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HORMONAL RESPONSES OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS TO STRESS

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HORMONAL RESPONSES OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS TO STRESS

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He taught me all the necessary
survival skills.
He raised my stress threshold
to a level that no other man could have.
And to him I owe my health, my success
my accomplishments, and above all--my
existence.
To my father with respect, love and passion.

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ABSTRACT

HORMONAL RESPONSES OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS TO STRESS

BY

Jahan Shah Eftekhar

Plasma concentration of ACTH, corticosterone , epinephrine and norepinephrine was measured by radioimmunoassay in 22-34 week old male spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats before and after exposure to various forms of stress. Blood samples were obtained through catheters chronically implanted in the left carotid artery and attached to a swivel above each cage, such that the animals were completely undisturbed except when exposed to experimental stress. Resting morning blood pressure (mean ± standard deviation) was 180 ± 24 mmHg in the SHR rats (n=14) and 107 ± 27 in the WKY (n=16) [$p<0.01$]. In one experiment blood was taken before and after the rats were exposed to 30 minutes of ether (n=4 SHR, 10 WKY) and/or 30 minutes vibration (n=5 SHR, 8WKY) stress in a randomly alternate sequence that allowed 4-7 days between each test. In a second experiment, samples were taken 15 minutes after the rats were manually restrained for 5 minutes (n=9 SHR, 8 WKY). Resting values were obtained both individually for each

test and also were pooled across all experiments.

Results of pooled morning resting level of the hormones were as follows: ACTH, 128±99 vs. 97±66 pg/ml; corticosterone, 96±101 vs. 96±77 ng/ml; epinephrine, 79±94 vs. 25±18 pg/ml ($p<0.01$); and norepinephrine, 262±137 vs. 149±58 pg/ml ($p< 0.005$) for SHR and WKY rats respectively. The post ether-stress level of the hormones were as follows: ACTH, 510±144 vs. 522±166 pg/ml; corticosterone, 368±47 vs. 396±95 ng/ml; epinephrine, 7433±6757 vs. 362±273 pg/ml ($p< 0.01$); and norepinephrine, 2298±2375 vs. 590±70 pg/ml ($p< 0.05$). Post vibration-stress findings were: ACTH, 400±120 vs. 450±112 pg/ml; corticosterone, 486±129 vs. 411±108 ng/ml; epinephrine, 713±378 vs. 185±92 pg/ml ($p< 0.025$); and norepinephrine, 680±156 vs. 413±135 pg/ml ($p<0.025$). Finally, the post manual-restraint results were: ACTH, 351±149 vs. 400±90 pg/ml ; corticosterone, 395±54 vs. 336±58 ng/ml ($p<0.05$); epinephrine, 524±453 vs. 114±56 pg/ml ($p<0.01$); and norepinephrine, 558±336 vs. 200±84 pg/ml ($p<0.005$).

The higher concentration of catecholamines found in SHR both at rest and in response to a variety of stresses indicates that these hypertensive rats have a higher level of sympathetic activity than their normotensive controls. However, comparable levels of ACTH and corticosterone suggest that SHR and WKY do not differ significantly in pituitary-adrenocortical function.

INTRODUCTION

The interrelationships among 'hypertension', 'stress' and 'salt intake' are complex and controversial. On one hand they seem to be causally related--that is, the occurrence of any of them in a person , is followed by the occurrence of at least one if not both of the other conditions; on the other hand, one sees evidence in the literature that tends to refute the causal association of these conditions. There are also discrepancies in the literature over the issues of: finding the one condition among the three which is the final cause of the other two; or finding the material order of causation among them--that is, which one comes first, which one next and which one last; or determining the organ/system of the body that can best serve to link these conditions; or to decide which level of organization--from subcellular to organismal or even societal, can best serve to advance our knowledge of these states. Finally, and perhaps the most important of all is the lack of a universal agreement over the exact boundaries which delineate the concepts of 'stress ' and 'hypertension'. This problem seems to be the major intensifier of the complexity of the issue--since not all the scientists who refer to the concept of (for example) 'stress', really refer to the same thing.

This thesis agrees in part with the simplified, mechanistic and pragmatic definition of stress proposed by Yates et al.,(1974). These

authors state that " a stress is any stimulus, internal or external, chemical, physical or emotional, that excites neurons of the hypothalamus to release corticotropin-releasing hormone (CRH) at rates greater than would occur at that time of the day in the absence of the stimulus. The CRH drives the pituitary to release ACTH, and ACTH stimulates adrenal cortex to secrete corticosteroids". However this thesis also includes activation of the sympathetic nervous system, either dependent or independent of the ACTH release mechanism as part of the comprehensive definition of stress.

The underlying hypothesis of this thesis is that stress activates humoral and neural mechanisms in the body that lead to elevated blood pressure (hypertension) and increased salt intake. More specifically the hypothesis states that stress stimulates the pituitary-adrenocortical axis, and the released ACTH molecule, then, either alone and/or through the adrenal steroids causes elevated salt appetite (Blaine et al., 1975; Denton et al., 1980) and heightened blood pressure (Scoggins et al., 1974; Kawasaki et al., 1978; Genest et al, 1978; McCaa, 1978; Rauh et al., 1979; Freeman, et al., 1980).

Spontaneously hypertensive rats (SHR), widely studied as an animal model of essential hypertension (Okamoto and Aoki, 1963; Okamoto et al., 1966; Okamoto, 1972) were chosen for these experiments because they also have a naturally elevated salt appetite (Catalanotto et al., 1972; Bernard et al., 1980).

The specific hypothesis tested in these experiments is that the high blood pressure and high salt intake of the SHR rats are due to an abnormally active pituitary-adrenocortical system manifested either by a high resting level of ACTH, or a heightened ACTH response to stress or

both . Although a role for catecholamines is not proposed in the hypothesis, their plasma levels are also measured for two reasons. First, to perform a more complete assessment of the stress response of the SHR rats; and second, because of the possible synergistic effect of the catecholamines on ACTH function (Ramey et al., 1951; Lohmeir and Guyton, 1981). Finally, as part of the methodological strategy, the experiment employs three types of stress, namely; ether, vibration and manual-restraint--in order to provide a variety of stress options--from physical (manual-restraint) to neural (ether) and to psycho-physical (vibration).

REVIEW OF THE LITERATURE

A- HYPERTENSION, PITUITARY-ADRENOCORTICAL AND SYMPATHETIC NERVOUS SYSTEM

Selye and his peers (1942) were among the early pioneers who demonstrated that the adrenal cortex plays a role in the physiology of hypertension. They showed that hypertension is easily inducible in baby chicks by a high dose of desoxycorticosterone acetate. A year later, consistent with this finding, Sarason (1943) published the results of an autopsy study on patients with essential hypertension . The report indicated presence of enlarged adrenals in most of these cadavers--implying a state of hyperactivity. Another early line of support for the association of hypertension and adreno-cortical activity is evident in the work of Plotz et al. (1952), who stated that a connection between high secretion of cortisol and high diastolic blood pressures has been known for quite a long time in most of the patients with various forms of Cushing's syndrome.

From the 5th decade of the 20th century onward, one sees more experimental work on the interrelationship of hypertension and the pituitary-adrenocortical system . Knowlton et al, (1952), were able to induce moderate to severe arterial hypertension in normal and adrenalectomized rats by administering cortisone acetate to them. The same glucocorticoid was also demonstrated to induce hypertension in

mice, one to two days after the first injection (Clark et al., 1968). Also in a study on methylprednisone, a potent cortisol analog, Krakoff et al. (1975) were able to bring forward evidence in favor of the role of cortisol in hypertension. They demonstrated that this substance increased the plasma renin activity and the pressor response to angiotensin II in the rat. They further showed that treatment of rats with angiotensin II inhibitor (Sar^1-Ala^8-Angiotensin II) caused a drop in blood pressure. Based on these findings, they proposed a role for glucocorticoids in general and cortisol in particular in the elevation of arterial pressure, and ascribed a precipitating function to the renin-angiotensin system.

The idea of searching for precipitating elements in explaining the role of the glucocorticoids in the etiology of hypertension is also not a new idea. Early in the 1950's, Ramey and his collaborators were able to demonstrate that the pressor response to norepinephrine could not be maintained in adrenalectomized dogs, and blood pressure fell back to shock level despite large doses of norepinephrine. However, when they added cortisol to the post surgical drug regimen of the dogs, the blood pressure responded favorably to the previously ineffective norepinephrine doses. Their conclusion was that cortisol may have a permissive role in the maintenance and elevation of blood pressure (Ramey et al, 1951).

There is also evidence in the literature on the interaction between stressful stimuli and adrenocortical activity in the maintenance of blood pressure. For example, Goldstein et al (1950) were able to observe that glucocorticoids (cortisol and cortisone) and not mineralocorticoids (deoxycorticosterone and aldosterone) were involved in the maintenance of blood pressure. They showed that adrenalectomized

dogs under severe muscular exercise failed to maintain blood pressure--whether they were injected with deoxycorticosterone or not.

Literature also shows controversial evidence regarding the role of corticosterone in the maintenance of blood pressure. Thorn et al. (1953) indicated that corticosterone raised blood pressure to hypertensive levels in patients that were bilaterally adrenalectomized for hypertension treatment. On the contrary, Peterson and Pierce (1960) based on their findings on the plasma level of corticosterone in man, have suggested that this hormone, although a weak Na^+ retainer and a modest glucocorticoid and pressor substance--is unlikely to have a major role in the maintenance of blood pressure. Their reasoning was based on the fact that this hormone is produced in a very small amount; 1.5-4.0 mg/day, by the adrenal cortex in man.

The experiments and findings cited above all have one thing in common--they all ascribe a role to the adrenal cortex and the concomitantly released glucocorticoids in the maintenance and/or origin of blood pressure. Nevertheless, the fact that the adrenal cortex is controlled by the anterior pituitary gland and the latter is in turn under the influence of the hypothalamus raises the question of what role or roles is the hypothlamus-pituitary-adrenal axis playing in hypertension, and more specifically, to what extent and how is the ACTH molecule involved in the etiology and/or maintenance of hypertension. In what follows, the focus of the reports and experiments will center around answering these questions.

Walser and colleagues (1955), based on experiments wherein they studied the effects of salt loading in conjunction with ACTH and corticosterone administration in Sprague-Dawley rats,

suggested that increase in blood volume is not a necessary sequel to salt and water retention, nor an inevitable consequence of adrenocortical steroid administration but an outcome of the interaction of both of them. Rauh and associates (1979) who studied the effects of continuous (5 days) infusion of ACTH on adrenocortical function, electrolyte metabolism and blood pressure of normotensive and hypertensive children, reported a continuous rise in the plasma cortisol and deoxycorticosterone, a transient kaliuresis and a continuous fall in serum K^+, retention of Na^+, and weight gain in all the patients. They observed that plasma aldosterone rose only transiently in both normo- and hypertensive groups. They also showed that the cumulative retention of Na^+ had a significant correlation with the rise of systolic blood pressure in both groups. Furthermore, they found evidence that ACTH on a low salt diet can cause a small rise in blood pressure. However, they were not able to show any significant correlation between the level of plasma cortisol, deoxycorticosterone, aldosterone and the rise in blood pressure.

Over three decades ago, Levitt et al. (1951) were able to show that cortisone and ACTH could induce a transient shift of water, sodium and chloride into the measurable extracellular fluid compartment in man. They observed that the maximum value was attained 8 to 9 days after the beginning of the therapy. Based on their experiment, they proposed that the rise in blood pressure could be related to the role of ACTH in volume expansion and salt retention. One should note that this suggestion is in contrast with Walser et al's recommendation cited previously.

Vazir et al. (1981), based on their experiments on Sprague-Dawley rats, showed that ACTH can induce functional transformation of glumerolusa cells to fasciculata-like cells in the adrenals, and that the

induced hypertension is related to the increased secretion of corticosterone and 18-hydroxy-deoxycorticosterone, and not to aldosterone or to 18-hydroxycorticosterone. They further observed a decrease in body weight and an increase in adrenal weight in all the experimental rats.

Haack et al. (1978) studied the effect of chronic ACTH treatment on blood pressure and urinary excretion of steroids in male Wistar rats. They observed that chronic ACTH administration caused a loss of body weight and increased blood pressure, while it had no effect on aldosterone production. This work is further supported by the research of Freeman et al. (1980) who studied the effects of continuous ACTH administration on blood pressure and water metabolism in male Sprague-Dawley rats. They were able to show an ACTH induced hypertension without prior sensitizing maneuvers such as unilateral nephrectomy or salt-loading. Furthermore, they observed that the induced hypertension was entirely reversible if the ACTH infusion was stopped. Also they explicitly affirmed that the volume expansion was not critically involved in the development of ACTH-induced hypertension--because both the experimental and control groups in the study were on positive Na^+ balance.

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Concomitant to the lines of the aforementioned works, the role of the autonomic nervous system in the regulation and maintenance of the essential hypertension has also been the subject of intensive study by other scientists. Guyton and his associates (1974), writing on the role of the autonomic nervous system in the regulation of arterial blood

pressure, pointed out that although the long term control of blood pressure is accomplished by the kidneys through modulation of salt and water excretion, the baroreceptor reflexes, the chemoreceptors in the walls of the great vessels , and the receptors of vasomotor centers are responsible for the short term control of blood pressure.

Centrally active antihypertensive drugs have been used for quite a long time as clinical evidence in favor of the role of the sympathetic nervous system in the maintenance and/or etiology of high blood pressure . A recent study by Wing et al. (1977) has shown that the antihypertensive drug, clonidine, which lowers blood pressure by a central nervous action, reduces the sympathetic tone and causes a drop in plasma norepinephrine level. This study affirms the role of norepinephrine in the maintenance of the high blood pressure. Further support for the role of norepinephrine comes from the work of Louis et al. (1974). These authors demonstrated a high correlation between plasma norepinephrine level and blood pressure on one hand, and the level of dopamine β-hydroxylase--an enzyme that converts dopamine to norepinephrine, on the other. In another study on the heart of young and old spontaneously hypertensive rats, Louis et al. (1969) showed that the cardiac epinephrine turnover time of the older hypertensive rats is much lower than the older control groups. This suggested to them that hypertension might be due to the concentration of epinephrine which stays longer in the cardiac vasculature. However, in contrast to this suggestion, Yamuri (1974) who studied the activity of autonomic nervous system in young spontaneously hypertensive rats (1-2 months age), observed an increased cardiac turnover of epinephrine compared to the normotensive controls.

