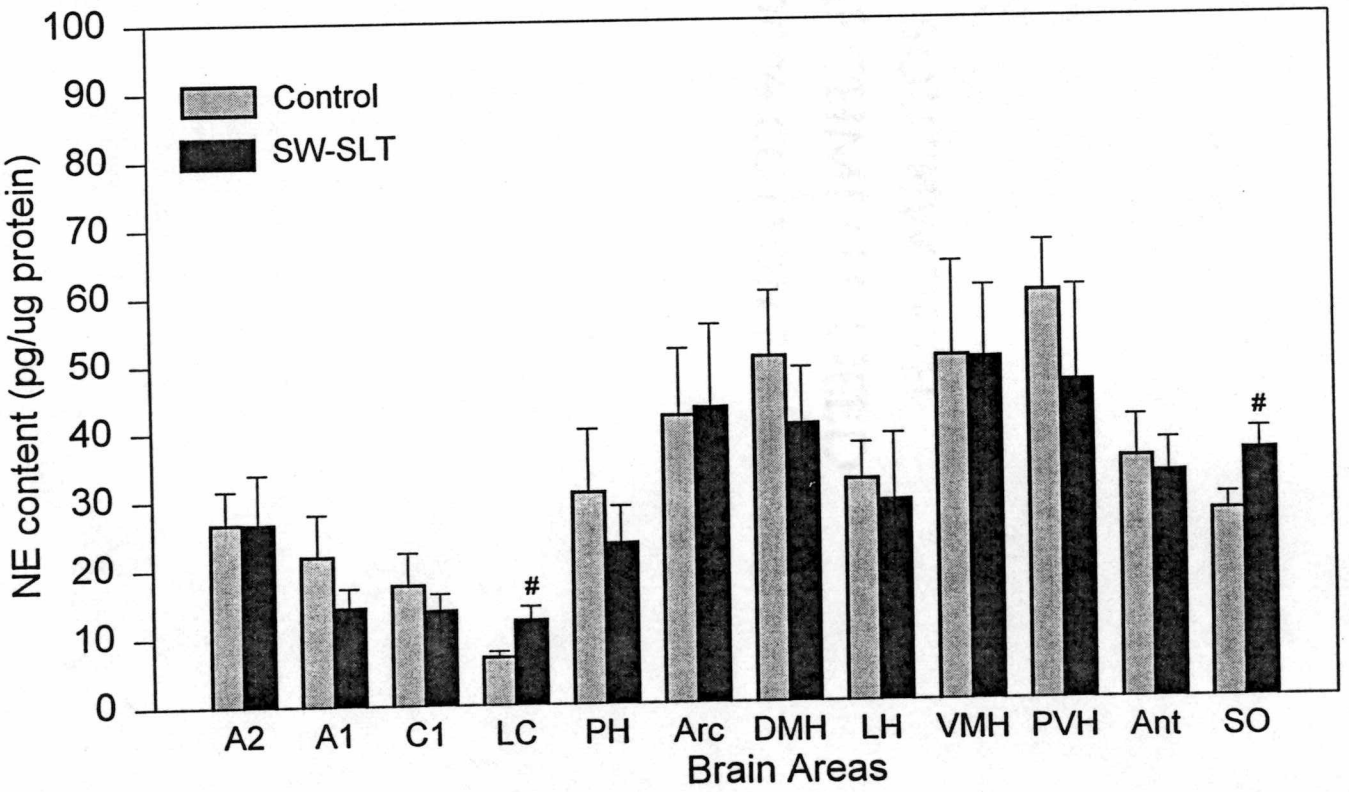

Figure 12. NE levels of the brain nuclei after 6 months of swim+salt (SW-SLT) treatment vs. nonintervention (#p<0.1).

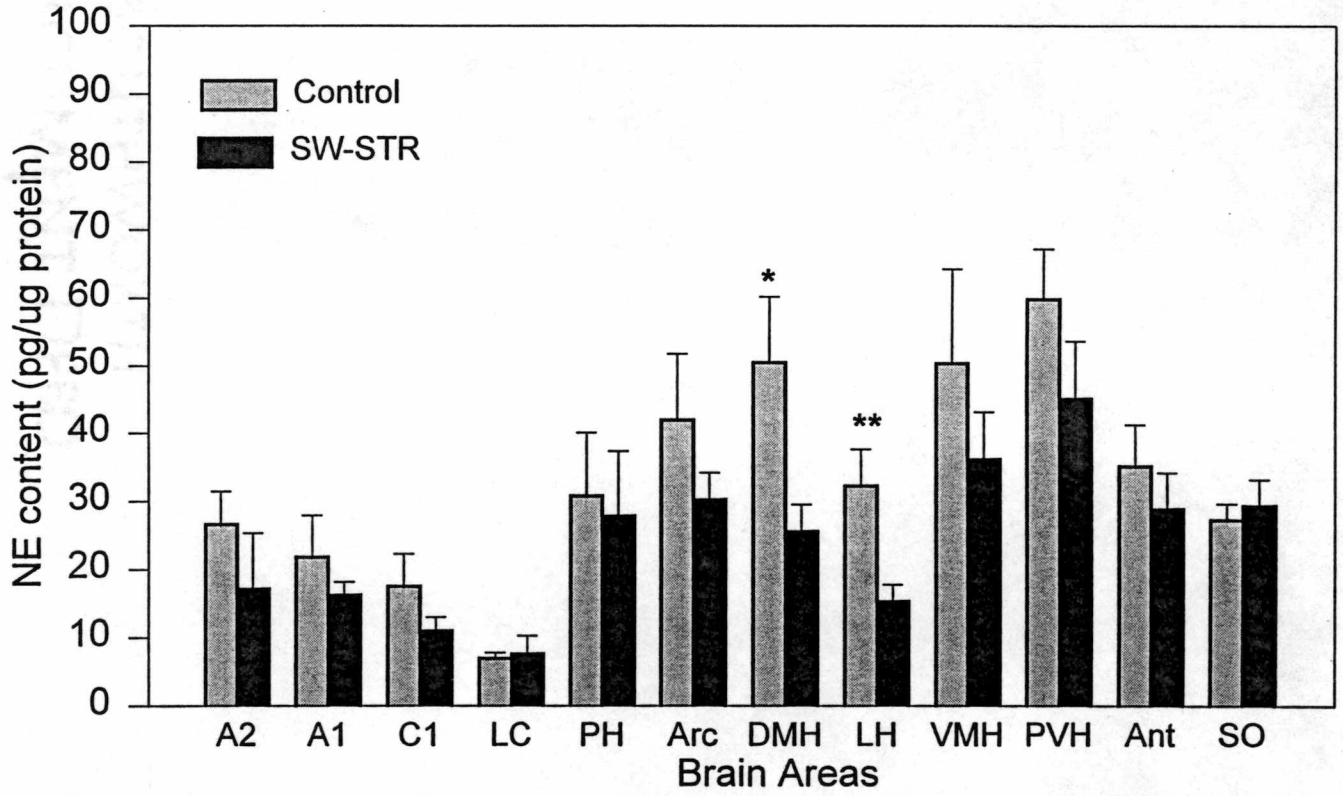

Figure 13. NE levels in the brain nuclei after 6 months of swim+stress (SW-STR) treatment vs. nonintervention (*p<0.05; **p<0.01).

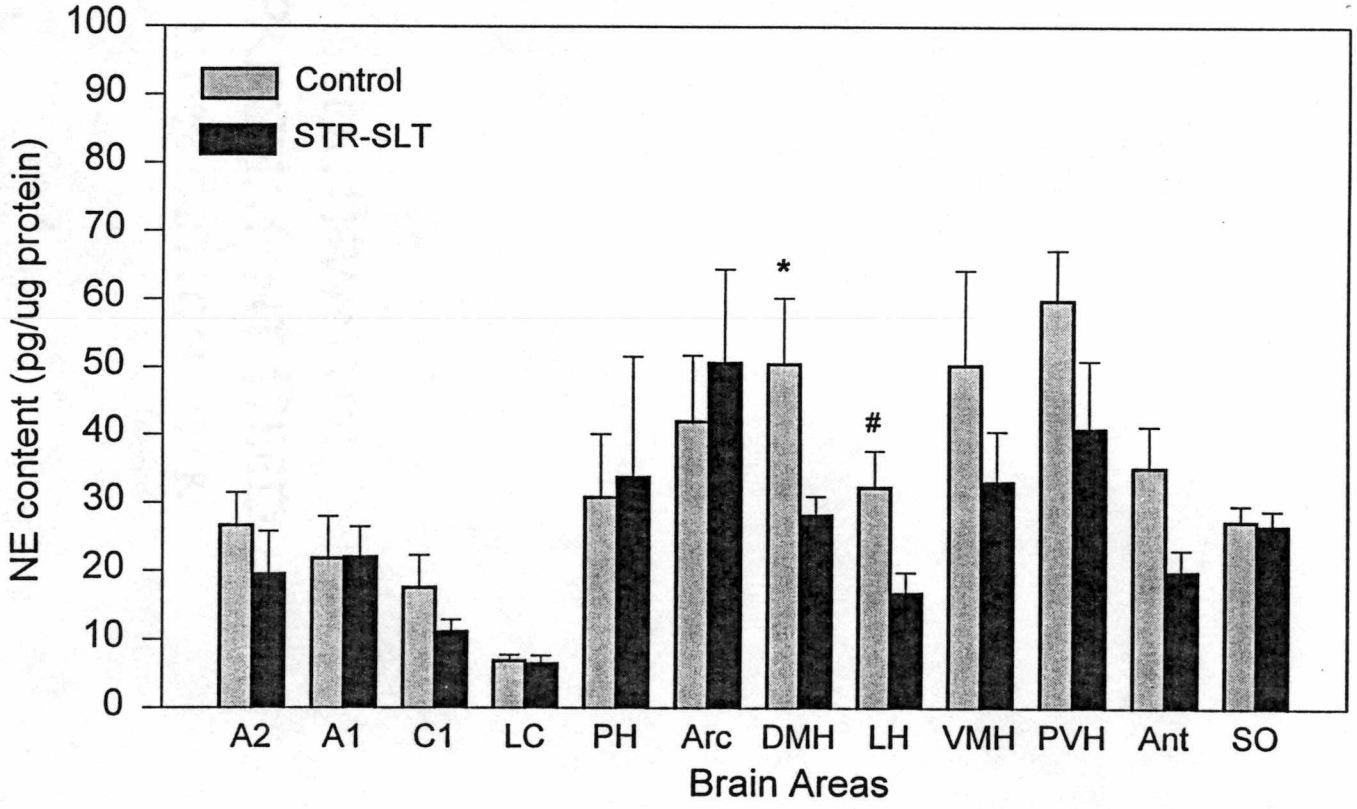

Figure 14. NE levels in the brain nuclei of BHR after 6 months of stress+salt (STR-SLT) treatment vs. nonintervention (*p<0.05; #p<0.1).

