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I am submitting herewith a dissertation written by Brian John Sanders entitled "Cardiovascular, Renal, and Neuroendocrine Responses to Behavioral Stress and Increased Dietary Sodium in the Borderline Hypertensive Rat." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

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CARDIOVASCULAR, RENAL, AND NEUROENDOCRINE RESPONSES TO BEHAVIORAL STRESS AND INCREASED DIETARY SODIUM IN THE BORDERLINE HYPERTENSIVE RAT

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Brian John Sanders

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DEDICATION

This dissertation is dedicated with love to my mother, Jean Tribler Sanders, for her many sacrifces and continual encouragement.

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ABSTRACT

The first filial (F_1) offspring of female spontaneously hypertensive rats (SHR) and male Wistar-Kyoto (WKY) normotensive rats has been shown to develop borderline hypertension (systolic blood pressure = 150 mmHg) in the absence of any environmental manipulation. It has therefore been called the borderline hypertensive rat (BHR). However, when exposed to psychological stress in the form of a shock-shock conflict paradigm or when placed on a high sodium diet the BHR develops severe hypertension (systolic blood pressure = 185 mmHg).

The present study sought to characterize the cardiovascular and renal responses of WKY and BHR during behavioral stress and to determine the influence that a high sodium diet exerts upon these responses.

The study utilized male WKY and BHR rats 8-16 weeks of age. Half of the subjects from each strain were placed on a high sodium diet for eight weeks while the other half remained on a normal diet. During the eighth week of being on their respective diets, all animals were exposed to five days of classical conditioning and were instrumented with chronic bladder, arterial and venous catheters. Cardiovascular measurements and renal function assessments were made on conscious animals during a control period and during one hour (40 trials) of classical conditioning. Blood samples were taken at the end of each

recording session for the determination of plasma catecholamine levels.

The combination of increased dietary sodium and behavioral stress produced no greater degree of hypertension in the BHR than exposure to each stressor separately. Hence, the cardiovascular reactivity of BHR on high sodium was much less than normal sodium controls during stress. With respect to WKY, a high sodium diet did not produce hypertension, but this treatment did prevent the adaptation of blood pressure across trial blocks of stress that was observed in WKY on a normal diet. Furthermore, WKY on a high sodium diet displayed a lack of reactivity to the tone on period during classical conditioning compared to other experimental groups. Finally, the plasma catecholamine data suggest that a high sodium diet reduces sympathetic nervous system functioning in WKY compared to BHR.

The renal function data suggest that the physiological stress of increased dietary sodium and classical conditioning interact in the BHR to decrease renal plasma flow and sodium excretion slightly . Data are discussed in terms of the responses of the BHR's hypertensive parent and the potential ability for the interaction of sodium, stress, and genetics to exacerbate the development of hypertension in the BHR.

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CHAPTER I

INTRODUCTION

Understanding the nature of the mechanisms involved in the etiology and maintenance of human essential hypertension is a major focus of health research. In addition to afflicting an estimated 23-60 million Americans, hypertension is a contributing factor in approximately 250,000 deaths each year (Report of Hypertension Task Force, 1979). Furthermore, 90-95% of all cases of hypertension cannot be attributed to an overt physiological problem, such as renal dysfunction or adrenal adenoma (Pickering, 1972). The enormity of the problem and elusiveness of an identifiable cause have made hypertension a vigorously researched and much debated disease.

Prior to becoming frankly hypertensive, most patients are categorized as having borderline hypertension. This phenomenon has been amply documented (Julius & Esler, 1975; Julius, 1977). Julius (1977) has advanced three characteristics of this condition. Perhaps the most salient feature is the actual blood pressure measurement, with typical systolic readings in the 150-160 mmHg range and diastolic values falling between 90 and 100 mmHg. Second, although most individuals display these values, they have periodic normotensive readings. Finally, there is no overt organ damage.

The hemodynamic patterns associated with both borderline and established hypertension are well defined. Borderline hypertensives

display an elevated cardiac output and normal total peripheral resistance. The "normalcy" of this resistance appears to be inappropriate, though. The normal physiological response to increased cardiac output is to lower resistance, thus maintaining pressure homeostasis. As the disease process continues, there is a progressive shift toward normalized cardiac output and elevated resistance. The constant physical insult of the pressure lability on the vasculature causes structural changes to occur that increase the wall to lumen ratio of vessels and hence permanently raise pressure (Folkow, Hallback, Lundgren, Sivertsson, & Weiss, 1973).