A further investigation was reported by Hallbach (1976) who studied the interaction of centeral autonomic hyperactivity and environmental stimuli in relation to their roles in the devolpment of hypertension in rat. The report supports the idea that genetic hypertension is mediated through central adrenergic hyperactivity--since mental stress imposed on young normotensive Okamoto rats resulted in a dramatic elevation of arterial pressure and in an earlier onset of hypertension. Additional support for Hallbach's study comes from the work of Weiss and colleagues (1974). They studied the effectiveness of early ß-adrenergic blockade treatment in reducing the increase in blood pressure that occurs with age in the spontaneously hypertensive rats, and suggested that increased sympathetic activity in early age may be the cause of spontaneous hypertension.

Page et al. (1958), in an effort to summarize the factors that regulate blood pressure and tissue perfusion, named cardiac output, blood volume, blood viscosity, vascular caliber, vascular elasticity, vascular reactivity, chemical substances and neural activity as the major precipitating factors. They further indicated that neurogenic sympathetic constrictor outflow is certainly a significant factor in essential hypertension even if not a necessary cause of it.

Finally, in a more recent review on the relationship between blood pressure, heart rate and plasma noradrenaline, Reid et al. (1978) wrote: "there are many possible explanations for the controversial and contradictory reports on the role of autonomic mechanisms in hypertension. Most of these directly or indirectly are the consequence of the use of plasma levels of noradrenaline as an index of sympathetic

activity". The authors highlighted the major reasons and causes of these controversies as the following: methodological problems of sensitivity, specificity, and reproducibility; site and conditions of blood sampling; variability in race, sex and age of the subjects; and the differences in clearance and metabolism of noradrenaline and the sensitivity of receptors to neurotransmitters. The work of these authors casts some light upon the comprehensibility of the reports of other reseachers like Hoobler et al (1954), who indicated that plasma concentration of catecholamines in patients with essential hypertension was not markedly different from concentrations in normal subjects. Or the contrasting work of Franco-Morselli et al (1978) who measured plasma cathecholamines in human essential hypertension and in DOCA salt hypertension of rat, and reported that there is a slight increase in plasma norepinephrine and a marked rise in the level of epinephrine in the hypertensive patients compared to control normotensives . These authors further were able to show a positive correlation between plasma epinephrine level and blood pressure--with plasma epinephrine appearing more elevated than norepinephrine. They remarked that epinephrine represents a more accurate index of sympathetic activity than norepinephrine, which is subjected to a more complex diffusion from nerve endings to the lumen of blood vessels.

To obtain a reliable estimate of the plasma norepinephrine level under various conditions of rest and exercise, Watson et al. (1978) used a forearm indwelling cannula in patients with mild to moderate essential hypertension. They observed that plasma norepinephrine was lowest during sleep (0.27±0.03 µg/l) and progressively increased through sitting, standing and walking, reaching the highest value during bicycle exercise

(2.32±0.47 µg/l). They further found a significant correlation between the log of plasma norepinephrine and blood pressure under different physical conditions of the experiment.

The literature also shows experiments and reports that propose to unveil the complex cellular and subcellular mechanisms for the interaction of hypertension and hypophysio-adrenocortical or autonomic nervous system. Kalsner (1969), who studied the mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta, concluded that corticosteroids are required for the vasoconstrictor action of norepinephrine. This study showed that in the absence of glucocorticoids, the vasopressor action of catecholamine hormones is diminished or lost and blood pressure is decreased. Kalsner proposed the following detailed mechanism: glucosteroids inhibit the enzyme catecholamine-o-methyltransferase (COMT) which is responsible for inactivating the catecholamine hormones at their action sites. If this enzyme is not inhibited by corticosteroids, catecholamines are metabolized so rapidly that they cannot accumulate in sufficient amounts to cause vasoconstriction.

Tobian et al. (1956) studied the effect of norepinephrine on the electrolyte composition of arterial smooth muscle in rats and concluded that regulation of ion distribution on the two sides of the cell membrane may be the basic determinant of the steady tension produced by these muscles. They showed that infusion of norepinephrine sufficient to cause an increased blood pressure is accompanied by a decrease in extracellular sodium and an increase in the sodium content of arterial smooth muscle.

On a closer look at the cellular mechanisms which account for the

correlation between sodium metabolism and peripheral vascular resistance, Blaustein (1977) proposed that the sodium electrochemical gradient across the vascular smooth muscle cell plasma membrane (sarcolemma) plays an important role in the regulation of cell calcium.

The author argued that since there is a significant resting tension in most resistance vessels, the ionized calcium must be maintained above the threshold level for their contractility. Accordingly, Ca^{++} transport in the sacrcolemma and, presumably, Na^{+}-Ca^{++} exchange mechanisms must be held in such a way as to hold the Ca^{++} inside at the required high level. Any change in the Na^{+} gradient will then be reflected by a change in Ca^{++} inside an in turn in the tension of the vessels and peripheral resistance. The author further suggested the possibility of a circulating agent, such as natriuretic hormone, affecting the Na+ gradient across the sacrcolemma, the Ca++ inside, and the tension of the vasculature as a consequence. Consistent with this report and in the same year, De Wardener(1977), in a review on natriuretic hormone, wrote that an increased concentration of natriuretic substances that are Na^{+} transport inhibitors is demonstrated in both man and animals during a high intake of Na^{+} and after acute volume expansion. These substances, while returning the sodium balance toward normal, might also cause hypertension.

Postnov et al (1976) noticed an altered permeability in the erythrocyte membrane of SHR rats for sodium and potassium ions compared to Wistar-Kyoto and Sprague-Dawley rats. They found a higher permeability of the SHR membrane for these two ions. The increased permeability, according to the authors, reflected a widespread cell membrane defect which would serve as a general cause for activating the mechanism for maintaining high blood pressure.

Finally, Luft et al. (1979) studied the plasma and urinary norepinephrine values at the extremes of sodium intake from 10-1500 mEq/24 hrs to determine the plasma and urinary norepinephrine values in man. They offered to each of the 14 subjects in the study a graded intake of sodium for three days--starting with 10 and proceeding to 300, 800 and 1500 mEq/24 hours. The results showed that mean arterial pressure of the subjects increased from 83.8±1 to 100.3±3 mmHg. Venous plasma norepinephrine decreased from 467±63 to 67±24 pg/ml, while urinary norepinephrine excretion decreased from 54.3±3.4 to 23.4±2.9 µg/24 hr. The calculations showed that urinary excretion of sodium was inversely correlated with the urinary norepinephrine values. This suggested to the authors that sympathetic nervous system activity may decrease with sodium loading in normal subjects and facilitate the excretion of massive salt loads which in turn will modulate the increase in blood pressure. Accordingly, it is possible that the high blood pressure of hypertensive patients and SHR rats is due to a defect in their ability to excrete high Na^+ loads under high norepinephrine concentration of plasma.

B- STRESS, PITUITARY-ADRENOCORTICAL AND SYMPATHETIC NERVOUS SYSTEM

Cannon (1929) identified the fight/flight response to stress and described the accompanying increase in sympathetic and adrenocortical activity. Selye in the 1930's described the general adaptation syndrome (GAS) in response to stress and demonstrated that this can be beneficial or harmful depending on the length of time that the emergency bodily responses are sustained. According to his view, animals are in the state of stress when they manifest a syndrome of three changes: hypertrophied

adrenals, atrophied lymphatic organs, and bleeding gastrointestinal ulcers. This syndrome of changes, he called "GAS". Stressors, according to his hypothesis, produce a generalized state of stress in the body. The state of stress in turn inaugrates a series of "alarm signals" which he postulated can act through the floor of the brain (presumbly the hypothalamus) to stimulate the sympathetic nervous system and the pituitary gland. Stimulation of the sympathetic nervous system stimulates the adrenal medulla which in turn increases the secretion of epinephrine. Concomitantly, stimulation of the anterior pituitary increases the secretion of ACTH, which acts on the adrenal cortex and enhances glucocorticoid secretion. The latter produces a hyperglycemic condition in the body--that is, it mobilizes the tissue fats and proteins, and increases the liver's gluconeogenesis. On the thymus gland, the glucocoticoids cause an atrophy, followed by a decreased number of lymphocytes and eosinophils. The former causes a condition of decreased immunity, while the latter decreases the allergic reactions of the body. Consequently, the corticosteroids enable the animals to resist or adapt to the stressors or the stresses (Selye, 1936).

Following the work of the above two authors, the ground was paved for further research by many interested authors on the link between stress and hypertension on one hand, and the role of the nervous and endocrine system in this relationship on the other. Friedmann and Paul (1952) influenced by the idea of dehydration as a stress condition found that vasopressin in large doses could elevate blood pressure in rats and rabbits. Ingle (1954) studied the role of the adrenal cortex in stress and found that corticosteroids have a permissive action in

stress--although their presence at a basal level is necessary to permit animals to cope with the stresses, their increase beyond the normal level does not add to the ability of the animals to adapt to the stress challenge. Anderson (1966) studied the hormones of peripheral blood in male Fischer rats after subjecting them to severe physiological stress. It was found that their blood contained ACTH-releasing and antidiuretic activity and that the hormones associated with this dual activity enter the general circulation by way of the portal vessels of the anterior pituitary gland. According to the author, the most severe stress, laparotomy under ether anaesthesia followed by rapid removal of blood from the aorta , provoked the highest ACTH-releasing and antidiuretic response compared to control hypophysectomized rats.

Riegle (1973) studied the effect of chronic stress on the adrenocortical responsiveness of young and aged male and female rats. He subjected the rats, twice daily, for two hours to restraint or ether stress for a period of 20 days. He observed that the adrenocortical responsiveness to restraint or ether vapor stress was decreased in all the stressed groups--being greater in the younger than the older rats. This suggested that the corticosteroid feedback from chronic stress activation of the adrenal cortex may result in incomplete inhibition of the adrenocortical control mechanisms. A year later, Buckingham and Hodges, being interested in the effects of ether anaesthesia as a stress factor, studied the interrelationships of the anterior pituitary and plasma corticosterone in adrenalectomized and stressed adrenalectomized rats. They correlated the changes in the pituitary and plasma ACTH, with the changes in plasma corticosterone before and after exposure of the rats to stress. They observed that adrenalectomy increased the ACTH level

of the plasma--presumbly due to removal of the feedback input from the adrenal. They also found that ether stress caused a small rise in the plasma ACTH level of the intact, and a profound increase in the plasma level of the adrenalectomized rats. However this exaggerated response was shown to be reduced to normal when physiological doses of corticosterone were given to the rats. The authors further observed that prolonged treatment with corticosterone was needed to abolish the stress response of the ACTH . They proposed that the synthesis and the basal level release of ACTH is directly controlled by the concentration of corticosteroids in the blood--but the corticosteroids exert only a delayed effect in modulating the stress induced release of the hormone.

Hallback and Folkow (1974) studied the cardiovascular responses to acute mental stress in spontaneously hypertensive rats. They compared the responses of 7 month old SHR rats that had a manifest hypertension ,with those of control prehypertensive (10-11 week old SHR) and normotensive rats. Blood pressure and heart rate were measured after light, noise and vibration stimuli. The tachycardia (due to accentuated sympathetic and centrally suppressed vagal discharge) was used as an index of neural stimulation. It was found that the mature SHRs responded more strongly than the other two groups, suggesting that they had a lower threshold for defense reactions. The authors proposed that the sympathetic hyperactivity in the SHR rats tended to trigger structural and vascular adaptive changes which resulted in a manifest hypertension. They further suggested that these changes were genetically produced and were not a sequel to hypertension--because they were also shown in the prehypertensive, but not in the renal hypertensive rats.

Hirata et al. (1975) measured the plasma levels of β-MSH and ACTH during acute stresses and after administration of metyrapone [2-methyl-1,2-bis-(3-pyridyl)-propanone], an adrenal steroidogenesis blocking compond, in man. Simultaneous measurements of plasma level of MSH and ACTH were made under insulin-induced hypoglycemic condition, and lysine-vasopressin or metyrapone injection as the stressors. It was observed that insulin hypoglycemia caused a marked increase in ACTH and a slight but significant rise in plasma MSH. Lysine-vasopressin however, caused a significant rise in plasma ACTH levels without a significant response from β-MSH. Finally, the metyrapone caused a rise in both β-MSH and ACTH levels. The serial blood sampling showed that peak rise of the hormones occurred together--pointing to the similar mechanism or pathway of release for both of them. Lysine-vasopressin, on the other hand, caused a significant rise in the ACTH level 15 minutes after the injection (ACTH rising from 91.3±13.2 to 165.7±19.7 pg/ml). This rise was followed by the rise in plasma cortisol from 8.0±1.3 to 19.6±3.3 µg/100 ml. Lastly, the metyrapone, which is an inhibitor of 11β-hydroxylation of adrenal steroidogenesis and causes the shift of the products from cortisol and corticosterone to deoxycorticosterone and 11-deoxycortisol, was shown in this experiment to remove the feedback inhibition on ACTH and in this way leading to a high level of plasma ACTH with the concomitant MSH secretion.

Le Mevel et al. (1978) observed the dynamic changes in plasma adrenocorticotropin after subjecting 90-day old male and female Sherman rats to neurotropic stress. The stress was composed of removing the rats from their cages and putting them in a glass jar for 3 minutes. It was observed that the stress increased the levels of ACTH, reaching

the maximum level of 27μ unit/ml (equivalent to 270 pg/ml), 5-10 minutes post-stress, and falling significantly after 15 minutes (while remaining constant for the next 40 minutes).

McCarty et al. (1978) studied the sympatho-adrenal activity of SHR and WKY rats during recovery from a 2-hour forced immobilization stress. They observed that SHR rats responded significantly to the stress by a higher level of norepinephrine, epinephrine and dopamine. While the plasma corticosterone response was not different in the two rat groups. This suggested to the authors that the sympathoadrenomedullary system of the SHR rats may be more sensitive to immobilization stress, and that this system remains in a heightened state for a longer period of time following the stressful stimulation.