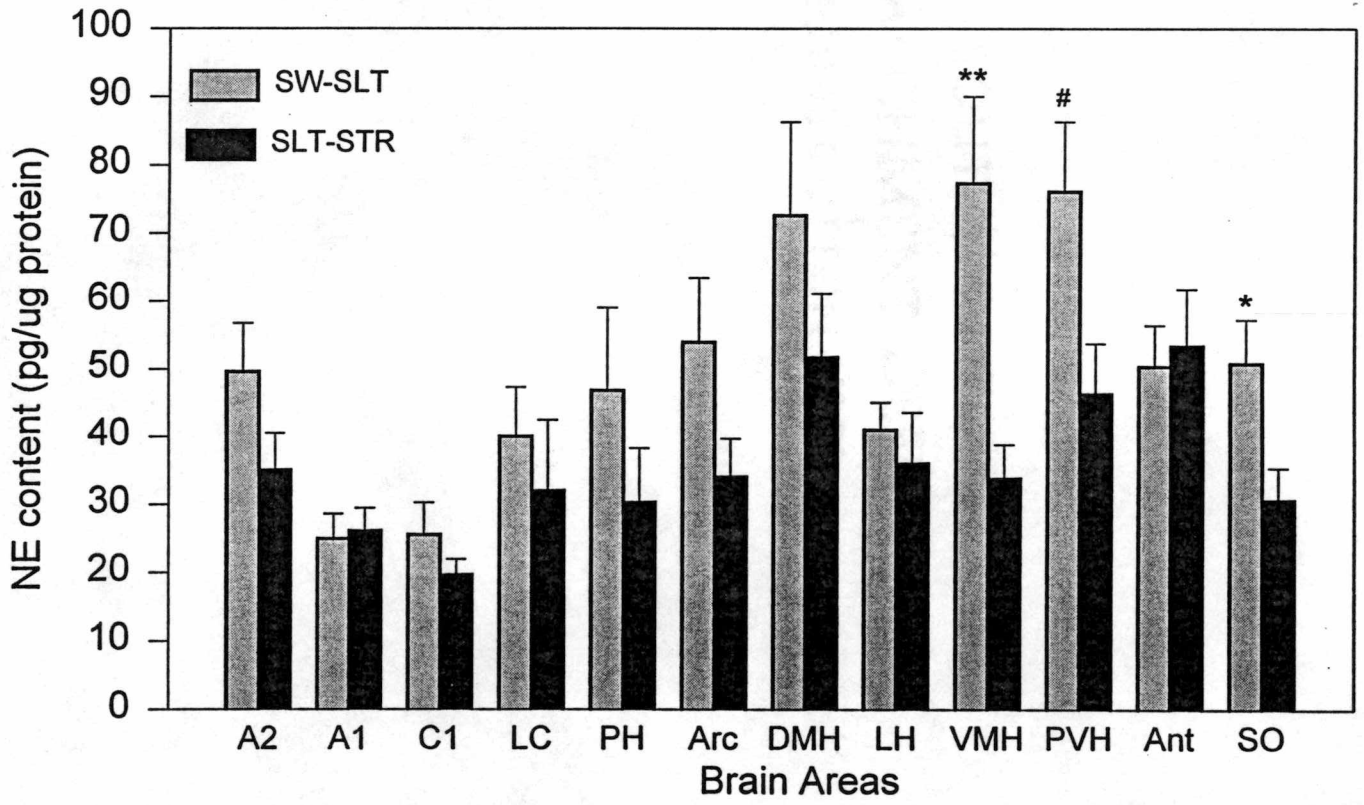

Figure 15. NE levels in the brain nuclei of BHR after 2 months of swim+salt (SW-SLT) vs. salt+stress (SLT-STR) treatment (*p<0.05; **p<0.01; #p<o.1).

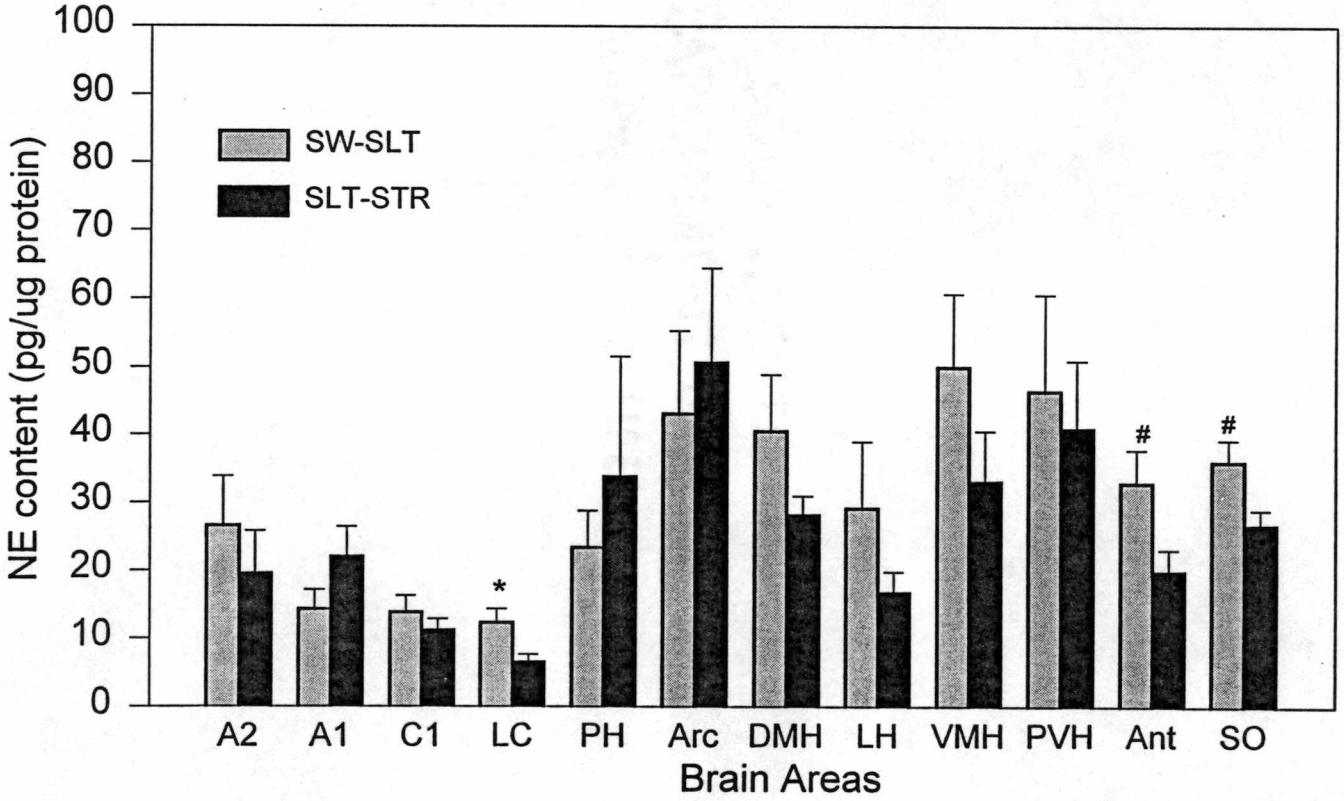

Figure 16. NE levels in the brain nuclei of BHR after 6 months of swim+salt (SW-SLT) vs. salt+stress (SLT-STR) treatment (*$p<0.05$; #$p<0.1$).