Hemodynamic patterns have also been studied in the spontaneously hypertensive rat (SHR) and its normotensive control, the Wistar-Kyoto (WKY) rat. The results of these studies, using electromagnetic flowometry, indicate that the hypertension in the adult SHR is due to elevated total peripheral resistance (TPR) compared to WKY rats (Iriuchijima, 1973a). This elevation in TPR was reportedly caused by enhanced sympathetic nervous system (SNS) activity (Iriuchijima, 1973b.).

Since the hemodynamic pattern that characterizes human essential hypertension is a dynamic one, shifting from elevated cardiac output to elevated TPR, a critical question concerns whether the SHR displays a similar phenomenon during the development of its hypertension. To address this question, Smith and Hutchins (1979) conducted a developmental study of hemodynamic variables in SHR and WKY. Hemodynamic functioning was determined in both strains at approximately

six, eleven, and eighteen weeks of age. The data from this experiment show that the six week old SHR has hypertension that was characterized by elevated cardiac index. By the eighteenth week, however, the hypertension was maintained by elevated TPR. Thus, it appears that the development of hypertension in the SHR follows a hemodynamic pattern similar to that observed in some cases of human essential hypertension.

The mechanisms by which blood pressure remains chronically elevated are undoubtedly many and complex. Individual physiological systems will be discussed in subsequent sections, but it is appropriate to appreciate in a broad sense some of the ways that the study of hypertension has been approached. Since the SNS is a major factor in many forms of hypertension, evaluation of its contribution has been a prominent feature of many attempts to elucidate the mechanisms involved in elevated blood pressure (Abboud, 1982). The various ways this approach has been pursued is illustrative of the complexity of the disorder.

One way in which the importance of the SNS in hypertension has been evaluated is by manipulation of the central nervous system (CNS) in ways which affect SNS functioning. A good example of this approach is the study of lesions of the nucleus of the solitary tract (NTS). The cells of the NTS receive the primary afferents from the baroreceptors and, in turn, project to higher cardiovascular centers such as the hypothalamus (Palkovits, 1981). Thus, the NTS is one of several regions in the medulla oblongata that is involved in cardiovascular system regulation. It has been shown, for example, that

electrolytic lesions of the NTS in rats produces a fulminating hypertension from which the animal dies within a few a hours (Doba & Reis, 1973). The hypertension was attributed to hyperactivity of the SNS that produced greatly elevated TPR. Using the cat, Nathan and Reis (1977) have demonstrated that medially located NTS lesions produce chronic labile hypertension, tachycardia, abolition of baroreflexes, and increased cardiovascular responsivity to environmental stimuli. The authors conclude that this surgical maneuver results in disinhibition of the SNS which produces the observed cardiovascular changes. Thus, disruption of this cardiovascular integrating center results in sympathoadrenal medullary hyperactivity with attendent blood pressure adjustments.

The converse approach to the one just described has also been employed. That is, surgical intervention in certain CNS structures reduces SNS activity and attenuates or prevents hypertension. One of the most successful paradigms in this regard has been the placement of electrolytic lesions in the anteroventral portion of the third ventricle (AV3V). This lesion has been shown to prevent or attenuate several forms of experimental hypertension: Goldblatt (Haywood, Fink, Buggy, Boutelle, & Brody, 1978), Grollman (Buggy, Fink, Johnson, & Brody, 1977), deoxycorticosterone-acetate (DOCA) (Fink, Buggy, Johnson, & Brody, 1977) and Dahl salt sensitive hypertension (Brody & Johnson, 1980).

Given the fact that the AV3V lesion protects against so many different forms of hypertension, the mechanisms involved in this

prophylaxis are obviously very complicated. Since a strong SNS component is a common denominator in most types of experimental hypertension, it is reasonable to posit that ablation of the AV3V region interferes with the sympathetic drive to the cardiovascular system. Indeed, it has been shown that descending neural projections from the AV3V region course through the ventromedial hypothalamus and median eminence region and are involved in the mediation of the cardiovascular responses to AV3V stimulation (Swanson, Kucharczyk, & Mogenson, 1978). Specifically, electrical stimulation of the ventromedial hypothalamus results in regional hemodynamic alterations almost identical to those observed during AV3V stimulation. Further, electrolytic ablation of this hypothalamic area removes the cardiovascular responses of AV3V stimulation (Fink, Haywood, Johnson, & Brody, 1978). These observations suggest that disruption of this neural pathway may be at least one mechanism by which the AV3V lesion exerts its protective effect.