In another experiment using forced immobilization and handling stress, Kvetnansky and colleagues (1978) attempted to measure the plasma levels of epinephrine, norepinephrine and dopamine ß-hydroxylase in Sprague-Dawley rats. They observed a statistically significant and a time-dependent rise in the level of norepinephrine and epinephrine during and after the stress. This suggested to them that plasma catecholamines may be a good measure for reflecting the degree of activation of the sympathoadrenal system during stress. Kvetnansky et al. (1979) in a further study of immobilization stress in SHR rats, measured the blood pressure, heart rate and plasma norepinephrine, epinephrine and corticosterone, before, during and after seven periods of immobilization stress (150 minutes of stress per day). They found a significantly higher level for the all measured hormones in the SHR rats compared to the WKY rats and in all the stress periods. However they also observed that the mean arterial pressure, being highest in the first period, gradually fell in

the successive later stages in the SHR rat. This suggested to them that the adaptive changes in the cardiovascular and sympathoadrenomedullary systems of the repeatedly stressed rats is greater in the SHR than the WKY rats.

Falkner et al. (1979) studied the cardiovascular response of normal adolescent children of hypertensive parents to mental stress, which consisted of performing arithmetic calculations within a short time period. The findings showed that the subjects with labile hypertension demonstrated a sustained increase in systolic and diastolic pressure and heart rate during stress. The analysis of the plasma level of catecholamines showed that the post-stress levels were higher in the labile hypertensive individuals and subjects that had at least one hypertensive parent. This suggested to the authors that increased adrenergic activity was the mediator in the response of the hypertension prone subjects.

Finally, Hausler et al. (1983) studied the ether-stress induced secretion of ACTH and corticosterone during the development of spontaneous hypertension in rats. They observed that the ACTH response was markedly enhanced in 4-weeks-old but not in 12 or 16 weeks old SHR rats. This suggested to them that the imbalance of the pituitary-adrenal axis may be the major cause of the onset and development of hypertension in SHR rats.

C- STRESS, SALT INTAKE AND HYPERTENSION

In the previous two sections the relationship of stress and high blood pressure was discussed. However the connection between stress and salt intake, and salt intake and high blood pressure remain to be explored.

Stressors cause the release of ACTH --and this is almost unanimously accepted by stress researchers (Selye et al., 1936; Kendall, 1971; Riegle et al., 1973; Yates et al., 1974; Le Mevel et al., 1978; Fagin et al., 1983; Hausler et al. 1983 and 1984). Denton and his colleagues (1980) and Blaine et al. (1975) observed high intakes of sodium, potassium and calcium chloride during pregnancy and lactation (presumably to compensate for the needs of the developing fetus) in rabbits and ewes. These authors were further able to demonstrate that the pregnancy hormones and exogenous ACTH by itself could produce similar results in control rabbits, and stress could produce results comparable to exogenous ACTH. Since aldosterone did not stimulate salt appetitre in rabbits and since the effect of ACTH occurred in adrenalectomized rabbits maintained with glucocorticoids and mineralocorticoids, the authors suggested that ACTH, either via a central or a peripheral neural mechanism or both, may be involved in the regulation of salt appetite. Weisinger et al. (1980), however, confirmed the role of ACTH in salt appetite, but pointed out that ACTH had no extra-adrenal effect in rats--that is, it produced its effect solely through the adrenal gland, thus indicating a species difference in ACTH action mechanisms.

Since ACTH is extensively used as a measure of stress in the animals (Selye, 1936; Yates et al., 1974), the link between stress and high salt appetite should not come as a surprise. Furthermore, the epidemiological finding that in societies with very low sodium chloride intake hypertension is almost totally absent (Shaper, 1967; Lovell, 1967; Prior et al., 1968; Truswell et al., 1972; Page et al., 1974 and

Tobian, 1975); or studies that have shown a higher incidence of hypertension in the acculturated/developed countries wherein people consume a high amount of salt in their diet (Lowenstein, 1961; MacMahon et al., 1973; Wilhelmsen et al., 1973; Hatano, 1975); or the salt restriction studies--wherein a subsequent drop in blood pressure has been observed (Allen, 1920; Perera, 1947; Dahl, 1972); or the diuretic therapy studies, wherein elimination of salt from the body has been accompanied by a reduction in the intensitity of hypertension (Dahl, 1972); or salt loading experiments that have indicated a concomitant rise in the blood pressure of animals (Dahl, 1958, 1960 and 1964); or the conviction that hypertension rests on processes in the body that increase the demand for salt (Fallis et al., 1962; Schecter et al., 1973); or the possibility that salt is a factor in hypertension only in the presence of stress--which would be the most consistent factor in modern and developing societies (Epstein, 1963; Cobb and Rose, 1973; Pickering, 1977; Eyer, 1975); or the possibility that high level of ACTH may cause hypertension--suggested by the experiments in which blood pressure was elevated in rats (Freeman et al., 1980) or sheep (Scoggins et al., 1974) or dogs (McCaa, 1978) or human (Rauh et al., 1979) all provided direct or indirect proof for the link among stress, hypertension and salt intake.

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In conclusion, a critical review of the literature on the interrelationship among hypertension, stress, salt intake and neuro-endocrine physiology shows a large number of experimental studies, many of which are in disagreement, either qualitatively or

quantitatively, regarding the exact causal interrelationship among these factors. Part of the problem is due to the complexity of the issues that are being studied (Page et al., 1958; Bernard, 1985) and part is due to the methodological variations in the design of the studies (Reid et al., 1978). On the extremes of the controversy, one sees Sjoerdsma (1972) writing that " wherees no convincing chemical evidence can be obtained to implicate excess activity of vasoconstrictor systems (such as sympetho-adrenal system) in pathogenesis or maintenance of the hypertension, most of the effective antihypertensive drugs act by lessening vasoconstrictor activity, especially that mediated by sympathetic nervous system". Or Doyle (1978) writing that " increased levels of circulating catecholamines in hypertension do not get us very far in solving the primary cause of raised blood pressure. What it does however, is to emphasize the central role of sympathetic nervous system in the maintenance of hypertension". Or Oglesby (1977) who wrote: " To date, however the epidemiologic evidence for a critical role of sodium chloride ingestion in human essential hypertension has been fragmentary and not persuasive to most students of hypertension". Or Dahl (1972) who writes: " The evidence that salt induces a permanent and fatal hypertension is direct, quantitative and unequivocal in the rat". Or Folkow (1982) who writes: " For nearly 100 years great efforts have been made to elucidate how human primary hypertension is initiated and maintained. Progress was greatly stimulated by Goldblatt's introduction of experimental secondary hypertension in 1930's; unfortunately enthusiasm for the developed unitarian models also delayed the realization that human primary hypertension is basically of multifactorial origin". This seems to be the state-of-the-art on the underlying mechanisms for the interrelationship among hypertension, stress, and salt appetite.

D- STRESS, HYPERTENSION AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS

Spontaneously hypertensive rats (SHR) were first developed by selective inbreeding of normotensive Wistar rats by the Kyoto group in Japan, under the direction of Okamoto and Aoki (Okamoto and Aoki, 1963; Okamoto et al., 1966). These rats show a 100% incidence of hypertension with a markedly high blood pressure and spontaneously elevated salt appetite (Catalanotto et al., 1972; Bernard et al., 1980). The presence of a very high frequency of hypertensive cardiovascular disease in them suggested that they may be good models for the study of hypertension in man (Okamoto, 1972).

Traditionally there have been two control groups for the SHR rats. The normotensive Wistar rats (NC) and Wistar-Kyoto strain of normotensive rats (WKY). These two control groups are not quite similar biochemically and physiologically, but together can act as a safe control for the SHR rats (Frohlich and Edward, 1977).

At the time of maturity, approximately 2-3 months of age, when the period of rapid development is over, the arterial blood pressure will become highly elevated in the SHR rats (183/126 mmHg), compared to WKY (134/90 mmHg) or with NC control rats (140/97 mmHg) [Pfeffer and Frohlich, 1973]. The SHRs at the age of 5-10 weeks are said to have developed a labile phase of hypertension. At the age of 3-4 months the hypertension becomes established , and at 4-6 months it is well established. They show signs of increased sympathetic discharge, reduced vagal tone, increased heart rate and cardiac output. (Folkow and Hallback, 1977). *In vitro* comparisons of SHR and WKY rats for the

mechanical and morphological properties of the resistance vessels show that in SHRs the hypertension is associated with increased peripheral resistance, which in turn is related to structural changes such as narrowed lumen, a thickened media and increased number of smooth muscle cell layers in these vessels (Mulvany et al., 1978). Also morphological studies on the endocrine organs of the SHR rats have demonstrated a condition of hyperactivity in both adeno hypophysio-adrenocortical and the adenohypophyseo-thyroidal systems, together with a hypersecretion of vasopressin and sympathetic overactivity. These changes are suggested by Tabei et al. (1972) to possibly participate in the pathogenesis of the SHR's hypertension.

Spontaneously hypertensive rats have a naturally elevated salt appetite (Catalanotto, et al., 1972; Bernard et al., 1980; and Mogenson et al., 1980)--but it is not quite clear if the occurrence of this appetite is secondary to hypertension or not. Catalanotto et al. (1972) suggested that the establishment of elevated salt appetite in the SHR happens after the establishment of hypertension (that is, after maturity). However, Mogenson, et al. (1980) who observed enhanced sodium appetite in immature (5 to 7-week old) SHR rats, suggested that it starts before the appearance of hypertension. This controversy may be due to the lack of homogeneity of the SHR (and WKY) population--that is, the breeding history and the suppliers of the rats could somehow participate in the generation of these controversies.

Studies of catecholamine turnover rate in SHR rats of different age have shown that under undisturbed conditions only the young SHRs show signs of increased sympathetic activity--whereas the turnover rate is unchanged or even reduced in mature SHRs when they compared

with the control rats (Folkow, 1975 and 1977). Consistent with this finding, Conway's group (1975)--based on the assumption of sympathetic hyperactivity in very young SHR rats, treated them with ß-adrenergic blocker, propranolol . It was found that the treatment to a great extent hindered the later development of hypertension. However, the same treatment had relatively no effect in the mature SHRs. This experiment not only supported Folkow's point of view, but further suggested to its authors that the adrenergic ß-blocking agents, beside their interference with sympathetic cardiac control and renin release, might also centrally damp the sympathetic discharge.

Hausler et al, (1984), in a search for anatomical differences in the anterior-pituitary of the 4-weeks-old SHR and WKY rats, quantitatively analysed the ACTH-immunoreactive cells. The findings showed a larger anterior lobe and more abundant immunoreactive cells in the SHRs compared to the control rats. This suggested to the authors that an enhanced availability of ACTH in the anterior pitruitary may explain the markedly enhanced stress-induced release of ACTH in these immature rats. They further mentioned that the instability of the hypothalamo-pituitary-adrenal axis may contribute to the development of genetically programmed hypertension.

E- CHRONIC CATHETERIZATION IN RAT

The nature of the experiments on hypertension and stress calls for undisturbed conditions of observation. The sympathetic nervous response of the rats to handling and minor disturbances is often immediate and usually masks the effect of the factors that the experimentors are trying to measure. Therefore considerable precaution

is recommended in the studies of stress hormones in general and catecholamines in particular (Kvetnansky et al., 1978). It is easily conceivable here that one of the most difficult situations encountered by an experimentor may be the problem of measuring the basal level (that is zero and undisturbed level of stress hormones in the rats or other experimental animals. Furthermore, the need for constantly monitoring the blood pressure of rats (or other lab animals) as an integral part of the hypertension/stress studies has always indicated the need for finding a sound method that will impose the least disturbance on the animals. The tail-cuff manometeric methods, plethysmography (Heymann and Salehar, 1949) and other methods of direct blood pressure measurement other than indwelling catheterization, however, have had the negative effects of altering the hemodynamics of blood under the influence of anaesthesia and surgical procedures, or damaging the vessels of small animals (Still et al., 1956).

In response to these problems, chronic catheterization of rats has gained increasing interest among stress/hypertension students. Catheters are usually constructed from polyethylene (or tygon) tubing with a shorter and narrower tip (as described later in the chapter on Materials, Methods and Procedures) to be inserted in the artery of concern--usually the carotid, abdominal aorta, femoral or tail artery of rat (Buckingham, 1976), with the distal end being externalized through the rat's skin--and supported within a shield--usually a metal spring (Kvetnansky et al., 1978 and 1979; MacCarty et al., 1978; Fagin et al., 1983).

In short, chronic cannulation (catheterization) of rats allows

for repeated blood sampling and pressure measurement on one hand (Fagin et al., 1983) and more accurate measurement of the resting circulatory level of stress hormones (Carruba et al., 1981) on the other. For this reason chronic cannulation is an integral element in the experimental design of this thesis research.

MATERIALS, METHODS AND PROCEDURES

1) PROTOCOL OF THE EXPERIMENT

The overall experiment was composed of two major parts; part one was designed to measure and study the hormonal and concomitant hemodynamic hypertensinogenic responses of rats to ether and vibration stresses (11:00 to 12:00 AM); and part 2, to examine the rats' response to manual-restraint stress (11:00 to 12:00 AM) and also to determine the resting level of the aforementioned responses between 11:00 to 12:00 PM.

Two groups (batches) of rats, as described in the next section, were used for the parts of the experiment. They were first physically examined and then housed in the laboratory. Their readiness for the stress experiments was based on two criteria: one, their response to two-bottle salt preference tests, and, second, their full maturity--evidenced by the plateau of their growth rate.

Rats (in groups of 6--half control and half experimental) were then chronically cannulated and connected to infusion pumps to facilitate blood sampling, blood pressure, heart rate and hematocrit measurement, and infusion processes.

Upon recovery from the surgery, usually 3-6 days after the cannulation, the rats were subjected to one of the aforementioned three types of stress, and blood samples were obtained from them before and

after the stress application. The blood samples were then stored for later radioimmunoassay of plasma epinephrine, norepinephrine, ACTH and corticosterone. Finally, the radioimmunoassay results and hemodynamic data were statistically analysed.