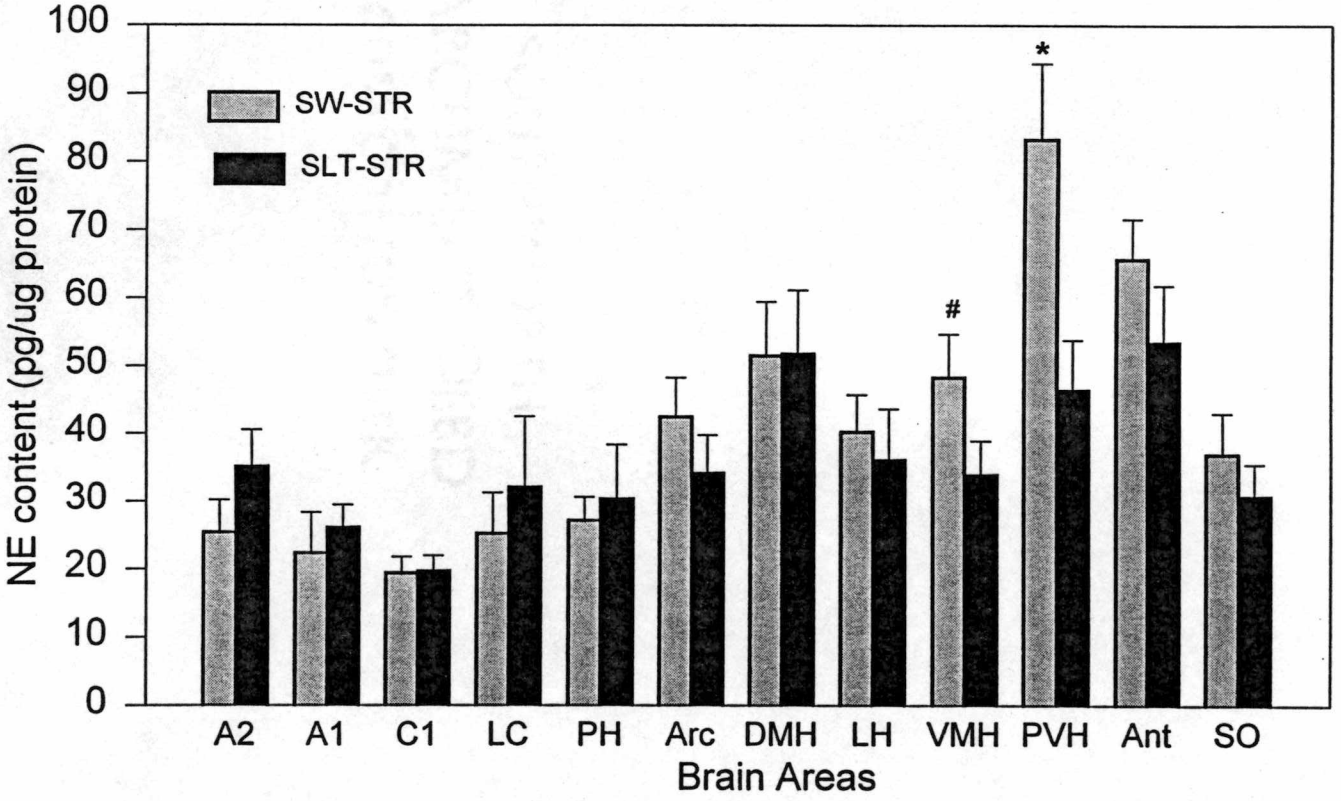

Figure 17. NE levels in the brain nuclei of BHR after 2 months of swim+stress (SW-STR) vs. swim+stress (SLT-STR) treatment (*p<0.05; #p<0.1).

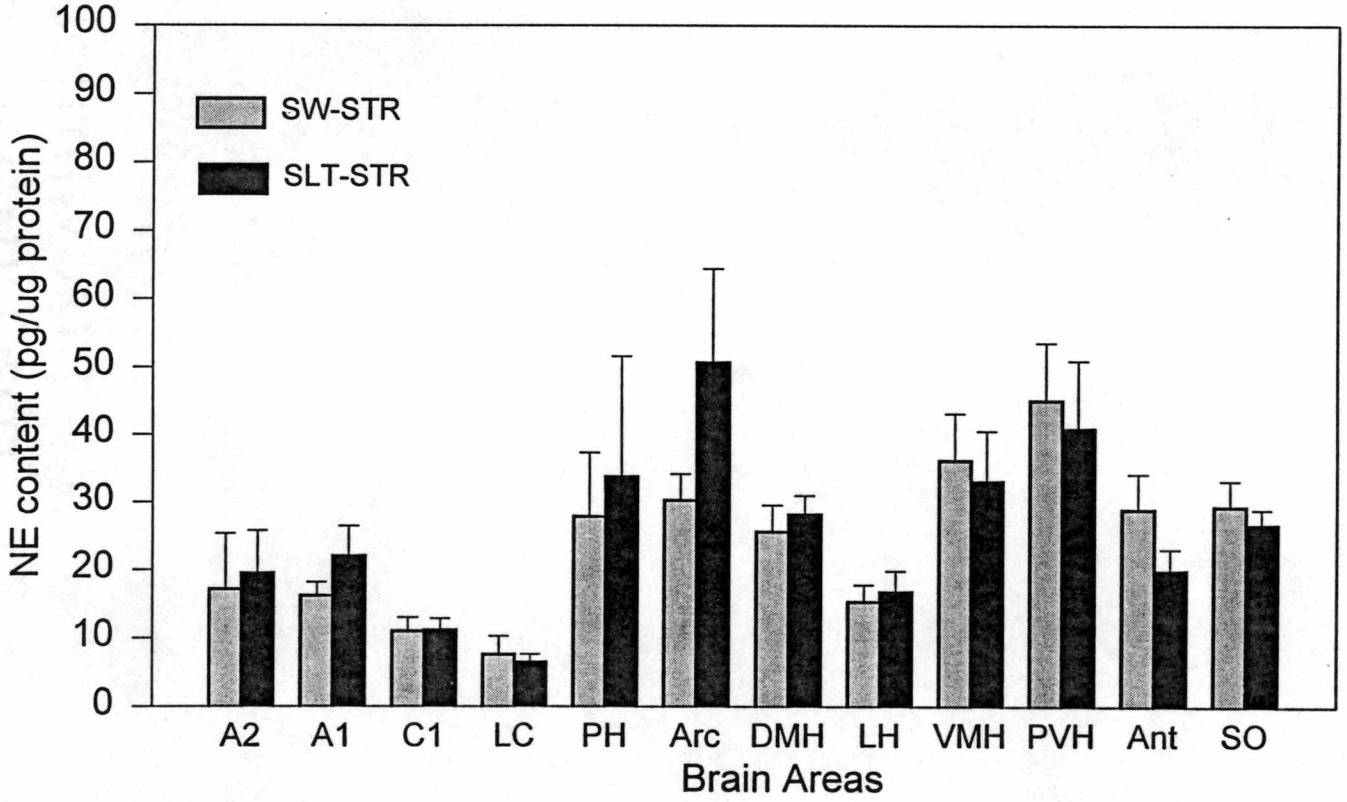

Figure 18. NE levels in the brain nuclei of BHR after 6 months of swim+stress (SW-STR) vs. salt+stress (SLT-STR) treatment.

CHAPTER 4

DISCUSSION

As discussed in the literature review, several nuclei in the brain stem and hypothalamus are important in coupling the behavioral response following environmental challenges with the physiological adaptations necessary to sustain the behavior. They also play important roles in the integration of neural and hormonal control of the cardiovascular system to maintain homeostasis. The central distribution of the NE cell groups and the connections between them cover several regions in the hypothalamus and medulla. Evidence has suggested the role of the central NE system in cardiovascular response to stressors.

Compound stressors (salt+stress, salt+swim, swim+stress) were used in this study. Previous research has shown that individual components (i.e., salt and stress) of the stressor are associated with chronic elevations in blood pressure in the BHR (Lawler et al, 1980; 1981; 1984; 1989, Sanders et al, 1989). Exercise might have the ability to counteract the effect of stress-induced cardiovascular and renal pathology (Cox et al, 1985; McCoy et al, 1993).

The first objectives of this study was to investigate the effect of a compound stressor (salt+stress) on NE levels in discrete brain nuclei known to play a role in blood pressure regulation. Results from this study revealed that the combination of salt plus stress reduced NE levels compared to BHR control animals. After two months of treatment, the VMH showed a significant decrease, while after six months, DMH, PVH, and Ant nuclei showed marginal decreases, and LH showed a significant decrease.

The second objective was to determine the attenuating role of exercise in the central response to either stress or a high salt diet. As previously discussed, the nature of exercise and its ultimate impact on the organism is determined by the intensity, duration and type of exercise. To a certain extent, exercise can be considered a stressor. Comparison of the neural and hormonal response between exercise and other stressors shows some similarities. For example, Dimsdale and Moss (1980) demonstrated that during public speaking, both plasma NE and Epinephrine (Epi) increased. The similar catecholamine response pattern also was demonstrated in animals (de Boer, 1989). In this study, plasma NE, Epi and corticosterone (CS) increases were

determined in individual rats subjected to either 20 regularly or irregularly scheduled white-noise stimulations (4 min, 100 dBA). Blood was frequently sampled during the first and twentieth noise exposure, and during a re-exposure after 24 hours. The NE response to noise was partially attenuated following irregular administration of noise. However, regular exposure produced increased NE levels prior to noise presentation and a subsequent decrease during stimulation. After 24 hr, noise evoked an exaggerated initial NE release in the regular group. The noise-elicited rise in Epi was completely abolished after 20 noise presentations irrespective of whether these were applied regularly or irregularly. Re-exposure after 24 hr again evoked a significant Epi response in both groups. In another study by Stratton et al (1983), serial hemodynamic and plasma catecholamine responses were compared among 10 healthy men during symptom-limited handgrip, cold pressor testing and symptom-limited supine bicycle exercise. During maximal supine exercise, plasma NE and Epi concentrations increased three to six times more than during either symptom-limited handgrip or cold pressor testing. Nieman et al (1989) reported that one 3 hour bout of exercise elicited elevation of NE and Epi in marathon runners.

In the present study, the exercise procedure was forced isothermic swimming, lasting for 90 minutes. Significantly higher levels of NE were present in exercise plus salt BHR when compared with salt plus stress BHR for several hypothalamic nuclei, as well as for the locus coeruleus. In addition, significantly higher levels of NE were present in exercise plus stress BHR when compared with salt plus stress BHR after two months of treatment, but not after six months. The fact that animals exposed to stressors combined with swim had higher central NE, especially during the first two months, indicates that early exercise evokes NE responses that may be associated with preventing the hypertension that would otherwise develop from these interventions (Lawler et al, 1980; 1984) via effects on central nervous system structures primarily in the hypothalamus. In short, the deleterious effects of salt and stress on central NE levels can be attenuated by pairing either with exercise.