The study of abnormalities in sympathetic functioning in genetic models represents another way hypertension has been investigated. One such model is the Dahl salt sensitive rat. There is evidence suggesting that sympathetic tone is exaggerated in this animal prior to the development of sodium-induced hypertension (Takeshita & Mark, 1978). Also, it appears that there is a decrease in baroreceptor sensitivity in prehypertensive Dahl rats (Gordon, Matsuguchi, & Mark, 1981). Both of these conditions might make this strain more reactive to environmental challenges, such as increased dietary sodium. Another

model in which the SNS seems to play a prominent role is the DOCA-salt rat. Hypertension in this model has been associated with abnormal functioning of the sympathetic nerve terminals and synthesis, uptake, turnover, and storage of norepinephrine (de Champlain, Krakoff, & Axelrod, 1967;1968; Krakoff, de Champlain, & Axelrod, 1967). Much more attention will be given to these models and potential mechanisms in a later section.

Since so many cases of essential hypertension lack an overt physical cause, much research has addressed the factors that may interact with an organism to produce hypertension. While genetic background is one variable that figures prominently in the equation, accounting for approximately 1/3 of the variance in human hypertension (Grollman, 1973), it is not solely responsible. A clear picture of the etiology of essential hypertension necessitates an appreciation and understanding of other variables that might interact with a predisposing genetic background. Indeed, in recent years several studies have addressed the role of sodium (NaCl) intake (Luft & Weinberger, 1982), diet (Samuel, McNamara, & Shapiro, 1984), or lifestyle (Marx & Kolta, 1978), in the development of essential hypertension. These and several other studies have led to the belief among many researchers that environmental factors can be crucial in the elaboration of this disease (Lawler, Cox, & Hubbard, 1986; Friedman, 1981).

The following review will focus on experimental attempts by investigators to pursue the involvement of environmental events in the

development of hypertension. The stressors that have been employed most often and those that will be addressed here are psychological stress, increased dietary sodium, and the combination of both. Attention will also be given to the various physiological systems that have been studied in efforts to elucidate responsible mechanisms.

Psychological Stress and Hypertension

Sensory Stimulation/Withdrawal

Attempts to induce hypertension in animals using sensory stimulation have generally employed loud noises, flashing lights, and oscillating cages, either alone or together. For example, in one early study rats were exposed to loud noise for 10 minutes each day for 10 months. Of 31 rats studied, 17 showed at least one systolic blood pressure (SBP) reading greater than 180 mmHg (Medoff & Bongiovanni, 1945).

Perhaps a better example of this type of experiment is found in a study by Smookler and Buckley (1969). Rats were subjected to 20 weeks of randomized flashing lights, motion and loud noises. After 12 weeks on this schedule, SBP values reached approximately 150-160 mmHg. Since measurements were not taken during a post-stress period, it is not known whether a permanent hypertension was produced by this manipulation.

An extension of this finding was provided by Perhach, Ferguson,

and McKinney (1975). These investigators used stressors similar to those employed by Smookler and Buckley (1969), but monitored blood pressures for 20 weeks following the termination of the stress. Although no pathological studies were carried out, SBP values remained elevated (c. 160 mmHg) during this follow-up period. It should be pointed out that these findings have not been replicated by other investigators. Indeed, the more typical repsonse is for SBP to rise during the stress and then fall to baseline levels during the follow-up period.

A different approach to the issue of exploring the role of sensory input in the development of hypertension has involved assessing the organism's response to withdrawal of sound. That humans require adequate sensory stimulation to maintain normal cognitive functioning (e.g., thinking, reasoning, emotional stability, etc.) has long been realized (Scott, Bexton, Heron, & Doane, 1959). While the human experience of sensory deprivation includes depression, hallucinations, and psychosis, this manipulation has been reported to produce hypertension in rats.

In one such study (Lockett and Marwood, 1973), it was found that when inbred Wistar rats that had been chronically exposed to increased levels of auditory stimulation were transferred in pairs to soundproof chambers, the mean arterial pressure (MAP) increased after 2 weeks and plateaued at approximately 180 mmHg by the 6th week of isolation. Lockett and Marwood (1973) also reported that the pituitary-adrenal cortical system is critical for the expression of sound withdrawal

hypertension. This conclusion was based on the observation that salt-maintained, adrenalectomized rats as well as hypophysectomized rats did not develop hypertension in response to this maneuver. Furthermore, sound deprivation produced hypertension in adrenalectomized rats supplemented with glucocorticoids, but not in hypophysectomized rats given the same treatment.