2) EXPERIMENTAL ANIMALS

Two groups of male rats composed of a total of 19 spontaneously hypertensive (SHR), 19 Wistar-Kyoto normotensive (WKY) and 6 Sprague-Dawley rats (SD), were used for different phases of the overall experiment. Group A (to be stressed by ether and vibration) was composed of 11 WKY and 9 SHR rats, all 16 weeks old before admission to the experiment; and group B (to be used for manual-restraint stress and evening blood sampling) was composed of 8 WKY, 10 SHR and 6 SD rats, all 15 weeks old before the admission. The SHR and WKY rats were all born on the same date and were purchased from Taconic Farms, Germantown, NY. Weight analysis of the rats at the time of their arrival showed that the group A SHR rats had an average weight of 274±7.5 (mean±1SD), ranging from 259 to 285 grams; and group A WKY rats, had an average weight of 292±11, ranging from 275-307 grams. The group B SHRs weighed 273±7.1 (range: 271-292 grams); and group B WKYs were 286±10.7 (range: 276-315 grams). The SD rats were not age-matched with their counterparts. However, they were weight-matched with the WKY rats.

3- HOUSING AND FEEDING CONDITION OF THE RATS

Each two rats of the same strain were placed in a 32X35X16 cm plexiglass and transparent cage with a barred -metal lid. Cages were

placed , 4 in each shelf, on a metal rack, in a window-less room that was partially isolated from the traffic of the main laboratory compartment. The cage bedding was made of sawdust, and distilled water and Teklad® rat chow was available ad libitum. Watering, feeding, change of bedding and weighing of the rats were performed once a week.

A diurnal light cycle was maintained (lights on from 0800-2000 hour), and room temprature was kept at or around 70 F°.

From the moment of their arrival into the laboratory, up to the end of the experiment, the rats were kept and cared for 2-3 months.

Starting from 15 to 20 days after their arrival and for a period of 1 to 2 weeks, a randomized two-bottle preference test for NaCL was performed on the rats. Due to the methodological nature of the test, one of the consequences was a transient alteration in the watering pattern of the rats during this period--because two bottles; one filled with distilled water and the other composed of a salt solution, were provided for each cage rather than one.

After surgical cannulation (as described later), the rats were housed singly; provided with honey and peanut butter in addition to their ordinary rat chow for the first 3 days post operation, and their externalized cannulae were connected to infusion pumps by Tygon tubes. The rats were kept alive between 5-13 days after surgery, depending on their physical health status as determined, first, by their weight, blood pressure and hematocrit reading ; and second by their physical appearance and feeding behavior. The appropriate stress tests were performed on the rats during this period and not sooner than 3-5 days after the surgery.

4- SALT PREFERENCE TEST

Two to three weeks after the arrival of each batch of rats into the laboratory, two-bottle preference tests were performed . The rationale for this test--an accessory part of the main experiment, was to make sure that the rats had developed salt appetite and that there was a difference in preference between the SHR and WKY rats before proceeding with the stress experiments.

Each rat was provided with the choice between a bottle of distilled water, and a bottle of salt (NaCl) solution. Each day the position of the flavor was changed randomly (being placed either to the left or right) so as to control for positional preference . Three different concentrations (0.03, 0.1 and 0.2 Molar) of the salt solution were assigned to each cage in a random basis and in such a way that each was supplied with all the different concentration of salt over a 6-day period (two successive days for each concentration).

Each day, between 11:00 to 12:00 AM, the consumption rate of the rats was measured and results were recorded. If the SHRs did not show a significantly higher preference for all the salt concentrations or at least the last two higher concentrations, then a second preference test was scheduled for 10 to 20 days later.

5- CHRONIC CATHETERIZATION

Catheters were chronically implanted in the left carotid artery of the rats to insure a direct measurement of blood pressure, serial blood sampling, and arterial drug injection such that the animals

were completely undisturbed except when exposed to experimental stress.

A- SURGICAL PROCEDURE

The surgery for catheterization was performed under aseptic conditions. All the surgical instruments were heat/pressure sterlized. The rats were anaesthetized with IM injection of Xylazine (Rompun®) , 7.5 mg/Kg body weight, together with Ketamine hydrochloride (Vetalar®), 37.5 mg/Kg body weight. For eye protection during surgery, Chloromycetin® ophthalmic ointment(with 1% chloramphenicol) was applied to the eye ball. The rats were then secured on a rodent surgery table-- hind-quarters higher than the fore-quarters. Two areas were shaved and cleaned with Betadine®; the first was dorsal between the ears and half-way down the neck, and the second, the ventral skin from the pharyngeal to the thorasic area. Then a horizontal incision, 1 inch in length, running cranio-caudally on the ventral mid-line of the neck, was made. All the exposed muscle layers were pulled to the sides and secured with the help of fine hooks. After localizing the left carotid artery, it was stripped from the surrounding connective tissues with exceptional care and with the help of the dissecting microscope. Then a 5 mm length of the artery was isolated from the general circulation with micro clamps, and the first 20 mm of a cannula (Plate 1a, Appendix A) as described below, was inserted cranio-caudally into the artery (Plate 1c, Appendix A). The Tygon cannula was composed of a terminal piece (1 inch in length, and 0.01X0.03 inches in inner and outer diameters, respectively). This piece was inserted into a slightly bigger tube, 18-20 inches in length, 0.02 X0.06 inches in inner and outer diameters. The first inch of the latter tube was turned into

a 0.5-0.7 cm loop in boiling water. Immediately before surgery, Dow Corning solution (10 cs) was flushed through the cannula in order to reduce friction and to facilitate the flow of solutions in it. The tube was then filled up with heparin-saline solution (50 units heparin /ml 0.9% NaCl solution).

After properly inserting the cannula, the two ends of the artery (the free cranial end, and the caudal end harboring the cannula) were both ligated with non-absorbable, transparent, nylon suture(Plate 1 d, Appendix A). A trocar was then attached to the distal(free) end of the cannula and was directed subcutaneously, from left-lateral neck direction to the cephalic aspects of the rat; peneterating through the skin somewhere close to the mid-point of the hypothetical line joining the two ears together.

Panalog® ointment (nystatin-neomycin sulfate thiostrepton) was then generously applied to the exposed surgical sites. The muscle layers were brought up together; the subcutaneous layers were first continuosly sutured--trapping the loop of the cannula inorder to retard unwanted and damaging pulls over the carotid artery; finally, the skin was closed up by interrupted sutures.

A supporting stainless steel spring (30 cm length; 3mm diameter) was then directed over the emerged catheter and its proximal part was first patched to one or two wound clips attached to either side of the protruded catheter (Plate 1e, Appendix A). Then, Caulk® repair material was generously applied to the first few millimeters of the spring, the clips and the skull's skin, and allowed to harden (Plate 1f, Appendix A). The distal end of the catheter was joined to a swivel--a hypodermic needle (no.23) attached to a 1ml plastic disposable syringe (Plate 1b, Appendix A).

The purpose of the spring and swivel was to protect the catheter from possible damage by the rats.

B- POST- SURGICAL PROCEDURES

The operated rats were weighed and housed individually in clean plastic cages with fresh bedding, while water and food were provided for them as previously described. To discourage unnecessary post-operative hypothermia, light bulbs were located adjacent to their cages for 2-3 hours. To prevent any unwanted septicemic conditions, 50 mg /kg/day chloramphenicol was injected intra-arterially into each rat for the first 3 days after surgery.

The patency of the arterial catheter was maintained by connecting the distal part of the swivel to infusion pumps, and constantly flushing the tubing systems. The infusion fluid was composed of 5% dextrose with 5 units/ml heparin. The pumps were adjusted such that 10 ml fluid was flushed through the catheters each day.

6- HEART RATE, BLOOD PRESSURE AND HEMATOCRIT MEASUREMENTS

Each day, starting with day one after catheterization, between 11:00 to 12:00 AM and under a quiet and undisturbed conditions, heart rate, blood pressure and hematocrit of the rats were measured. In this process the distal end of the catheter was attached to an Electromedic® pressure transducer which was connected to a Gilson® polygraph. Calibration was performed with the aid of a mercury sphygmomanometer.

The heart rate was measured and the blood pressure was calculated from the polygraph record. Mean arterial pressure was calculated from the systolic and diastolic pressures using the formula:

$$MAP(\text{ mmHg}) = [2(\text{diastolic})+(\text{systolic})]/3$$

The hematocrit value, an index of the animal's health, proper blood volume and suitable cell count, was measured by aspiration of a drop of blood from the catheter onto a heparinized and clean microhematocrit capillary tube and centrifuging it with the international micro-tube centrifuge® (model M8).

7- BLOOD SAMPLING PROCEDURES

Before and during the stress tests, blood samples were taken directly from the catheter without handling the rats. The purpose of sampling was to measure the circulating level of ACTH, corticosterone, epinephrine and norepinephrine. To insure proper flow of the blood during the test, 30-45 minutes before the zero sampling (i.e. resting level), 1-2 units of fibrinolysin (Plasmin®) dispersed in 0.2-05 ml of distilled water was infused into each rat through the catheter.

A total of 1.5 ml of blood was drawn into a heparinized syringe, of which 1.0 ml was transferred to an iced glass tube for ACTH and corticosterone assay, and 0.5 ml was put into an EDTA-containing iced tube for catecholamine assay. Tubes were then centrifuged at 3,000 g, 4°C , for 5 minutes. The clear aliquot of plasma was then transferred to iced plastic tubes--those, to be used for ACTH/corticosterone assay, containing 150 µl Trasylol solution (containing 600 KIU). The samples were then frozen with dry ice and stored at -40°C until assayed for the hormones.

In order to avoid anemic conditions that could jeopardize the health

of the rats or act as incidental source of stress, the blood cells (i.e. the precipitate portion of the centrifuged blood samples) were thoroughly mixed with 0.6-1.0 ml heparin-saline solution and returned to the rats. Immediately after the cell return, 0.5 to 1.0 ml of heparin-saline solution was infused to each rat to clear the catheter and restore fluid volume.

If a catheter remained occluded, further fibrinolysin treatment or catheter repair was performed. If this was successful the rat was rescheduled for the appropriate stress test and blood sampling not sooner than 1-2 days. If not successful, then he was removed from the experiment.

Furthermore, rats showing signs of sickness evidenced by their poor hematocrit count (less than 23% cells), very low blood pressure (less than 95 mmHg in the WKY and 130 mmHg in the SHR), or severe weight loss, were given a few days rest--if not recovered, then they were also removed from the experiment.

8- STRESS TESTS

A- ETHER STRESS

Group-A rats were used for this test. After a zero time blood sampling under non-stressful conditions each rat was placed for 2-5 minutes in a glass jar saturated with ether vapor (to achieve anaesthesia). The rats were then taken out of the glass jar, but continued to be exposed to ether by placing the muzzle into a nose cone containing ether-soaked cotton. The rats were kept under close surveillance during this stage so as to avoid respiratory depression. The time period of exposure to ether

stress was 30 minutes. At the end of the time period a blood sample was taken. After sampling, the rats were returned to their cages and kept warm to fully recover. The subsequent stress test was not performed sooner than 3-4 days after the previous experiment. The ether and vibration stress tests were performed in a randomly alternate sequence.

B- VIBRATION STRESS

After taking resting blood samples the candidate group A rats, were placed in a plexiglass cage , transferred to an Eberbach® 6000 shaker, and shaken at low speed (180 oscillations/minute). After the end of the 30-minute stress test, a blood sample was drawn and the rats were returned to their cages.

C- MANUAL-RESTRAINT STRESS

Subsequent to resting level blood sampling, the group-B WKY, SHR and SD rats were held tightly in the hand and restrained from any movement for 5 minutes. Post-stress blood sampling was performed 15 minutes after their release, that is, 20 minutes after the beginning of the manual-restraint stress.

These rats were further used for measuring the resting evening level of their hormones, unless their physical status called for their removal from the experiment.

9- RADIOIMMUNOASSAY

All the samples were radioimmunoassayed with the collaboration of Dr. Raymond Nachreiner,Animal Health Diagnostic laboratory, College of Veterinary Medicine, Michigan State University.

A- CATECHOLAMINES

CAT-A-KIT^{TM} (The Catecholamines Radioenzymatic Assay Kit[^{3}H]), from Upjohn Company, which incorporates a modified Passon and Peuler methodology (1973) was employed for the RIA of epinephrine and norepinephrine. The sensitivity of the assay method for epinephrine was 10 pg/ml, and for norepinephrine it was 18 pg/ml. The intra-assay coefficient of variation was 11.9% and 11.4%; the inter-assay precision was 10% and 18%; and the accuracy of recovery was 88-106% and 93-116% for epinephrine and norepinephrine respectively. Parallelism in 1:2 and 1:4 dilutions resulted in 104% and 115% recovery for epinephrine, and 109% and 118% recovery for norepinephrine.

B- CORTISOL AND CORTICOSTERONE

The Corticosterone (H3) Kit, from Radioassay Systems Laboratories was used in the RIA of plasma corticosterone. The antiserum used in this kit produces 100% cross reactivity with corticosterone and 5.3% cross reactivity with cortisol. Parallelism of the corticosterone assay for 1:2 and 1:4 dilutions included 114% and 100% recovery respectively. The interassay coefficient of variation with rat serum is around 14%.

Due to the reactivity of the corticosterone assay with cortisol it was necessary to also measure the plasma level of cortisol. For this purpose, [^{125}I] Cortisol Radioimmunoassay Kit (GammaCoat^{TM}) from Travenol-Genentech Diagnostics, which has 100% cross reactivity of the antiserum with cortisol, was used. The procedure was modified for use with bovine plasma by taking larger sample volumes (20 µl), adding an additional 0.2 mg 8-anilino-1-naphthalene sulfonic acid per sample tube,

and incubating at 37C for 2 hours before decanting the tubes. The Specificity tests of the antiserum indicated 65.8%, 3.8% and 2.1% cross-reactivity for prednisolone, prednisone and corticosterone, respectively. Cross reactivity was less than 1% for cortisone, deoxycorticosterone and dexamethasone, progestrone and betamethasone. Precision on replicated quality control samples indicated less than 10% intra and interassay coefficients of variation. Sensitivity, as calculated from the standard curve at 90% of total trace binding was 3.8 ng/ml.