The primary source of exercise's effect centrally is during an early phase, since most of the changes were confined to the 2-month treatment group. These results may indicate that central NE levels are critically important during the early phase of a chronic stressor. Subsequently, since previous studies in this laboratory have shown that

blood pressure elevations are chronic during either stress (Lawler et al, 1980) or salt (Lawler et al, 1984), it may be that other factors intervene to maintain the hypertension produced by the environmental stressor. The role of exercise could thus be to reduce the effect of a central trigger that would otherwise initiate hypertension.

These exercise-induced alterations in NE levels could be attributed to any of several mechanisms. For example, a reduction of NE content could be produced by a decrease in synthesis, which can be estimated by measuring the enzyme phenylethanolamine N-methyltransferase (Saavedra and Torda, 1980). Another possibility is that the decrease in NE content reflects an increase of release, which leads to the depletion of NE storage. The efflux or release of NE can be accessed by *in vivo* microdialysis (Abercrombie et al, 1988; Saavedra and Torda, 1980). Another method, which measures the turnover of the NE to evaluate the activity of the noradrenergic system, has been used in other animal models (Brodie et al, 1966; Nakata et al, 1993). The present results, though intriguing, do not allow us to choose a probable cause for the differences observed.

The present study examined a number of important nuclei

that were sensitive to chronic compound stressors, including A2, A1, LC, C1, DMH, PVH, Ant, VMH, and SO. However, the present experimental design does not give us enough information to assess the functional alterations in the NE system of the brain. The change in NE content induced by chronic compound stressors could indicate other related adaptive changes. For instance, the observed alterations in NE levels may be associated with changes in the sensitivity of noradrenergic receptors. For example, an increased sensitivity of the C1 cell group (RVLM region) was found after expoure to excessive salt intake (Pawloski-Dahm and Gordon, 1993). Microinjections of L-glutamate augmented blood pressure response in rats fed high salt diet. Klangkalya et al (1988) reported that low affinity p-[3H] aminoclonidine (an alpha 2-adrenoceptor agonist) binding in the anterior hypothalamic region was increased significantly in SHR after one or two weeks of high salt loading. Considering the fact that high salt diet reduced hypothalamic NE turnover in the same animal model (Chen et al, 1988), they suggest that reduction in NE release induces a compensatory increase in alpha-2-adrenoceptor number in the anterior hypothalamic area of SHR. DiBona and Jones (1991) reported that 6 weeks of high salt diet increased the responsiveness of the alpha-receptors in the brain of BHR.

Thus, decreased or increased NE content might not reflect the excitation or inhibition of noradrenergic function. Theories that explain the neurochemical basis of adaptation to stress should take receptor function into account. Research on the correlation of the content and the responsiveness of the noradrenergic receptors would give us more detailed information.

One aspect of this study that is somewhat disturbing, however, is a comparison of the direction of differences found when comparing chronic salt plus stress to control in the present study versus that found in a previously published study from our laboratory (Mitchell and Lawler, 1987). In that previous study, BHR were exposed to a repeated single stressor (tail shock) for four months and the NE content in the hypothalamus was eventually increased, while in the current study, the salt plus stress treatment tended to reduce NE content, producing marginal and significant differences in several hypothalamic nuclei. This discrepancy may reflect the different stress procedures and stress durations utilized in the two studies. Researchers have not obtained consistent results in NE levels after exposure to stressors using various animal models and different stress protocols. For example, Corder and his

coworkers (1992) reported that repeated footshock or chronic high salt alone did not affect the concentration of neuropeptide Y (NPY), whereas the combination of shock and salt produced significant elevations in NPY, which could interact with the NE response. Kvetnansky et al (1977) reported increased NE concentration in the hypothalamus after chronic stress, whereas Adell et al (1990) found no significant changes. Since our previous study found that reductions, no differences, or increases could be found in NE levels, depending on when they are determined relative to treatment onset (and, presumably, stage of hypertension development), patterns determined at several time points may be necessary.

In conclusion, compound stressors alter NE levels in selected brain stem and hypothalamic nuclei in BHR. Two or six months of swim+stress, swim+salt or stress+salt treatment tends to reduce the NE levels in most of the selected nuclei. The duration of the stressors was associated with changes in central NE levels. Animals after two months of exposure to stressors have higher NE levels than after six months of stress. This study in BHR suggests that the brain noradrenergic system is involved in the adaptation to environmental stress and the regulation of

blood pressure. Exercise may evoke central NE responses which could have protective effects leading to the prevention of hypertension.

The complex data of this study do not provide a simple and definitive explanation regarding the role of the central NE system in the development of stress-induced hypertension and adaptive responses to stress in general. This is partially due to the complexity of the neural and hormonal systems involved in the response to various environmental stressors. However, the NE alteration in discrete nuclei after experimental treatment does provide important information concerning the impact of environmental stress on the central nervous system thought to play an essential role in the development of hypertension. This information also gives us some insight into the role of exercise in intervening stress-induced alterations of NE levels, as well as stress-induced hypertension. To understand the mechanisms in detail, however, further investigations, focusing on receptor change, and the involvement of other neurotransmitters and modulators, and their interactions, are necessary.

BIBLIOGRAPHY

Abercrombie ED, Keller Jr, RW, Zigmond J. Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: pharmacological and behavioral studies. Neuroscience. 1988;27:897-904.

Abboud FM. The sympathetic nervous system in hypertension. Clin Exp Hypertens. 1984;6:43-60.

Adell A, Garcia-Marquez C, Armario A, et al. Effects of chronic stress on serotonin and norepinephrine in the rat brain. Biogen Amines. 1990;7:19-26.

Agarwal SK, Calaresu FR. Monosynaptic connection from caudal to rostral ventrolateral medulla in the baroreceptor reflex pathway. Brain Res. 1991;555:70-74.

Agarwal SK, Gelsema AJ, Calaresu FR. Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. Am J Physiol. 1989;257:R265-270.

Agarwal SK, Gelsema AJ, Calaresu FR. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. Am J Physiol. 1990;258:R127-138.

Alexander N. Psychosocial hypertension in members of a Wistar rat colony. Proc Soc Exp Biol Med. 1974;146(1):163-169.

Amendt KJ, Czachurski J, Dembowsky K, Seller H. Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J Autonom Nerv Syst. 1979;1:103-117.

Arends JJ, Wild JM, Zeigler HP. Projections of the nucleus of the tractus solitarius in the pigeon (Columba livia). J Comp Neurol. 1988;278:405-429.

Bachelard H, Harland D, Gardiner SM, et al. Regional haemodynamic effects of noradrenaline injected into the hypothalamic paraventricular nuclei of conscious, unrestrained rats: possible mechanisms of action. Neuroscience. 1992;47:941-957.

Bandler R, Tork I. Midbrain periaqueductal grey region in the cat has afferent and efferent connections with solitary tract nuclei. Neurosci Lett. 1987;74:1-6.

Banks D, Harris MC. Lesions of the locus coeruleus abolish baroreceptor-induced depression of supraoptic neurons in the rat. J Physiol (London). 1984;355:383-398.

Benarroch EE, Balda MS, Finkielman, et al. Neurogenic hypertension after depletion of norepinephrine in anterior hypothalamus induced by 6-hydroxydopamine administration into the ventral pons: role of serotonin. Neuropharmacology. 1983;22:29-34.

Blaustein MP, Hamlyn JM. Sodium transport inhibition, cell calcium, and hypertension. The natriuretic hormone/Na+-Ca2+ exchange/hypertension hypothesis. Am J Med. 1984;77:45-59.

Blessing WW, Li YW. Inhibitory vasomotor neurons in the caudal ventrolateral region of the medulla oblongata. Prog Brain Res. 1989;81:83-97.

Blessing WW, Reis DJ Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: relationship to the area containing A1 noradrenaline cells. Brain Res. 1982;253:161-171.

Blessing WW, Reis DJ. Evidence that GABA and glycine-like inputs inhibit vasopressor neurons in the caudal ventrolateral medulla of the rabbit. Neurosci Lett. 1983;37:57-62.

Blessing WW. Depressor neurons in rabbit caudal ventrolateral medulla act via GABA receptors in rostral medulla. Am J Physiol. 1988;254:H686-692.

Brodie BB, Costa E, Dlabac A, et al. Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J Pharmacol Exp Therapeut. 1966;154:493-498.

Brody, MJ, Varner, KJ, Lewis, SJ. Central nervous system and the pathogenesis of hypertension. Hypertension. 1989;18 (Suppl III):III-7-III-12.

Bunag RD, Eferakeya AE. Immediate hypotensive aftereffect of posterior hypothalamic lesions in awake rats with spontaneous, renal, or Doca hypertension. Cardiovas Res.

1976;10:663-671.

Byrum CE, Guyenet PG. Afferent and efferent connections of the A5 noradrenergic cell group in the rat. J Comp Neurol. 1987;261:529-542.

Cabot JB. Sympathetic preganglionic neurons: cytoarchitecture, ultrastrucutre, and biophysical properties. In Loewy AD and Spyer KM (Edit). Central Regulation of Autonomic Functions. pp.22-34, Oxford University Press, New York, Oxford, 1990.

Carrive P, Bandler R, Dampney RAL. Anatomical evidence that hypertension associated with the defense reaction in the cat is mediated by a direct projection of the midbrain periaqueductal grey to the subretrofacial nucleus of the medulla. Brain Res. 1988;460:339-345.

Chalmers J, Arnolda L, Kapoor V, Llewellyn-Smith I, Minson J, Pilowsky P. Amino acid neurotransmitters in the central control of blood pressure and in experimental hypertension. J Hypertens. 1992;10:S27-S37.