C- ACTH ASSAY

The ACTH kit from Immuno Nuclear Corp, which uses rabbit anti-porcine ACTH antibody, and shows a good cross-reactivity with human ACTH was employed in the ACTH assay. Previous pilot studies of ACTH parallelism in dilutions of rat serum which had been used to determine the equivalence of rat ACTH and the human ACTH in WHO standards, showed that a 1:2 dilution of the rat sera with ACTH-free human plasma resulted in 106%, 106% and 129% recoveries. Dilution with borate buffer resulted in 88% and 132% recovery. In our experiments in which pooled rat serum containing 163 pg/ml ACTH was used, recovery ranged from 74-98%; parallelism was 81% for 1:2 dilution, 86 to 109% for 1:4 dilution, and 117% for 1:8 dilution. The interassay coefficient of variation of the pooled plasma was 17%.

10- STATISTICAL ANALYSIS

Results of all the experiments were presented as mean±1SD unless otherwise specified. Paired comparisons were made for the blood samples

taken before and after stress exposure within each group. The significance of the difference between the groups was measured by mixed design analysis of variance (P=0.05) and/or one-tail student *t* test (P=0.005).

11- CALIBRATING ACTH/CORTICOSTERONE CONCENTRATION

Addition of 0.15 ml of Trasylol to the a tubes used for ACTH and corticosterone would have caused an unrealistic picture of their plasma concentration had no correction factor been introduced. The reported RIA concentration of these hormones would have been lower than their actual value, and this difference would have been more pronounced in the case of SHR rats that naturally possess a higher hematocrit value than their counterparts. To correct this problem, the following formula was applied to all the reported values of these two hormones:

Actual Conc./ml= (Reported RIA Conc.)/[(1-Hct%)+0.15]

Where:

Hct= Hematocrit value, expressed in percentage form

RIA= Radioimmunoassay

RESULTS

1- GROWTH RATE

Figure and Table 1 show the pooled growth data of 20 WKY and 20 SHR rats. As shown, the SHR rats reached physical maturity (in terms of body weight) at an earlier age, that is, between 135-151 days of life. Thereafter their weight stayed relatively constant, close to 355 grams. However the WKY rats not only reached maximum weight at a later age, that is between 195-205 days of life, but get bigger and heavier than their SHR counterparts. The maximum weight attained by the WKY rats was 493±16 grams.

2- BLOOD PRESSURE

Figure and Table 2 represent the findings on blood pressure of the three rat groups (age=22-34 wks.). The morning (11:00 AM) mean arterial pressure represents the pooled blood pressure , before any stress test and 3 to 5 days after the original surgery. The evening (11:00 PM) pressure, however, is based on the non-pooled, 2-5 days post-stress experiment samplings from the animals.

As indicated, the morning pressure of the WKY, SD and SHR rats was 107±27, 142±21 and 180±24 mmHg respectively. Mixed analysis of

Figure 1. The growth rate of SHR and WKY rats for a period of 130 days, from day 86 up to day 216.

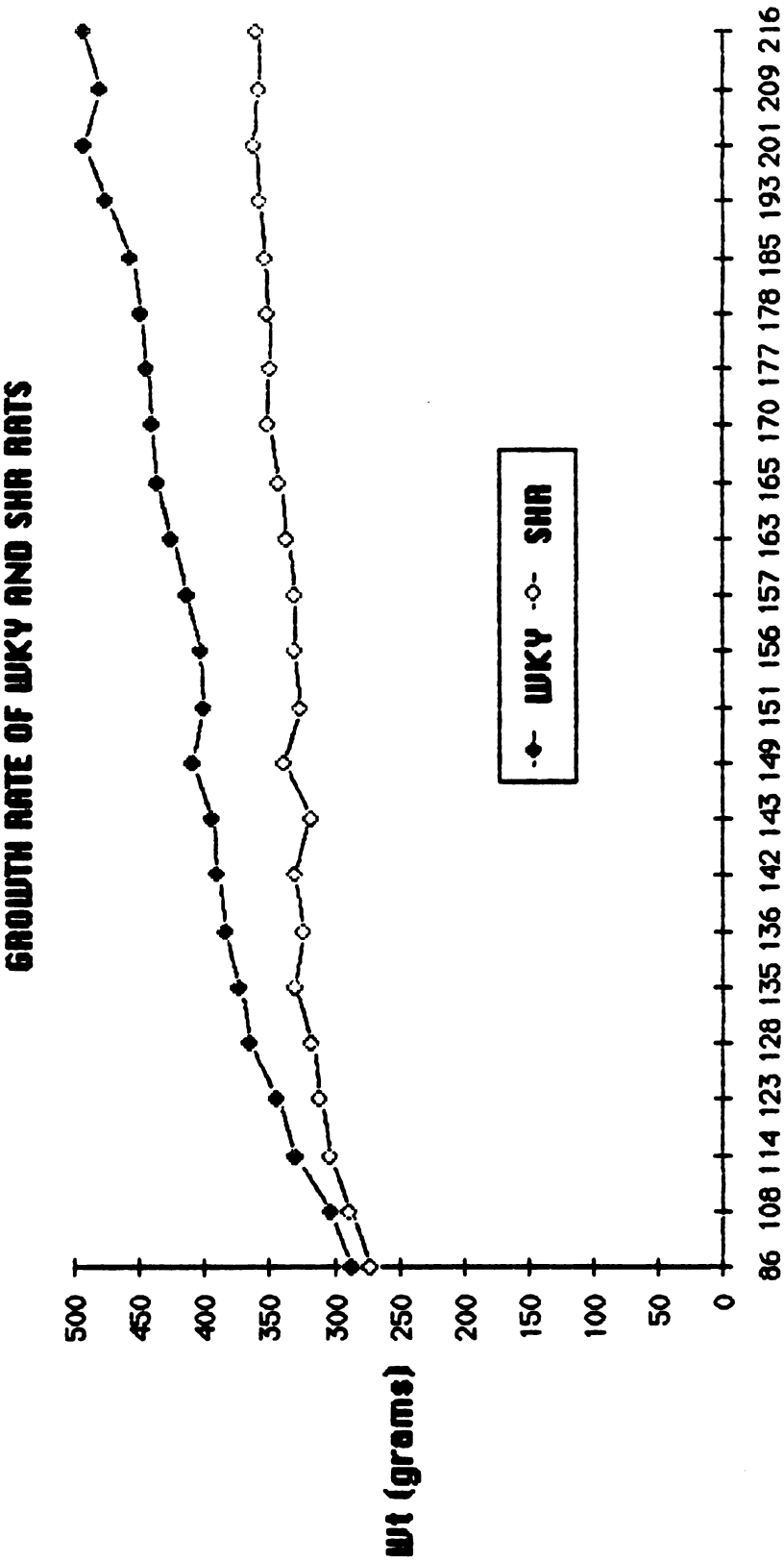
GROWTH RATE OF WKY AND SHR RATS
Wt (grams)
500
450
400
350
300
250
200
150
100
50
0
86 108 114 123 128 135 136 142 143 149 151 156 157 163 165 170 177 178 185 193 201 209 216
AGE (DAYS)
WKY
SHR

FIGURE 1

TABLE 1

GROWTH RATE OF WKY AND SHR RATS

AGE (DAYS)	WEIGHT(grams)±SE		NUMBER OF RATS	
	WKY	SHR	WKY	SHR
86	286±3	273±2	8	8
108	304±8	289±8	8	8
114	331±4	304±2	8	8
123	345±3	311±1	12	12
128	366±8	317±8	20	20
135	373±8	330±1	12	12
136	385±4	323±6	8	6
142	390±7	330±3	12	12
143	394±5	318±5	12	7
149	410±5	339±2	12	12
151	400±8	325±4	8	8
156	403±7	330±2	12	12
157	414±7	330±3	8	8
163	425±8	336±2	12	12
165	436±7	342±4	6	6
170	441±5	350±3	18	18
177	444±11	348±2	9	7
178	449±8	350±7	4	6
185	457±5	352±4	9	8
193	475±5	358±2	9	6
201	493±12	362±3	7	6
209	479±16	358±3	7	7
216	492±25	360±6	4	3

Figure 2. Morning (11:00 AM) and evening (11:00 PM) resting mean arterial pressure of the SHR, WKY and SD rats.

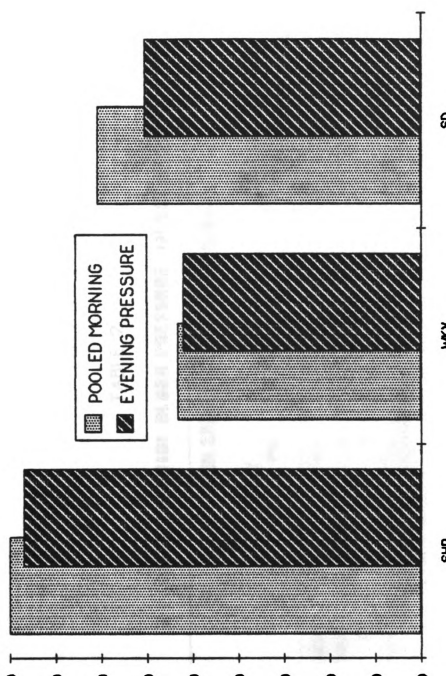
BLOOD PRESSURE
POOLED MORNING
EVENING PRESSURE
mm Hg
180
160
140
120
100
80
60
40
20
0
SHR
WKY
SD
RAT GROUPS

FIGURE 2

TABLE 2

MEAN ARTERIAL BLOOD PRESSURE IN RAT GROUPS

	MEAN ARTERIAL PRESSURE (mm Hg)					
	WKY		SD		SHR	
	AM	PM	AM	PM	AM	PM
MEAN	107	104	142*	121□	180*	174*
S.Dev.	27	6	21	19	24	19
n	16	6	6	5	14	7

*= p< 0.0005 □= P<0.05

variance and t-test of the results showed a significant difference between the rat groups. The SHRs had a significantly higher blood pressure compared to both SD and WKY groups ($p<0.0005$). The SD's blood pressure was also found to be significantly higher than the WKY's ($p<0.0005$).

The evening blood pressure of all the groups showed a moderate decline compared to morning observation. Being 104±6, 121±19 and 174±19 mmHg for WKY, SD and SHR rats. Mixed analysis of variance and t-test of the observation indicated a significant difference between the findings. The SHR group possessed a significantly higher evening blood pressure than the other two groups ($p<0.0005$). The SD rats, on the other hand, had a significantly higher pressure compared to WKY rats ($p<0.05$).

3- ETHER STRESS

A- ACTH AND CORTICOSTERONE

Figure 3 shows the ACTH response of WKY and SHR rats to 30 minutes of ether stress . The resting level of ACTH was 66 ±19 pg/ml in WKY and 73±9 pg/ml in SHR rats. Exposure to ether raised the level of ACTH significantly in both rat groups. The plasma level reached 522±166 pg/ml in WKY and 510±144 pg/ml in the SHR rats. Paired, t-test analysis of the results showed no significant difference between the two rat groups before and after the ether stress (table 3). When the results are expressed in terms of percent change , the WKY rats showed a 718 % increase and SHR rats showed a lower level of response, 616%. However the difference was not statistically significant.

Figure and Table 3 also show the plasma level of corticosterone before and after ether stress. As indicated , both rat groups

Figure 3. Plasma level of ACTH and corticosterone before and after 30 minutes ether stress in WKY and SHR rats.

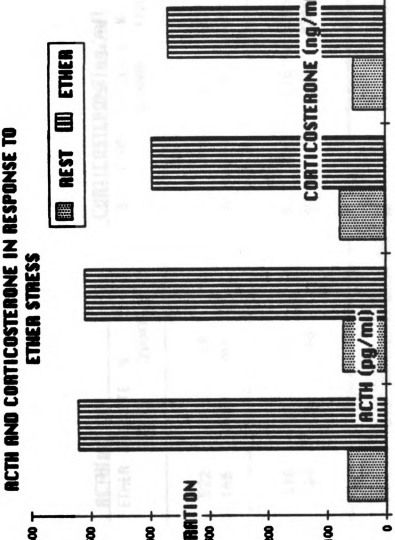
ACTH AND CORTICOSTERONE IN RESPONSE TO ETHER STRESS
REST
ETHER
CONCENTRATION
600
500
400
300
200
100
0
ACTH (pg/ml)
CORTICOSTERONE (ng/ml)
WKY
SHR
WKY
SHR

FIGURE 3

TABLE 3

ACTH AND CORTICOSTERONE IN RESPONSE TO ETHER STRESS

	ACTH(pg/ml)				CORTICOSTERONE(ng/ml)			
	REST	ETHER	ABSOLUTE CHANGE	% CHANGE	REST	ETHER	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	66	522	457	718	77	396	319	1371
S.Dev.	19	166	151	260	68	95	42	1902
SHR								
MEAN	73	510	437	616	53	368	315	1755
S.Dev.	9	144	150	240	45	47	44	2286

WKY (n= 10); SHR (n= 4)

responded by a significant increase in the level of plasma corticosterone, with the WKY rats going from 77±68 ng/ml to 396±95 ng/ml , and the SHR rats from 53±45 to 368±47 ng/ml . On a percentage basis the SHR rats showed a 1755% increase , while the WKY rats showed a 1371% increase. This difference was not statistically significant.

B- EPINEPHRINE AND NOREPINEPHRINE

The epinephrine and norepinephrine response of the rats to ether stress is sketched in Figure and Table 4. As it is shown, there was no significant difference in the resting level of epinephrine between the SHR (34±25 pg/ml) and the WKY (29±26 pg/ml). After 30 minutes of ether stress the SHR level rose to 7433±6757 pg/ml, an increase of 229081%, and the WKY rose to 362± 273 pg/ml, an increase of 9485%. The difference was statistically significant ($p<0.01$).

The basal levels of norepinephrine in the WKY (170±72 pg/ml) and the SHR rats (176±40 pg/ml) were not significantly different. Stress caused 313% and 1938% increase in the basal level of the hormone in the SHR and WKY rats (not statistically significant). The final (30-minute) plasma level of this hormone, significantly different in both rat groups--was 590±245 pg/ml in the WKY and 2298±2375 pg/ml in the SHR rats ($p<0.05$).

4- VIBRATION STRESS

A- ACTH AND CORTICOSTERONE

The ACTH response of the rat groups to vibration stress is shown in Table and Figure 5. There was no significant difference in the basal ACTH

Figure 4. Plasma level of epinephrine and norepinephrine before and after 30 minutes ether stress in WKY and SHR rats.

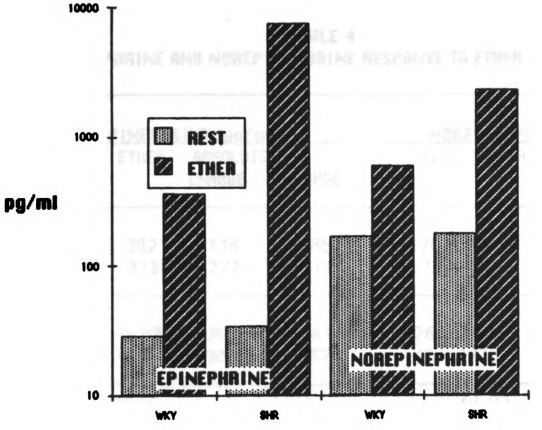
EPINEPHRINE AND NOREPINEPHRINE RESPONSE TO ETHER STRESS
10000
1000
100
10
pg/ml
REST
ETHER
EPINEPHRINE
NOREPINEPHRINE
WKY
SHR
WKY
SHR

FIGURE 4

TABLE 4
EPINEPHRINE AND NOREPINEPHRINE RESPONSE TO ETHER STRESS

	EPINEPHRINE (pg/ml)				NOREPINEPHRINE (pg/ml)			
	REST	ETHER	ABSOLUTE CHANGE	% CHANGE	REST	ETHER	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	29	362	334	9485	170	590	420	313
S.Dev.	26	273	277	5517	72	245	269	246
SHR								
MEAN	34	7433	7399	229081	176	2298	2122	1938
S. Dev.	25	6757	3901	387157	40	2375	1412	1602
p Value		<0.01				<0.05		

WKY (n= 7); SHR (n=3)

Figure 5. Plasma level of ACTH and corticosterone before and after 30 minutes vibration stress in SHR and WKY rats.

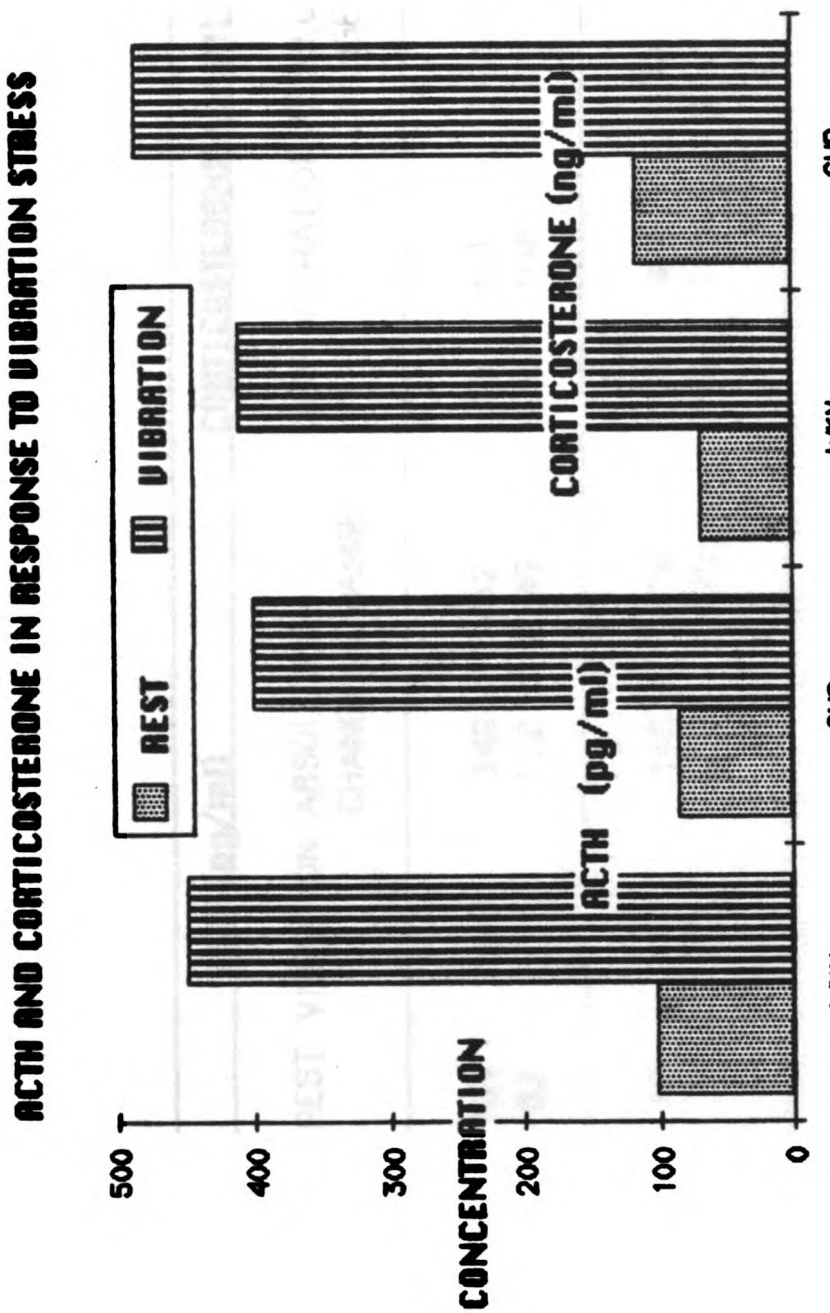
ACTH AND CORTICOSTERONE IN RESPONSE TO VIBRATION STRESS
REST
VIBRATION
CONCENTRATION
500
400
300
200
100
0
ACTH (pg/ml)
CORTICOSTERONE (ng/ml)
WKY
SHR
WKY
SHR

FIGURE 5

TABLE 5

ACTH AND CORTICOSTERONE IN RESPONSE TO VIBRATION STRESS

	ACTH (pg/ml)				CORTICOSTERONE (ng/ml)			
	REST	VIBRATION	ABSOLUTE CHANGE	% CHANGE	REST	VIBRATION	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	101	450	349	462	69	411	342	1051
S.Dev.	82	112	122	242	78	108	131	803
SHR								
MEAN	85	400	315	570	116	486	369	881
S.Dev.	71	120	156	375	96	129	189	1067

WKY (n=8); SHR (n=5)

level between SHR (101±82 pg/ml) and WKY (85±71 pg/ml). Both rat groups reacted similarly to vibration stress in terms of percent change and the post-stress plasma value of the hormone. The post-stress level was 450±112 pg/ml (462% increase) in WKY, and 400±120 pg/ml (570% increase) in the SHR.

Figure and Table 5 also show the corticosterone response of the rats. As it is seen there was no significant difference in the resting level between the SHR (116±96 ng/ml) and WKY (69±78 ng/ml). Vibration stress also produced a similar change in the corticosterone level. In both groups the level rose between 900-1050%. The post-stress hormone level in the WKY rats was 411±108 and in SHR rats was 486±129.

B- EPINEPHRINE AND NOREPINEPHRINE

Figure and Table 6 display the plasma level of epinephrine and norepinephrine before and after exposing the SHR and WKY rats to 30 minutes of vibration. The basal level of epinephrine in SHR rats was almost 6-times higher than the WKYs (176±122 versus 22±13 pg/ml)--however, due to variability among the samples, this difference was not statistically significant. After the vibration stress, the epinephrine level reached 185±92 pg/ml in the WKY and 713±378 pg/ml in the SHR rats ($p<0.025$)-- a 1557% increase in the WKYs and 951% in the SHRs.

Contrary to the findings on epinephrine, the norepinephrine results showed a significant difference in the resting level of the hormone between the rat groups, with 151±44 pg/ml for the WKYs and 426±130 pg/ml for the SHRs ($p< 0.005$). Upon subjection to 30 minutes stress, both rat groups showed an almost identical rise in their plasma norepinephrine

Figure 6. Plasma epinephrine and norepinephrine level before and after 30 minutes vibration stress in WKY and SHR rats.

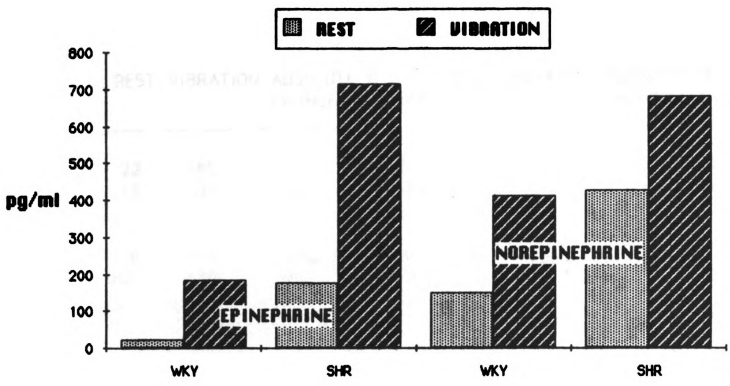
EPINEPHRINE AND NOREPINEPHRINE IN RESPONSE TO VIBRATION STRESS
REST
VIBRATION
800
700
600
500
pg/ml 400
300
200
100
0
EPINEPHRINE
NOREPINEPHRINE
WKY
SHR
WKY
SHR

FIGURE 6

TABLE 6

EPINEPHRINE AND NOREPINEPHRINE RESPONSE TO VIBRATION STRESS

	EPINEPHRINE(pg/ml)				NOREPINEPHRINE(pg/ml)			
	REST	VIBRATION	ABSOLUTE CHANGE	% CHANGE	REST	VIBRATION	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	22	185	162	1557	151	413	263	190
S.Dev.	13	92	91	2220	44	135	146	119
SHR								
MEAN	176	713	538	951	426	680	264	90
S.Dev.	122	378	258	469	130	156	49	48
p Value		< .025			< .005	< .025		

WKY (n= 5); SHR (n= 3)

level--which brought the final concentration of the hormone to 413±135 pg/ml in the WKYs and 680±156 in the SHRs ($p<0.025$).

5-MANUAL RESTRAINT STRESS

A- ACTH AND CORTICOSTERONE

Table and Figure 7 present the findings on ACTH and corticosterone before and 15 minutes after a 5-minute manual-restraint stress. The SHR rats had a significantly higher basal level of ACTH (182±115 pg/ml) compared to SD rats (85±27 pg/ml) [$p<0.05$]. Stress caused the highest increase in the WKY (266± 103 pg/ml), and the least in SHRs (169±155 pg/ml). On a percentage basis the WKY and SD rats responded almost identically (288±239% vs. 299±165%) to the stress. The SHR rats, however, responded less strongly (179±230%). The final, post-stress level of ACTH was highest in the WKY group (400±90 pg/ml), lowest in the SDs (310±79 pg/ml) and moderate (351±79 pg/ml) in the SHRs. Statistical analysis of the post-stress observation showed that only the difference between the WKY and SD rats was significant ($p<0.05$).

The corticosterone results show that the pre-stress level of the hormone in the WKY rats (146±72 ng/ml) was significantly higher than the SD rats (80±37 ng/ml) [$p<0.05$]. However the SHR response to stress was much stronger than the other two groups(1202±1354% increase). SD and WKY rats responded by 486±418% and 185±146% change, respectively. Mixed analysis of variance and t-test of the results of percent-change showed that both the difference between WKY and SD rats, and SHR and WKY rats were significant ($p<0.05$). The post-stress level of corticosterone,

Figure 7. Plasma ACTH and corticosterone before and 15 minutes after a 5-minute manual restraint stress in WKY, SHR and SD rats.

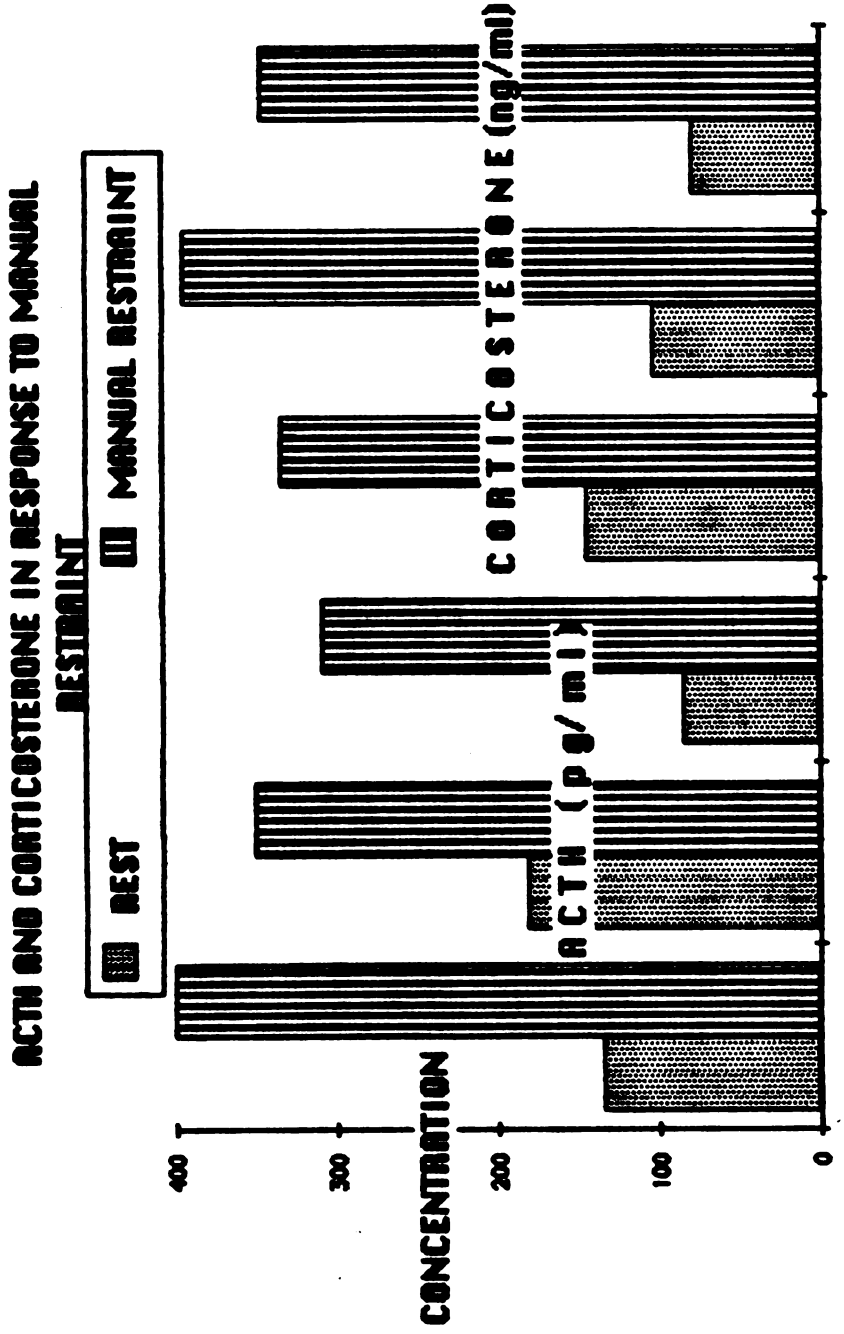
ACTH AND CORTICOSTERONE IN RESPONSE TO MANUAL RESTRAINT
REST
MANUAL RESTRAINT
CONCENTRATION
400
300
200
100
0
ACTH (pg/ml)
CORTICOSTERONE (ng/ml)
WKY
SHR
SD
WKY
SHR
SD