Chalmers JP, Pilowsky PM. Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. J Hypertens. 1991;9:675-94.

Chen, Y-F; Meng, Q-C; Wyss, JM, et al. High NaCl diet reduces hypothalamic norepinephrine turnover in hypertensive rats. Hypertension. 1988;10:55-62.

Cieroba ZJ, Li YW, Blessing WW. Characteristics of the caudal ventrolateral medullary neurons antidromically activated from rostral ventrolateral medulla in the rabbit. Brain Res. 1992;582:196-207.

Ciriello J. Caverson MM, Polosa C. Function of the ventrolateral medulla in the control of blood pressure. Brain Res Rev. 1986;11:359-391.

Ciriello, J., Rohlicek, CV, Polosa, C. Aortic baroreceptor reflex pathway: A functional mapping using [3H]2-deoxyglucose autoradiography in the rat. J Auton Nerv Syst. 1983; 8:111-128.

Connor JA. Neural pacemakers and rhythmicity. Ann Rev Physiol. 1985;47:17-28.

Connor HE, Drew GM. Do adrenaline-containing neurones from the rostral ventrolateral medulla excite preganglionic sympathetic cell bodies? J Auton Pharmacol. 1987;7:87-96.

Coote JH, Hilton SM, Perez-Gonzalez JF. Inhibition of the baroreceptor reflex on the stimulation in the brain stem defence centre. J Physiol (London). 1979;288:549-560.

Coote JH. The organization of cardiovascular neurons in the spinal cord. Rev Physiol Biochem Pharmacol. 1988;110:147-285.

Corder R, Castagne V, Rivet, JM, et al. Central and peripheral effects of repeated stress and high NaCl diet on neuropeptide Y. Physiol Behav. 1992;52:205-210.

Corley KC, Mauck HP, Shiel FO. Cardiac responses associated with "yoked-chair" shock avoidance in squirrel monkeys. Psychophysiology. 1975;12:439-444.

Corley KC, Mauck HP, Shiel FO, et al. Myocardial dysfunction and pathology associated with environmental stress in squirrel monkey: effect of vagotomy and propranolol. Psychophysiology 1979;16:554-560.

Corley KC, Shiel FO, Mauck HP, et al. Myocardial degeneration and cardiac arrest in squirrel monkey: physiological and psychological correlates. Psychophysiology. 1977;14:322-328.

Cowley AW Jr. Long-term control of arterial blood pressure. Physiol Rev. 1992;72:231-300.

Cox RH, Hubbard JW, Lawler JE, et al. Exercise training attenuates stress-induced hypertension in the rat. Hypertension. 1985;7:745-751.

Cravo SL, Morrison SF, Reis DJ. Differentiation of two cardiovascular regions within caudal ventrolateral medulla. Am J Physiol. 1991;261:R985-994.

Cunningham ET, Nissen R, Renaud LP Norepinephrine injections in diagonal band of Broca selectively reduced the activity of vasopressin supraoptic neurons in the rat. Brain Res. 1993;610:152-155.

Cunningham ET, Sawchenko PE. Anatomical specificity inputs to the paraventricular and supraoptic nuclei of the rat

hypothalamus. J Comp Neurol. 1988;274:60-76.

Dahlstrom A, Fuxe K. Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol Scand. 1964;62(Suppl 232):1-55.

Dampney RAL, Czachurski J, Dembowsky K, et al. Afferent connections and spinal projections to the vasopressor region in the rostral ventrolateral medulla of the cat. J Autonom Nerv Syst. 1987;20:73-86.

Dampney RAL, Goodchild AK, Robertson LG, Montgomery W. Role of the ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. Brain Res. 1982;249:223-235.

Dampney RAL, Goodchild AK, Tan E. Vasopressor neurons in rostral ventrolateral medulla of the rabbit. J Autonom Nerv Syst. 1985;4:239-254.

Dampney RAL, Moon EA. Role of ventrolateral medulla in vasomotor response to cerebral ischemia. Am J Physiol. 1980;239:H349-358.

Dampney RAL. Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 1994;74:323-364.

De Boer SF, Koopmans SJ, Slangen JL, Van der Gugten J. Effects of fasting on plasma catecholamine, corticosterone and glucose concentrations under basal and stress conditions in individual rats. Physiol Behav. 1989;45:989-994.

De Novellis V, Stotz-Potter EH, Morin SM et al. Hypothalamic sites mediating cardiovascular effects of microinjected bicuculline and EAAs in rats. Am J Physiol. 1995;269:R131-R140.

De Wardener HE and MacGregor GA. The relation of a circulating sodium transport inhibitor (the natriuretic hormone?) to hypertension. Medicine (Baltimore). 1983a;62:310-326.

De Wardener HE and MacGregor GA. The role of a circulating inhibitor of Na+-K+-ATPase in essential hypertension. Am J Nephrol 1983b;3:88-91.

DeJong W, Zandberg P, Palkovits M, Bohus B. Acute and chronic hypertension after lesions and transection of the rat brain stem. In DeJong W, Provost AP and Shapiro AP (Ed). Progress in Brain Research, Vol. 47) Hypertension and Brain Mechanisms. Elsevier Scientific Publishing Co., New York, 1977.

Dembowsky K, Czachurski J, Seller H. Some properties of the caudal ventrolateral medulla in the cat. Prog. Brain Res. 1989;81:143-157.

DiBona GF, Jones SY. Central alpha2-adrenoceptor responsiveness in borderline hypertensive rats. J. Hypertens. 1991;9:543-547.

DiBona GF; Jones SY. Acute environmental stress overrides cardiac volume receptor reflex in borderline hypertensive rats. J Hypertens. 1995;13:63-68.

Dimsdale JE, Moss J. Short-term catecholamine response to psychological stress. Psychosom Med 1980;42:493-497.

Dorward PK, Andresen MC, Burke SL et al. Rapid resetting of the aortic baroreceptors in the rabbit and its implications for short-term and longer term reflex control. Circ Res. 1982;50:428-439.

Drolet GJ, Chalmers J, Blessing W. Vasodepressor neurons in the medulla alter cardiac contractility and cardiac output. Hypertension (Dallas). 1993;21:210-215.

Eckberg DL, Abboud FM, Mark AL. Modulation of carotid baroreflex responsiveness in man: Effects of posture and propranolol. J Appl Physiol. 1976;41:383.

Ekelund LG, Haskell WL, Johnson JL, et al. Physical fitness as a predictor of cardiovascular mortality in asymptomatic North American men. The Lipid Research Clinics Mortality Follow-up Study. N Engl J Med. 1988;319:1379-1384.

Feldberg W, Guertzenstein PG. A vasodepressor effect of pentobarbitone sodium. J Physiol. 1972;224:83-103.

Feldberg W, Guertzenstein PG. Vasodepressor effect obtained by drug acting on the ventral surface of the brain stem. J Physiol (Lond). 1976;258:337-355.

Feldberg W. The ventral surface of the brainstem: a scarcely

explored region of pharmacological sensitivity. Neurosci. 1976;1:427-441.

Ferrario CM, Averill DB. Do primary dysfunctions in neural control of arterial pressure contribute to hypertension? Hypertension. 1991;18(Suppl I):I-38-I-51.

Ferrari AU, Gordon FJ, Mark AL. Primary impairment of cardiopulmonary baroreflexes in Dahl salt-sensitive rats. J Hypertens Suppl. 1984;2:S401-S403.

Folkow B. Sympathetic nervous control of blood pressure. Role in primary hypertension. Am J Hypertens. 1989;2:103S-111S.

Folkow B, Rubenstein EH. Cardiovascular effects of acute and chronic stimulation of the hypothalamic defense area in the rat. Acta Physiol Scand. 1966;68:48-57.

Gelsema AJ, Roe MJ, Calaresu FR. Neurally mediated cardiovascular responses to stimulation of cell bodies in the hypothalamus of the rat. Brain Res. 1989;482:67-77.

Gelsema AJ, Schoemaker RG, Ruzicka M, et al. Cardiovascular effects of social stress in borderline hypertensive rats. J Hypertens 1994;12:1019-1028.

Gieroba ZJ, Li, YW, Wesselingh SL, Blessing WW. Transneuronal labeling of neurons in rabbit brain after injection of herpes simplex virus type 1 into the aortic depressor nerve. Brain Res. 1991;558:264-272

Gordon FJ. Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. Am J Physiol 1987;252:R628-R633.

Granata A, Kumada M, Reis DJ. Sympathoinhibition by A1-noradrenergic neurons is mediated by neurons in the C1 area of the rostral medulla. J Autonom Nerv Syst. 1985;14:387-395.

Granata AR, Ruggiero DA, Park DH, Hoh TH, Reis DJ. Brain stem area with C1 epinephrine neurons mediates baroreflex vasopressor responses. Am J Physiol. 1985;248:H547-567.

Gray SD. Spontaneous hypertension in neonatal rat. Clin Exp Hypertens. 1984;6:755-781.

Guertzenstein PG, Silver A. Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. J Physiol. (Lond) 1974;242:489-503.

Guevara-Aguilar R, Donatti-Albarran OA, Solano-Flores LP, Wayner MJ. Nucleus of the tractus solitarius projections to the olfactory tubercle: an HRP study. Brain Res Bull. 1987;18:673-675.

Guyenet PG, Cabot JB. Inhibition of sympathetic neurons by catecholamines and clonidine: mediation by an alpha-adrenergic receptor. J Neurosci. 1981;1:908-917.

Guyenet PG, Haselton JR, Sun MK. Sympathoexcitatory neurons of the rostral ventrolateral medulla and the origin of the sympathetic vasomotor tone. Prog Brain Res. 1989;81:105-116.

Guyenet PG, Stornetta RL. Inhibition of sympathetic discharges by epinephrine and alpha-methylepinephrine. Brain Res. 1983;235:271-283.

Guyton AC, Richardson TQ, Langston JB. Regulation of cardiac output and venous return. Clin Anesth. 1964;3:1-34.

Harris MC, Loewy AD. Neural regulation of vasopressin-containing hypothalamic neurons and the role of vasopressin in cardiovascular function. In Loewy AD and Spyer KM (Ed). Central Regulation of Autonomic Functions. pp.225-246, Oxford University Press, New York, Oxford, 1990.

Harris MC. Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurons in the rats. J Endocrinol. 1979;82:115-125.

Hatton DC, Buchholz RA, Fitzgerald RD. Autonomic control of heart rate and blood pressure in spontaneously hypertensive rats during aversive classical conditioning. J Comp Physiol Psychol. 1981;95:978-990.

Heesch CM, Abboud FM, Thames MD. Acute resetting of carotid sinus baroreceptors, II. Possible involvement of electrogenic Na^+ pump. Am J Physiol 1984;247:H833-839.

Helke CJ, Sayson SC, Keeler JR, Charlton CG. Thyrotropin-releasing hormone-immunoreactive neurons project from the ventral medulla to the intermediolateral cell column: partial coexistence with serotonin. Brain Res. 1986;381:1-7.

Hendley ED, Ohlsson WG, Musty RE. Interstrain aggression in hypertensive and/or hyperactive rats: SHR, WKY, WKHA, WKHT. Physiol Behav 1992;51:1041-1046.

Hendley ED, Ohlsson WG. Two new inbred rat strains derived from SHR: WKHA, hyperactive, and WKHT, hypertensive, rats. Am J Physiol 1991;261:H583-H589

Henry JP, Liu YY, Nadra WE, et al. Psychosocial stress can induce chronic hypertension in normotensive strains of rats. Hypertension 1993;21:714-723.

Henry JP, Meehan JP, Stephens PM. The use of psychosocial stimuli to induce prolonged systolic hypertension in mice. Psychosom Med 1967;29:408-432.

Henry JP. The induction of acute and chronic cardiovascular disease in animals by psychosocial stimulation. Int J Psychiatry Med 1975;6:147-158.

Herbert H, Moga MM, Saper CB. Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. J Comp Neurol. 1990;293:540-580.

Hokfelt T, Fuxe K, Goldstein M. Immunohistochemical studies on monoamine-containing cell systems. Brain Res 1973;62:461-469.

Holets VR. The anatomy and function of noradrenaline in the mammalian brain. In: The Pharmacology of Noradrenaline in the Central Nervous System. Heal DJ and Marsden CA (Ed). Oxford Medical Publications, Oxford University Press, New York, 1990.

Huang BS, Leenen FH. Dietary Na+ and cardiopulmonary baroreflex control of renal sympathetic nerve activity in SHR. Am J Physiol. 1995;268:H61-H67.

Jansen, ASP, Nguyen XV, Karpitskiy V, et al. Central command neurons of the sympathetic nervous system: Basis of the flight-or-fight response. Science. 1995;270:644-646.

Jeske J, Reis DJ, Milner A. Neurons in the barosensory area of the caudal ventrolateral medulla project monosynaptically on to sympathoexcitatory bulbospinal neurons in the rostral ventrolateral medulla. Neurosci. 1995;65:343-353.

Joint National Committee. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med. 1993;153:154-183.

Jordan D, Mifflin SW, Spyer KM. Destruction of noradrenergic neurons in rabbit brainstem elevates plasma vasopressin, causing hypertension. Science. 1982;217:661-663.

Jordan D, Mifflin SW, Spyer KM. Hypothalamic inhibition of neurones in the nucleus tractus solitarius of the cat is GABA mediated. J Physiol. (Lond.) 1988;399:389-404.

Jordan D. Autonomic changes in affective behaviour. In Loewy AD and Spyer KM (Ed). Central Regulation of Autonomic Functions. pp.349-366. Oxford University Press, New York, Oxford, 1990.

Klangkalya B, Spripairojthikoon W, Oparil S, Wyss, JM. High NaCl diet increases anterior hypothalamic alpha sub(2)-adrenoceptors in SHR. Brain Res. 1988;415:77-84.

Kobinger W. Central blood pressure regulation. Chest. 1983;83:297-300.

Krichheim HR. Systemic arterial baroreceptor reflexes. Physiol Rev. 1976;56:100-116.

Kvetnansky R, Palkovits M, Mitro A, et al. Catecholamines in individual hypothalamic nuclei of acutely and repeatedly stressed rats. Neuroendocrinology. 1977;23:257-267.

Lakka TA, Venalainen JM, Rauramaa R, Salonen R, Tuomilehto J and Salonen JT. Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. N Engl J Med 1994;330:1549-1554.

Lawler JE, Barker GF, Hubbard JW, Allen, MT. The effect of conflict on tonic levels of blood pressure in the genetically borderline hyperensive rat. Psychophysiology. 1980;17:363-370.

Lawler JE, Barker GF, Hubbard JW, Schaub RG. Effect of stress on blood pressure and cardiac pathology in rats with borderline hypertension. Hypertension. 1981;3:496-505.

Lawler JE, Barker GF, Hubbard JW, et al. Blood pressure and plasma renin activity responses to chronic stress in

borderline hypertensive rat. Physiol. Behav. 1984;32:101-105.

Lawler JE, Edgemon IP. The borderline hypertensive rat (BHR): A model for studies of the role of the environment in hypertension. In R.M. McCarty, D.A. Blizard, and R.L. Chevalier (Eds), Development of the hypertensive phenotype: Basic and clinical studies. New York: Elsevier. 1998.

Lawler JE, Naylor SK, Abel MM. Predictability of foot shock differentially affects the phasic blood pressure of SHR, BHR, and WKY rats. Physiol Behav 1993(a);54:369-374.

Lawler JE, Naylor SK, Abel MM, Baldwin DR. A chronic high-salt diet fails to enhance blood pressure reactivity to a tone associated with footshock in SHR, BHR, and WKY rats. Physiol Behav 1993(b);54:941-946.

Lawler JE, Obrist PA, Lawler KA. Cardiovascular function during pre-avoidance, avoidance, and post-avoidance in dogs. Psychophysiology. 1975;12:4-11.

Lawler JE, Sanders BJ, Chen Y-F, et al. Hypertension produced by a high sodium diet in the borderline hypertensive rat (BHR). Clin Exp Hypertension. 1987;A9:1713-1732.

LeDoux JE, Sakaguchi A, Reis DJ. Behaviorally selective cardiovascular hyperreactivity in spontaneously hypertensive rats: evidence for hypoemotionality and enhanced appetitive motivation. Hypertension. 1982;4:853-863.

Li SG, Lawler JE, Randall DC, Brown DR. Sympathetic nervous activity and arterial pressure responses during rest and acute behavioral stress in SHR versus WKY rats. J Auton Nerv Syst. 1997;62:147-154.

Li YW, Wesselingh SL, Blessing WW. Projections from rabbit caudal medulla to C1 and A5 sympathetic premotor neurons, demonstrated with phaseolus leucoagglutinin and herpes simplex virus. J Comp Neurol. 1992;317:379-395.

Lightman SL, Todd K, Everitt BJ. Ascending noradrenergic projections from the brainstem: Evidence for a major role in the regulation of blood pressure and vasopressin secretion. Exp Brain Res. 1984;55:145-151.

Linden W, Stossel C, Maurice J. Psychosocial interventions

for patients with coronary artery disease: a meta-analysis. Arch Intern Med. 1996;156:745-752.

Lindvall, O, Bjorklund A. The organization of the ascending catecholamine neuron system in the rat brain: revealed by the glyoxylic acid fluorescence method. Acta Physiol Scand Suppl. 1974;412:1-48.

Lindvall O, Bjorklund A. Dopamine- and norepinephrine-containing neuron systems: their anatomy in the rat brain. In Emson PC (Ed). Chemical Neuroanatomy. Pp 229-255. Raven Press, New York, 1983.

Lisa M, Filippelli A, Marmo E, Wible JH Jr, DiMicco JA. Microinjection of muscimol into posterior hypothalamus blocks cardiovascular response to experimental stress in rats. Pharmacol Res. 1989;21(Suppl. 1):9-10.

Loewy AD. Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. Brain Res. 1981a;222:129-133.

Loewy AD. Descending pathways to sympathetic and parasympathetic preganglionic neurons. J Auton Nerv Syst. 1981b;3:265-275.

Loewy AD, McKellar S. Serotonergic projections from the ventral medulla to the intermediolateral cell column in the rat. Brain Res. 1981;211:146-152.

Ludbrook MG, Ferrari A, Gregorini L, et al. Carotid baroreceptor reflex in normotensive and hypertensive subjects. Clin Sci Mol Med. 1976;51:343-345.

MacMahon S, Peto R, Cutler J, Collins R, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet 1990;335:765-774.

Maiorano G, Contursi V, Saracino E, Ricapito M. Physical exercise and hypertension. New insights and clinical implications. Am J Hypertens 1989; 2(2 Pt 2):60S-64S

Mansi JA, Drolet G. Chronic stress induces sensitization in sympathoadrenal responses to stress in borderline ypertensive rats. Am J Physiol. 1997;272:R813-R820.

McAllen RM. Action and specificity of ventral medullar vasopressor neurons in the cat. Neurosci. 1986a;18:51-59.

McAllen RM. Localization of neurons with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. Neurosci. 1986b;18:43-49.

McCoy DE, Steele JE, Cox RH, et al. Swim training alters renal and cardiovascular responses to stress in borderline hypertensive rats. J Appl Physiol 1993;75:1946-1954.