FIGURE 7

TABLE 7
ACTH AND CORTICOSTERONE IN RESPONSE TO MANUAL RESTRAINT STRESS

	ACTH (pg/ml)				CORTICOSTERONE (ng/ml)			
	REST	STRESS	ABSOLUTE CHANGE	% CHANGE	REST	STRESS	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	135	400*	266	288	146*	336	190	185
S.Dev.	77	90	103	239	72	58	51	146
n	7	7	7	7	8	8	8	8
SHR								
MEAN	182*	351	169	179	105	395□	290□	1202*
S.Dev.	115	149	155	230	122	54	108	1354
n	8	8	8	8	9		9	9
SD								
MEAN	85	310	225	299	80	348	269	486*
S.Dev.	27	79	84	165	37	97	122	418
n	6	6	6	6	6	6	6	6

*= P<0.05; □=P<0.025

395±54 ng/ml in SHR, and 336±58 ng/ml in WKY rats were shown to be significantly different ($p<0.05$). Also it was shown that the increase in plasma corticosterone level in the SHRs was significantly higher than the WKYs'--290±108 ng/ml, compared to 190±51 ng/ml ($p<0.025$).

B- EPINEPHRINE AND NOREPINEPHRINE

The epinephrine and norepinephrine response to manual-restraint stress is illustrated in Table and Figure 8. The SHR rats had a significantly higher resting level of epinephrine (65±38 pg/ml) compared to WKY rats (24±12 pg/ml), and not only reached to the highest post-stress level (524±453 pg/ml) but also their reaction in terms of absolute change (455±455 pg/ml, $p<0.01$) and % change (1153%) was greater than the other two rat groups. The WKY rats on the other hand, consistently showed a lower epinephrine level compared to SD and SHR rats.

With regard to norepinephrine, there was a significant resting level difference between the rat groups. The SD rats had the highest basal level (281±86 pg/ml) which was significantly different ($p<0.005$) from that of the SHR (240±83 pg/ml) and WKY (131± 57 pg/ml). SHR rats, on the other hand had the highest post-stress level (558±336 pg/ml) which was significantly different from the WKY (200±84 pg/ml) and SD (387±141 pg/ml) [$p<0.005$]. The absolute post-stress increase in the level of norepinephrine in the SHRs was significantly higher than that of the WKYs--305±315 pg/ml versus 69±109 pg/ml ($p<0.025$).

Figure 8. Plasma epinephrine and norepinephrine level before and 15 minutes after a 5-minute manual restraint stress in WKY, SHR and SD rats.

EPINEPHRINE AND NOREPINEPHRINE IN RESPONSE TO MANUAL RESTRAINT

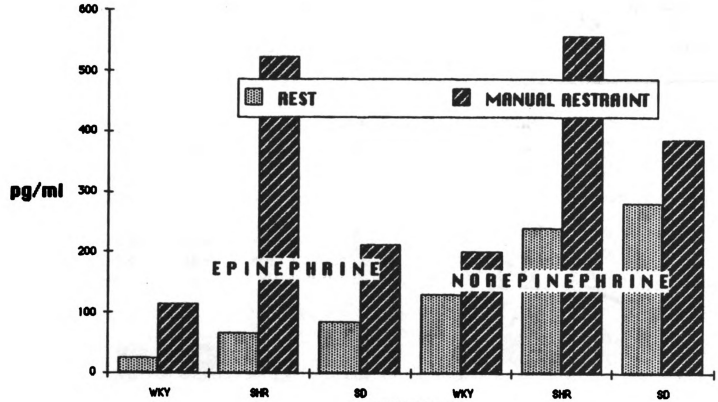

FIGURE 8

TABLE 8

EPINEPHRINE AND NOREPINEPHRINE IN RESPONSE TO MANUAL RESTRAINT

	EPINEPHRINE (pg/ml)				NOREPINEPHRINE(pg/ml)			
	REST	STRESS	ABSOLUTE CHANGE	% CHANGE	REST	STRESS	ABSOLUTE CHANGE	% CHANGE
WKY								
MEAN	24	114	90	456	131	200	69	86
S. Dev.	12	56	50	294	57	84	109	117
n	9	9	9	9	9	9	9	9
SHR								
MEAN	65¥	524◇	455□	1153	240¥	558¥	305□	141
S.Dev.	38	453	455	1957	83	336	315	125
n	11	10	10	10	10	11	10	10
SD								
MEAN	85¥	212*	150	242	281¥	387¥	148	69
S.Dev.	59	125	104	209	86	141	163	79
n	5	6	5	5	5	6	5	5

*=p<0.05; □=p<0.025; ◇=p<0.01; ¥=p<0.005

6- EVENING AND MORNING RESTING LEVEL OF HORMONES

Table and Figure 9 show that there was no significant difference between the rat groups in the resting level of stress hormones. The ACTH level (167±132 pg/ml) was highest in the SHRs, and lowest in the WKY rats (111±99 pg/ml). The corticosterone level was highest in WKY (142±64 ng/ml) and lowest in SD (93±30 ng/ml). Epinephrine was highest in the SD group (60±30 pg/ml), and lowest (27±13 pg/ml), in the WKYs. Norepinephrine was highest (245±144 pg/ml) in the SHRs and lowest (125 ±31 pg/ml) in the WKY rats.

Table and Figure 10 show the 11:00 AM, pooled level of the studied stress hormones in SHR and WKY rats. The pooled sample was determined by pooling together all the resting level results of the three stress experiments. As indicated the SHR rats had a significantly higher level of epinephrine (79±94 pg/ml vs. 25±18 pg/ml: $p<0.01$) and norepinephrine (262±137 pg/ml vs. 149±58 pg/ml: $p<0.005$). There was no significant difference in the ACTH level (128±99 pg/ml vs. 97±66 pg/ml) and corticosterone (96±101 ng/ml vs. 96 ± 77 ng/ml) between WKY and SHR rats.

Comparison of Table 9 and 10 indicates that except for norepinephrine and epinephrine in SHR rats, the level of other measured hormones was higher at 11:00 PM than at 11:00 AM. However, the intra-group comparison between the morning and evening hormone level for each rat (not reported here) showed that except for corticosterone wherein a drop was observed, all other hormones tended to increase in the evening.

Figure 9. Evening (11:00 PM) resting plasma level of ACTH, corticosterone, epinephrine and norepinephrine in WKY, SHR and SD rats.

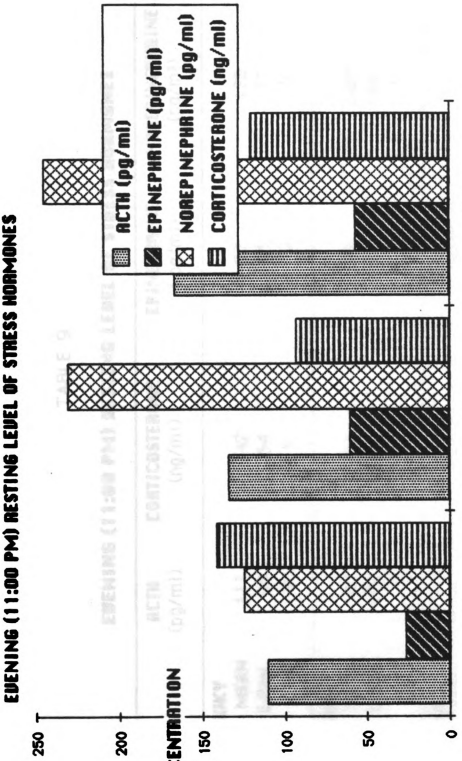
EVENING (11:00 PM) RESTING LEVEL OF STRESS HORMONES
CONCENTRATION
250
200
150
100
50
0
WKY
SD
SHR
ACTH (pg/ml)
EPINEPHRINE (pg/ml)
NOREPINEPHRINE (pg/ml)
CORTICOSTERONE (ng/ml)

FIGURE 9

TABLE 9

EVENING (11:00 PM) RESTING LEVEL OF STRESS HORMONES

	ACTH (pg/ml)	CORTICOSTERONE (ng/ml)	EPINEPHRINE (pg/ml)	NOREPINEPHRINE (pg/ml)
WKY				
MEAN	111	142	27	125
S.Dev.	99	64	13	31
n	6	6	5	5
SHR				
MEAN	167	121	57	245*
S.Dev.	132	51	41	144
n	8	8	8	8
SD				
MEAN	134	93	60*	231
S.Dev.	139	30	30	104
n	4	4	4	4

*= P< 0.05

Figure 10. Pooled morning (11:00 AM) resting plasma level of ACTH, corticosterone, epinephrine and norepinephrine in SHR and WKY rats.

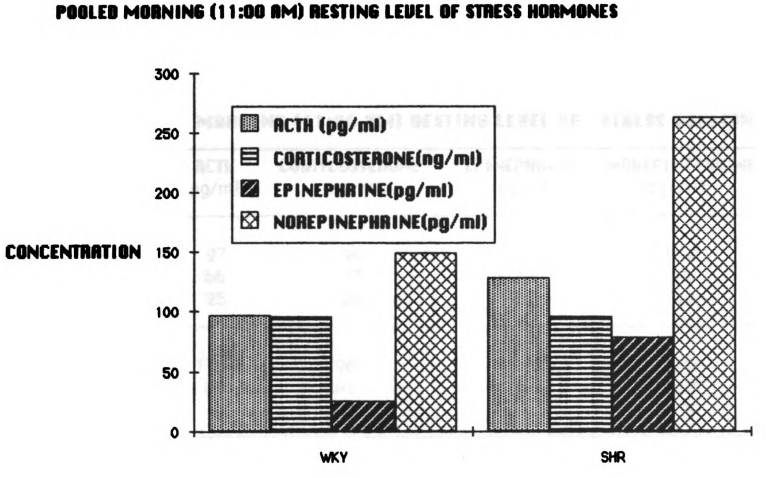
POOLED MORNING (11:00 AM) RESTING LEVEL OF STRESS HORMONES
CONCENTRATION
300
250
200
150
100
50
0
ACTH (pg/ml)
CORTICOSTERONE(ng/ml)
EPINEPHRINE(pg/ml)
NOREPINEPHRINE(pg/ml)
WKY
SHR

FIGURE 10

TABLE 10

POOLED MORNING (11:00 AM) RESTING LEVEL OF STRESS HORMONES

	ACTH (pg/ml)	CORTICOSTERONE (ng/ml)	EPINEPHRINE (pg/ml)	NOREPINEPHRINE (pg/ml)
WKY				
MEAN	97	96	25	149
S.Dev.	66	77	18	58
n	25	26	21	23
SHR				
MEAN	128	96	79	262
S.Dev.	99	101	94	137
n	17	18	17	16
p Value			< 0.01	< 0.005

7- SALT PREFERENCE TESTS

Table 11, 12 and 13 are in reference to NaCl preference tests performed on the SHR and WKY rats prior to the surgery and stress tests. The first table pertains to the group A rats (used for ether and vibration stress). Twenty four (12 SHR and 12 WKY) rats, 24 weeks old, were used for this experiment. The results show that this group of SHR rats had a higher preference and appetite for salt compared to WKY rats. This behavioral difference is statistically shown to be significant for 0.1 and 0.2 molar NaCl solutions.

Table 12, shows the results of the preference test on eighteen, 16-week old, group B rats. As it is indicated, the SHR rats only showed a significantly higher preference for and intake of salt solution at the highest concentration, that is, 0.2 molar NaCl. However, two weeks later the results of the next preference test on the same rats showed that the SHRs had a much higher salt appetite than the WKY rats for all the salt concentrations (Table 13). Furthermore, examination of the WKY preference for salt indicates that there was a decrease in their preference from 16 to 18 weeks. Thus the appearance of a significant difference between the two group was mainly due to a decrease in the WKY rather than an increase in the preference of SHR rats.