McCubbin JW. Carotid sinus participation in experimental renal hypertension. Circ Res. 1958;4:791-797.

McNeill TH, Sladek JR. Simultaneous monoamine histofluorescence and neuropeptide immunohistochemistry distribution of catecholamine varicosities and magnocellular neurosecretory neurons in the rat supraoptic and paraventricular nuclei. J Comp Neurol. 1980;193:1023-1033.

Melby CJ, Sanders BJ, Johnson AK. The effects of exercise training on salt-induced hypertension in the borderline hypertensive rat. Clin Exp Hypertens [A] 1991;13:865-872.

Mifflin SW, Kunze EM. Rapid resetting of low pressure vagal receptors in the superior vena cava of the rat. Circ Res. 1982;51:241-249.

Mifflin SW, Spyer KM, Withington-Wray DJ. Baroreceptor inputs to the nucleus tractus solitarius in the cat: modulation by the hypothalamus. J Physiol. (Lond.). 1988;399:369-387.

Mills EH, Minson JB, Chalmers JP. Effects of intrathecal administration of methysergide, phenotolamine, and pindolol on pressor responses to electrical stimulation of the rostral ventrolateral medulla. J Cardiovasc Pharmacol. 1988;11:456-460.

Minson JB, Chalmers JP, Caon AC, Renaud B. Separate areas of rat medulla oblongata with populations of serotonin- and adrenalin-containing neurons alter blood pressure after L-glutamate stimulation. J Auton Nerv Syst. 1987;19:39-50.

Mitchell VP, Lawler JE. Norepinephrine content of discrete brain nuclei in acutely and chronically stressed borderline hypertensive rats. Brain Res Bull. 1989;22:545-547.

Miura M, Onai T, Takayama K. Projections of upper structure to the spinal cardioacceleratory center in cats: an HRP study using a new microinjection method. J Auton Nerv Syst. 1983;7:119-139.

Miyawaki T, Kawamura H, Komatsu K, Yasugi T. Chemical stimulation of the locus coeruleus: inhibitory effects on hemodynamics and renal sympathetic nerve activity. Brain Res. 1991;568:101-108.

Moore RY, Card JP. Noradrenaline-containing neuron systems. In Bjorklund A and Hokfelt T (Ed). Handbook of Chemical Neuroanatomy, vol. 2. Classical transmitters in the CNS, Part I. pp. 123-156. Elsevier, Amsterdam, 1984.

Moreira ED, Ida F, Krieger EM. Extent of rapid baroreceptor resetting first hours of hypertension. Am J Physiol. 1989;257:H711-716.

Morpurgo C. Pharmacological modifications of sympathetic responses elicited by hypothalamic stimulation in the rat. Br J Pharmacol. 1968;34:532-542.

Munch PA, Andresen MC, Brown AM. Rapid resetting of aortic baroreceptors in vitro. Am J Physiol. 1983;244:H672-680.

Nakata T, Beerard W, Kogosov E, Alexander N. Cardiovascular change and hypothalamic norepinephrine release in response to environmental stress. Am J Physiol. 1993;264:R784-R789.

National High Blood Pressure Education Program Working Group. National High Blood Pressure Education Program Working Group report on primary prevention of hypertension. Arch Intern Med. 1993;153:186-208.

Nieman DC, Berk LS, Simpson-Westerberg M, et al. Effects of long-endurance running on immune system parameters and lymphocyte function in experienced marathoners. Int J Sports Med. 1989;10:317-323.

Nieman DC, Nehlsen-Cannarella SL, Markoff PA, et al. The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infections. Int J Sports Med 1990;11(6):467-473

Nissen R, Hu B, Renaud LP. Regulation of spontaneous

phasic firing of rat supraoptic vasopressin neurones in vivo by glutamate receptors. J Physiol. (Lond) 1995:484:415-424.

Nissen R, Cunningham JT, Renaud LP. Lateral hypothalamic lesions alter baroreceptor-evoked inhibition of rat supraoptic vasopressin neurones. J Physiol. (Lond.). 1993;470:751-766.

Okamoto K, Aoki K. Development of spontaneously hypertensive rats. Jpn Circ J. 1963;27:282-289.

Onai T, Saji M, Miura M. Functional subdivisions of the nucleus tractus solitarii of the rat as determined by circulatory and respiratory responses to electrical stimulation of the nucleus. J Auton Nerv Syst. 1987;21:195-202.

Onai T, Saji M, Miura M. Projections of supraspinal structures to the phrenic motor nucleus in rats studied by a horseradish peroxidase microinjection method. J Auton Nerv Syst. 1987;21:233-239.

Onai T, Takayama K, Miura M. Projections to areas of the nucleus tractus solitarii related to circulatory and respiratory responses in cats. J Auton Nerv Syst. 1987;18:163-175.

Oparil S, Chen Y-F, Berecek KH, et al. The role of central nervous system in Hypertension. In Laragh JH and Brenner BM (Edit), Hypertension: Pathophysiology, Diagnosis, and Management 2nd Edition. Raven Press, New York, 1995.

Otake K, Nakamura Y, Ezure K. Projections from the commissural subnucleus of the solitary tract onto catecholamine cell groups of the ventrolateral medulla. Neurosci Lett. 1993;149:213-216.

Paffenbarger RS Jr, Hyde RT et al. Physical activity, other life-style patterns, cardiovascular disease and longevity. Acta Med Scand Suppl. 1986;711:85-91.

Palkovits M, Brownstein MJ. Locus coeruleus. Adv Cell Neurobiol. 1983;4:81-103.

Palkovits M. Topography of chemically identified neurons in the central nervous system: A review. Acta

Morphologica Acad Sci Hung. 1978;26:211-290.

Paton JF, Rogers WT, Schwaber JS. The ventrolateral medulla as a source of synaptic drive to rhythmically firing neurons in the cardiovascular nucleus tractus solitarius of the rat. Brain Res. 1991;561:217-229.

Pawloski-Dahm CM, Gordon FJ. Increased dietary salt sensitizes vasomotor neurons of the rostral ventrolateral medulla. Hypertension. 1993;22:929-933.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. New York: Academic. 1982.

Pilowsky PM. Central control of blood pressure. European Heart J. 1992;13:2-9.

Reis DJ. Experimental central neurogenic hypertension from brainstem dysfunction: evidence for a central neural imbalance hypothesis of hypertension. Res Nerv Ment Dis. 1981;59:229-257.

Ricardo JA, Koh ET. Anatomical evidence of direct projections from the nucleus of the solitary track to the hypothalamus, amygdala, and other forebrain structures in the rat. Brain Res. 1978;15:1-26.

Riche D, De Pommery J, Menetrey D. Neuropeptides and catecholamines in efferent projections of the nuclei of the solitary tract in the rat. J Comp Neurol. 1990;293:399-424.

Roder S, Ciriello J. Caudal ventrolateral medullary projections to the nucleus of the solitary tract in the cat. Neurosci Lett. 1992;134:161-164.

Romaniuk A. Representation of aggression of and flight reactions in the hypothalamus of the cat. Acta Biol Exp. 1965;25:177-186.

Room P, Postema F, Korf J. Divergent axon collaterals of rat locus coeruleus neurons: demonstration by fluorescent double labeling technique. Brain Res. 1981;221:219-230.

Rosencrans JA, Watzman N, Buckley JP. The production of hypertension in male albino rats subjected to experimental stress. Biochemical Pharmacology. 1967;15:1707-1718.

Ross CA, Ruggiero DA, Joh TH, Park DH, Reis DJ. Adrenaline synthesizing neurons in the rostral ventrolateral medulla: A posible role in tonic vasomotor control. Brain Res. 1983; 273:356-365.

Ross CA, Ruggiero DA, Park DH, et al. Tonic vasomotor control by the rostral ventrolateral medulla: effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. J Neurosci. 1984;4:474-494.

Ross CA, Ruggiero DA, Reis DJ. Afferent projections to cardiovascular portions of the nucleus of the tractus solitarius in the rat. Brain Res. 1981;223:402-408.

Ross CA, Ruggiero DA, Reis DJ. Projections from the nucleus tractus solitarii to rostral ventrolateral medulla. J Comp Neurol. 1985;242:511-534.

Rudnick KV, Sackett DL, Hirst S, Holmes C. Hypertension in a family practice. Can Med Assoc J. 1977;117:492-497.

Saavedra JM, Torda, T. Increased brain stem and decreased hypothalamic adrenaline-forming enzyme after acute and repeated immobilization stress in the rat. Neuroendocrinology. 1980;31:142-146.

Sanders BJ, Cox RH, Lawler JE. Cardiovascular and renal responses to stress in borderline hypertensive rat. Am J Physiol. 1988;255:R431-438.

Sanders BJ, Lawler JE. The borderline hypertensive rat (BHR) as a model for environmentally-induced hypertenison: a review and update. Neurosc Biobehav Rev. 1992;16:207-217.

Sandvik L, Erikssen J, Thaulow E, et al. Physical fitness as a predictor of mortality among healthy, middle-aged Norwegian men. N Engl J Med. 1993;328:533-537.

Sasek CA, Helke CJ. Enkephalin-immunoreactive neuronal projections from the medulla oblongata to the intermediolateral cell column: relationship to substance P-immunoreactive neurons. J Comp Neurol. 1989;287:484-494.

Saum WR, Brown AM, Tuley FH. An electrogenic sodium pump and baroreceptor function in normotensive and spontaneously hypertensive rats. Circ Res. 1976;39:497-505.

Sawchenko PE, Swanson LW. The organization of noradrenergic pathways from brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res Rev. 1982;4:403-414.

Schafer SG, Kaan EC, Christen MO, Low-Kroger A, Mest HJ, Molderings GJ. Why imidazoline receptor modulator in the treatment of hypertension? Ann N.Y. Acad Sci. 1995;763:659-672.

Schwaber JS, Kapp BS, Higgins GA, Rapp PR. Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. J Neurosci. 1982;2:1424-1438.

Sinclair AM, Isles CG, Brown I, Cameron H, Murray GD, Robertson JW. Secondary hypertension in a blood pressure clinic. Arch Intern Med. 1987;147:1289-1293.

Smith ML, Thames MD. Cardiac receptors: discharge characteristics and reflex effects. In Armour JA and Ardell JL (Ed). Neurocardiology. Oxford University Press, New York, Oxford, 1994.

Smith OA, Astley CA, DeVito JL, et al. Functional analysis of hypothalamic control of the cardiovascualr response accompanying emotional behavior. Fed Proc. 1980;39:2487-2494.

Smith PG, Poston CW, Mills E. Ontogeny of neural and non-neural contribution to arterial pressure in spontaneously hypertensive rats. Hypertens. 1984;6:54-60.

Somogyi P, Minson JB, Morilak D, Llewellyn-Smith I, McIlhinney JR, Chalmers J. Evidence for an excitatory amino acid pathway in the brainstem and for its involvement in cardiovascular control. Brain Res. 1989;496:401-407.

Spacer CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. J Comp Neurol. 1982;210:163-173.

Spencer SE, Sawyer WB, Loewy AD. Cardiovascular effects produced by L-glutamate stimulation of the lateral hypothalamic area. Am J Physiol. 1989;257:H540-H552.

Spencer SE, Sawyer WB, Wada H, Platt KB, Loewy AD. CNS projections to the pterygopalatine parasympathetic preganglionic neurons in the rat: a retrograde transneuronal viral cell body labeling study. Brain Res. 1990;534:149-169.

Spyer KM. Neural organization and control of reflex. Rev Physiol Biochem Pharmacol. 1981;88:24-124.

Spyer KM. Neural mechanisms involved in cardiovascular control during affective behaviour. Trends Neurosci. 1989;12:506-513.

Spyer KM. The central nervous organization of reflex circulatory control. In Loewy AD and Spyer KM (Ed). Central Regulation of Autonomic Functions. pp.168-188, Oxford University Press, New York, Oxford, 1990.

Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. Brain Res. 1989;491:156-162.

Stratton JR, Halter JB, Hallstrom AP et al. Comparative plasma catecholamine and hemodynamic responses to handgrip, cold pressor and supine bicycle exercise testing in normal subjects. J Am Coll Cardiol. 1983;2:93-104.

Sun MK, Guyenet PG. Effect of clonidine and GABA on the discharge of medullospinal sympathoexcitatory neurons in the rat. Brain Res. 1986;368:1-19.

Sun MK, Hachett JT, Guyenet PG. Sympathoexcitatory neurons of rostral ventrolateral medulla exhibit pacemaker properties in the presence of a glutamate-receptor antagonist. Brain Res. 1988;438:23-40.

Sved AF, Felsten G. Stimulation of the locus coeruleus decreases arterial pressure. Brain Res. 1987;414:119-132.

Swanson LW, Sawchenko PE, Berod A, et al. An

immunohistochemical study of the organization of catecholamine cells and terminal field in the paraventricular and supraoptic nuclei of the hypothalamus. J. Comp.Neurol. 1981;196:271-284.

Takayama KJ, Okada J, Miura M. Evidence that neurons of the central amygdaloid nucleus directly project to the sites concerned with circulatory and respiratory regulation in the ventrolateral nuclei of the cat: a WGA-HRP study. Neurosci Lett. 1990;109:241-246.

Takeda K, Bunag RD. Chronic propranolol treatment inhibits sympathetic nerve activity and keeps blood pressure from rising in spontaneously hypertensive rats. Hypertens. 1980;2:228-235.

Terreberry RR. Neafsey EJ. Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus. Brain Res. 1983;278:245-249.

Thor KB, Helke CJ. Catecholamine synthesizing neuronal projections to the nucleus tractus solitarii of the rat. J Comp Neurol. 1988;268:264-280.

Thor KB, Helke CJ. Serotonin- and substance P-containing projections to the nucleus tractus solitarii of the rat. J Comp Neurol. 1987;265:275-293.

Ungerstedt U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol Scand. 1971;367(Suppl.):1-48.

van der Kooy D, Koda LY, McGintyJF, et al. The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat. J Comp Neurol. 1984;224:1-24.

Vasl SV. Theory of stress and health. In Cooper CL (Ed): Handbook of Stress, Medicine, and Health. CRC Press. 1995.

Veras-Silva AS, Mattos KC, Gava NS, et al. Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats. Am J Physiol. 1997;273:H2627-H2631.

Voisin DL, Chapman C, Poulain DA, Herbison AE. Extracellular GABA concentrations in rat supraoptic

nucleus during lactation and following hemodynamic changes: an in vivo microdialysis study. Neurosci. 1994;63:547-558.

Wasman M, Flynn JP. Directed attack from the hypothalamus. Arch of Neurol. 1962;6:220-227.

Wible JH Jr, Luft FC, DiMicco JA. Hypothalamic GABA suppresses sympathetic outflow to the cardiovascular system. Am J Physiol. 1988;254: R680-687.

Willette RN, Punnen S, Kreiger AJ, Sapru HN. Differential regulation of regional vascular resistance by the rostral and caudal ventrolateral medulla in the rat. J Auton Nerv Syst. 1987;18:143-151.

Willette RN, Punnen S, Kreiger AJ, Sapru HN. Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. Brain Res. 1984;321:169-174.

Williams RR, Hunt SC, Hasstedt SJ, et al. Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure? Hypertension 1991;18(Suppl. 3):I29-I37.

Wilson TW. History of salt supplies in West Africa and blood pressures today. Lancet. 1986;1:784-786.

Woo ND, Mukherjee K, Ganguly PK. Norepinephrine levels in paraventricular nucleus of spontaneously hypertensive rats: role of neuropeptide Y. Am J Physiol. 1993; 256: H893-H898.

Yang R-H, Jin H-K, Chen Y-F, et al. Dietary Ca^{+} supplementation prevents the exaggerated responsiveness of anterior hypothalamic alpha-2-adreoceptors in NaCl-loaded spontaneously hypertensive rats. J Cardiovasc Pharmacol. 1989;13:162-267.

Yardley CP, Andrate JM, Weaver LC. Evaluation of cardiovascular control by neurons in the dorsal medulla of rats. J Auton Nerv Syst. 1989; 29:1-12.

Yusof AP and Coote JH. Excitatory and inhibitory actions of intrathecally administered 5-hydroxytryptamine on sympathetic nerve activity in the rat. J Auton Nerv Syst. 1988;22:229-236.

Zanchetti, A. Hypothalamic control of circulation. In S. Julius and M. Esler (Eds.) The Nervous System in Arterial Hypertension. Charles Thomas Publishing, Springfield, IN, 1976.

Zheng, G-P, Li, S-G, Wang, C-S, et al. Renal functions after exposure to different durations of stress in BHR. Soc Neurosci Abst. 1994;24,225.10.

Brain Punch & HPLC Working Sheet Project_____

Brain Punch Punch Medium_____(ITSD)_____

Animal ID_____ Date_____ Animal ID_____ Date_____

Plate	Punch	Comment	Vial	Contns	Plate	Punch	Commnet	Vial	Contns
A-1	___	_____	A2	___	A-1	___	_____	A2	___
A-2	___	_____	A1	___	A-2	___	_____	A1	___
C1	___	_____	C1	___	C1	___	_____	C1	___
LC-1	___	_____	LC	___	LC-1	___	_____	LC	___
CL-2	___	_____	Post	___	LC-2	___	_____	Post	___
H1	___	_____	Arc	___	H1	___	_____	Arc	___
H2	___	_____	DMH	___	H2	___	_____	DMH	___
H3	___	_____	LH	___	H3	___	_____	LH	___
H4	___	_____	VMH	___	H4	___	_____	VMH	___
H5	___	_____	PVH	___	H5	___	_____	PVH	___
H6	___	_____	Ant	___	H6	___	_____	Ant	___
H7	___	_____	SO	___	H7	___	_____	SO	___

Plate for Punches (Paxinos & Watson The Rat Brain, 2nd Ed.)
p75 A2, A1; p69 C1; p59 LC; p32 Post, Arc; p31 DMH, LH, VMH; p25 PVH, Ant; p23 SO

HPLC Column Type_____

Mobile Phase_____ ITSD_____ Loop_____

Flow Rate_____ Pressure_____ Appl E_____ Offset_____ Temp_____

TH_____ PK WD_____AR Rej_____ ATT_____ Calculation Mode_____

Animal ID_____ Date_____ Animal ID_____ Date_____

Comment Commnet

BRAIN PUNCH RECORD

Punch Date	Animal ID	Comment	HPLC Date & Comment

VITA

Guanping Zheng was born in JiangYun, Hunan Province, China on November 25, 1959. After graduating from JiangYun First Middle/High School, he worked at a youth farm for two years. He entered Hunan Normal University in August of 1979 and received his Bachelor's degree in Biology in June, 1983. He continued his graduate study in Hunan Normal University and obtained his Master's degree in Psychology in August, 1986. Then he taught psychology and other courses at Hunan Normal University. He also served as a director of the General Psychology Division in the Department of Education and was a council member of Hunan Psychology Association. In August, 1991, the author entered the Doctoral Program in Experimental Psychology at the University of Tennessee, Knoxville. In August of 1996 he entered the Master's Program in Technology at East Tennessee State University to study computer graphics. The doctoral degree was received in May, 1998.