TABLE 11

SALT PREFERENCE OF 24 WEEK OLD, GROUP A RATS

	PREFERENCE %			NaCl INTAKE (ml)			WATER INTAKE (ml)		
MOLARITY	0.03	0.10	0.20	0.03	0.10	0.20	0.03	0.10	0.20
WKY									
MEAN	55	47	29	28	27	15	23	28	37
SE	2	7	4	2	6	2	1	3	2
SHR									
MEAN	71	91	91	35	72	80	15	7	8
SE	10	3	1	5	9	4	6	2	1
P value	NS	0.0005	0.0005	NS	0.005	0.005	NS	0.0005	0.0005

WKY (n= 12); SHR (n=12)

TABLE 12

SALT PREFERENCE OF 16 WEEK OLD, GROUP B RATS

	PREFERENCE %			NaCl INTAKE (ml)			WATER INTAKE (ml)		
MOLARITY	0.03	0.10	0.20	0.03	0.10	0.20	0.03	0.10	0.20
WKY									
MEAN	80	87	51	44	66	35	12	9	28
SE	7	3	6	5	6	6	3	2	3
SHR									
MEAN	86	92	78	54	70	57	9	7	14
SE	4	3	7	6	7	8	2	2	4
P value	NS	NS	0.025	NS	NS	0.05	NS	NS	0.025

WKY (n= 8); SHR (n=10)

TABLE 13

SALT PREFERENCE OF 18 WEEK OLD, GROUP B RATS

	PREFERENCE %			NaCl INTAKE (ml)			WATER INTAKE (ml)		
MOLARITY	0.03	0.10	0.20	0.03	0.10	0.20	0.03	0.10	0.20
WKY									
MEAN	40	65	41	25	36	25	31	20	34
SE	14	5	6	11	4	5	7	3	3
SHR									
MEAN	86	89	80	48	70	61	8	7	15
SE	3	5	3	5	9	5	2	3	2
P value	0.005	0.01	0.005	0.05	0.01	0.0005	0.005	0.025	0.005

WKY (n=8); SHR (n=10)

DISCUSSION

Comparison of the results of the stress tests within each rat group (Tables 3, 4, 5, 6, 7 and 8) shows that there was a significant post-stress rise in the level of ACTH, corticosterone, epinephrine and norepinephrine, compared to their basal (resting) level. These findings confirm the role that these hormones play in the physiology of stress (Cannon, 1929; Selye, 1936; Anderson, 1966; Kendall, 1971; Kvetnansky et al., 1978; McCarty et al., 1978; Le Mevel et al., 1978; Kvetnansky et al., 1979; Hausler et al., 1983).

Results of the evening blood sampling (Table 9) and pooled morning sampling (Table 10) for the resting level of the stress hormones show no significant difference between the SHR and WKY rats regarding the concentration of ACTH and corticosterone. However the SHR group showed a significantly higher basal level of epinephrine and norepinephrine compared to WKY group under both evening and morning sampling conditions. This latter finding is on one hand consistent with the reports that suggest a precipitating role for the sympathetic nervous system in general and sympathoadrenomedullary system in particular, in the hypertension-stress interrelationship (Louis et al., 1974; Hallback, 1976; Wing et al., 1977; Franco-Morselli et al., 1978; McCarty et al., 1978; Falkner et al., 1979), and on the other, in contrast with McCarty et al. (1978 and 1979) who were not able to show any resting difference between the two rat groups.

Furthermore, the compatibility of our resting catecholamine levels with Carruba et al's finding (1981) suggests that the chronic cannulation method is indeed one of the most reliable methods for measuring the plasma level of stress hormones in conscious rats.

The results of the three stress tests (Tables 4, 6 and 8) indicate that the SHR rats showed a significantly higher plasma level of epinephrine and norepinephrine compared to both control groups in response to ether, vibration and manual-restraint stresses. This exaggerated stress response gains support in the literature from other researchers who also have demonstrated a much higher post stress level for the above two catecholamines (Hallback et al., 1974; McCarty et al., 1978; Kvetnansky et al., 1978 and 1979; Falkner et al., 1979). Furthermore, the results of the three stress tests show a much greater post stress rise (exemplified in the form of % change from basal level) for epinephrine than for norepinephrine. A possible explanation for this finding is based on the work of Franco-Morselli et al (1978). These authors measured the plasma catecholamines of human subjects with essential hypertension and also DOCA-salt hypertensive Wistar rats. Their results suggested to them that epinephrine is released at a much faster rate from the adrenal medulla, or is involved to a greater extent than norepinephrine in the short term stress responses of the body. Moreover, our catecholamine results are also consistent with the behavioral responses of the SHR rats before, during and after the stress experiments. The SHR rats were more sensitive (hyperreactive) to noise, cage opening, handling and minor disturbances in their environment. This behavioral observation is consistent with McMurtry et al's report (1981) who attributed these behaviors to sympathetic hyperactivity.

The findings on the level of ACTH after the stress tests (Tables 3, 5 and 7) suggest no significant difference between the WKY and SHR groups. These results are consistent with Hausler et al's findings (1983), who reported a comparable ACTH level in 16-week old SHR and WKY rats after ether stress. However, as is shown in Tables 9 and 10, the pooled morning and evening basal ACTH level indicate that the SHR rats show a slightly higher resting plasma concentration compared to the WKY rats (128 vs. 97 pg/ml for morning, and 167 vs. 111 pg/ml for the evening samplings). Furthermore, the observed, consistent, trend for a lower post stress plasma ACTH level in the SHR rats (Tables 3, 5 and 7) may be regarded as an indication of their higher adaptibility to the stress conditions (in terms of raising the ACTH level). But since these differences are not statistically significant they preclude us from any solid positive judgement. Finally, it is worth mentioning that our pooled resting ACTH level for the SHR rats (Table 10) falls in the range that was reported by Hausler et al, (1983). These authors showed a range of ACTH level from 100-300 pg/ml, for 4, 8, 12 and 16 weeks SHR rats.

The findings on the stress level of corticosterone (Tables 3, 5 and 7) indicate a large degree of variation from experiment to experiment, within each rat group, and between the rat groups. The pooled morning level--an estimate of the actual resting concentration, shows that both rat groups have an identical plasma corticosterone level--that is, about 96 ng/ml (Table 10). However, WKY rats showed a slightly higher evening level (Table 9). Yamori et al, (1973), consistent with our finding, also reported no significant difference in the basal level of corticosterone between SHR and normotensive Wistar-Kyoto rats. They obtained their plasma samples by decapitation and/or tail tip cutting method, and reported '119±29 ng/ml'

as the basal plasma concentration of this hormone in the SHR rats.

The post ether stress level of corticosterone (Tables 3) shows that the WKY group had a slightly higher corticosterone level compared to the SHR rats. However, the post vibration and manual-restraint stress results show that SHR rats had a higher corticosterone level--being statistically significant with regard to the latter stress. The inconsistency of the corticosterone findings is also reflected in the literature, with McMurtry et al., and DeVito et al (1981) reporting a significantly lower basal level of this hormone in the SHR rats (140 ng/ml in the SHR vs. 210 ng/ml in the WKY rats); or a much higher level for it after the immobilization, heat and ether stresses (McMurtry et al., 1981); or even a drop in the level of this hormone after immobilization and ether stresses in the SHR rats (Yamori et al., 1973).

In summary, our findings support a higher activity of the sympathetic and sympathoadrenomedullary system in SHR rats, whereas the comparable levels of ACTH and corticosterone suggest that the two rat groups do not significantly differ in pituitary-adrenocortical function. The question that remains to be answered is to what extent these findings support or oppose the underlying and specific hypotheses of these experiments? The SHR rats used in these experiments, like all other progenies of the SHR were hypertensive--that is, they had a much higher resting blood pressure level (Table and Figure 2), and also had a spontaneously elevated salt appetite (Tables 11 and 13). Do the aforementioned results mean that the hypertension and elevated salt appetite of the SHR rats is not due to increased pituitary-adrenocortical activity?

There exist a few possibilities that still may fully or partially

support the specific hypothesis of this thesis and offer an answer to the questions of the former paragraph. One is based on the work of Hausler's group (1983) who were able to demonstrate a markedly enhanced ether-stress induced ACTH release in the 4 week old SHRs compared to the WKY, but not demonstrating the same exaggerated difference in 12 or 16 week old SHR rats. Furthermore, Hausler's group (1984) were also able to demonstrate a significantly larger anterior lobe of the pituitary and a higher total number of ACTH-immunoreactive cells in the 4-weeks old SHR rats compared to the control WKY groups. Based on these findings they suggested that an enhanced stress-induced ACTH in the young SHR rats during early development of hypertension is due to an increased sensitivity of the hypothalamic-pituitary-adrenal axis that may be linked to the development of hypertension, but not to its maintenance. According to these reports and based on our findings, it is arguable that if ACTH is to play a role in the salt appetite-hypertension interrelationship of the SHR rats, it may play it before the maturity of these rats. Nevertheless, our experiment not only was not designed for testing this possibility, but it also did not allow the use of the rats that were younger than 22 weeks.

A second line of support for the specific hypothesis of these experiments comes from the work of Lohmeier and Guyton (1981) who showed that norepinephrine potentiates the role of ACTH in hypertension. Or the reports of Ramey et al. (1951) and Kaisner (1969) who have shown that glucosteroids and norepinephrine have a synergistic action in the maintenance of blood pressure. Accordingly, since we have been able demonstrate a significantly higher level of epinephrine and norepinephrine in response to all the stresses that we studied and also at the resting conditions, and since we also observed a moderately (but not significantly)

higher basal level for ACTH in the SHR rats, then still the possibility is open that the combined synergistic action of ACTH (and corticosterone) and norepinephrine may be responsible for the manifest hypertension and elevated salt appetite of these rats.

Finally, it was part of the intention of these experiments to also investigate which of the two rat groups, WKY or SD can better serve as the control animals for the SHR rats in the study of salt appetite-stress-hypertension interrelationships. Our findings regarding the basal and post stress levels of the stress hormones (Tables 7 and 8) and also the blood pressure of the rat groups (Table 2), support the belief that WKY rats are indeed a better control groups in this respect.

In conclusion, the comparable levels of ACTH and corticosterone demonstrated in our experiments show no significant difference in the pituitary-adrenocortical function of the SHR and WKY rats. However, the higher concentration of catecholamines found in the SHR rats both before and in response to a variety of stresses indicates that the hypertensive rats have a higher sympathetic activity than the normotensives. The basis for the higher salt appetite in the SHR rats remains open for further investigation.

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APPENDIX

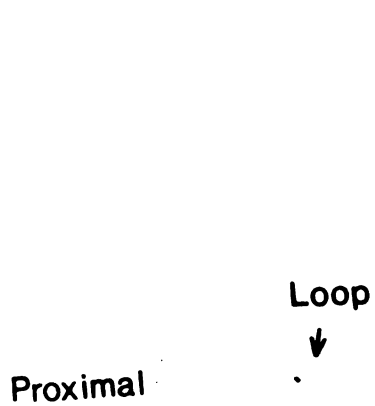
(PLATES)

PLATE 1a. Structure of cannula used for catheterization. The proximal end (smaller tube) enters the left carotid artery. The distal end comes out from the skin of cephalic neck area.

Plate 1b. Swivel and spring used to protect catheter from damage.

PLATE 1

a



b

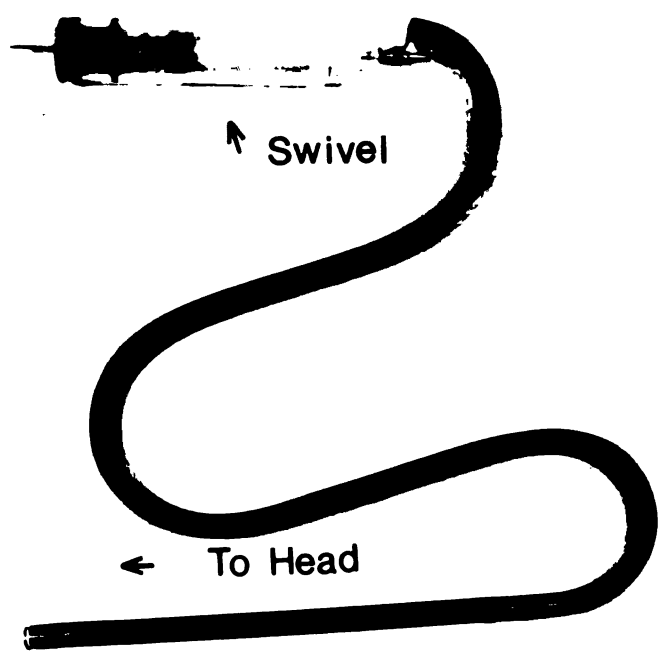
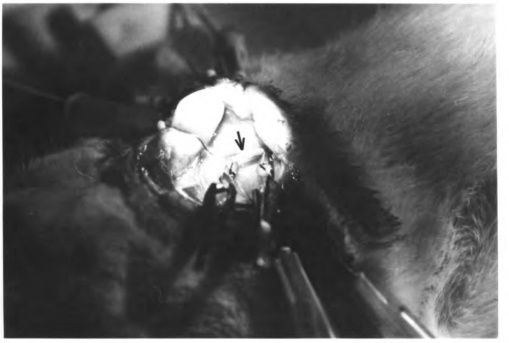


Plate 1c. Site of surgery for cannulation. The pointer is pointing at the left carotid artery.

Plate 1d. Cannulation of the left carotid artery.

PLATE 1

c



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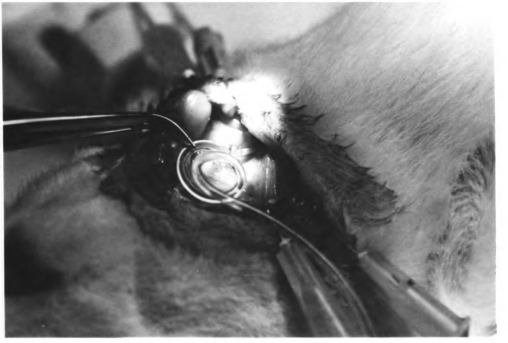
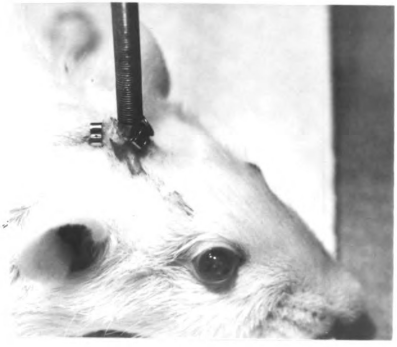


Plate 1e. Extroversion of the cannula through the supporting spring.

Plate 1f. Application of Caulk® repair material to the proximal end of the spring

PLATE 1

e



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