Another study of interest is that by Hallback (1975). This experiment demonstrates that isolating spontaneously hypertensive rats (SHR) results in lower SBP values in these animals. Reductions in sympathetic arousal and cardiovascular defense reactions were suggested as possible mechanisms for this prophylaxis. Furthermore, when young SHR were isolated in a dark room until 12 weeks of age, the rate of development of hypertension was delayed by about 2 weeks, although final values approached those of control subjects (Lais, Bhatnagar, and Brody, 1974). That the blood pressure responses of the SHR to sound deprivation differ from those of the WKY strains suggests that these two strains (SHR and WKY) may process and integrate sensory information differently.

Psychosocial Stress Models

James Henry and his colleagues have conducted a series of elegant experiments which elucidate the critical involvement of social interaction in the elaboration of essential hypertension. The basic tenet of this line of inquiry centers around the notion that animals

coexist in a fragile social environment which is based upon an established dominance hierarchy. This group reasoned that experimental manipulations which create a social environment conducive to territorial challenge or which threaten an animal's position in the hierarchy might provide a vehicle with which to study the potential hypertensive effects of socially stressful interactions.

The original study by this group examined the effects of chronic exposure to four different types of psychosocial stress on the development of hypertension in CBA mice (Henry, Meehan, & Stephens, 1967). These stressors included mixing males from different colonies, crowding, exposing males and females to threats from a predator, and creating a situation of territorial conflict using a system of interconnecting boxes. Each of these manipulations produced SBP values of about 160 mmHg in males and 140-150 mmHg in females. It was also determined that the early social experiences of the mice were important in the hypertensive response because mice which were group housed following birth developed a less severe hypertension when exposed to territorial stress than those subjects which were isolated immediately following birth. These researchers concluded that of the four types of stressors studied, territorial challenge provided the greatest amount of stress and produced the most substantial increase in blood pressure.

Henry and his associates extended these findings by conducting a study of adrenomedullary activity following chronic exposure to territorial stress (Henry, Stephens, Axelrod, & Mueller, 1971). In this study, 4 month old CBA mice were exposed to 6 months of

psychosocial stress. It was found that this treatment produced a significant increase in SBP as compared with mice that remained in isolation (170 mmHg vs. 126 mmHg). Further, stressed mice had heavier adrenal glands and showed increased levels of norepinephrine and epinephrine as well as elevated monoamine oxidase and thyroid hormone activity levels. These data suggest that heightened sympathoadrenal medullary activity plays a pivotal role in the genesis of this type of hypertension.

Henry, Stephens, and Santisteban (1975), in a continuation of this research, studied the effects of various durations of psychosocial stress on the development of myocardial and vascular pathology. Psychosocial stress was created by mixing previously isolated male mice with females which had been grouped housed. Subjects were removed and studied after being in the interconnecting box system for periods of time ranging from 2 days to 9 months.

The results of this study demonstrated that the severity of vascular and myocardial pathology increases as the duration of the stress increases. Specifically, 6 months of stress produced increases in arteriosclerotic lesions, myocardial fibrosis, and heart weight to body weight ratios. However, a reduction in SBP and pathology was observed if the mice were returned to isolation following 5 months of stress. Exposure to stress for 9 months produced vascular and myocardial pathology and hypertension that was sustained after the subjects were returned to isolation.

Learning Models

Several studies have employed classical conditioning paradigms in order to explore the relationship between blood pressure and psychological events. Classical conditioning, which is usually aversive for these purposes, involves the repeated pairing of a conditioned stimulus (e.g., a tone) with an unconditioned stimulus (e.g., a shock). Over time, the tone will elicit an elevation in blood pressure in the absence of the shock. This is the conditioned response (CR). Thus, the CR is considered a psychological response in that the organism is not presented with an aversive event, but responds to an auditory cue that has been frequently associated with such an event.

Shapiro and Melhado (1958) subjected rats to 6 hour conditioning sessions, 3 times per week for 25 weeks. Their results indicate that there were no persistent blood pressure elevations. Another study, using dogs, reported that only one subject showed a sustained blood pressure elevation in a 13 month period during which there were no conditioning sessions (Dykman & Gantt, 1960). Unfortunately, since the only blood pressure measurements taken were during the conditioning sessions, it was impossible to determine the extent to which the pressor response generalized to other environments.

Although classical conditioning paradigms have not been successful in creating permanent hypertension, this approach has been useful in exploring subtle physiological responses to behavioral events. For example, Randall, Cottrill, Todd, Price, & Wachtel (1982) examined

cardiac output (CO) and hemodynamic changes during rest and classical conditioning in the conscious monkey. Using a dye-dilution technique with indocyanine green, it was observed that the conditioned response displayed by the conscious monkey is attributable to an increased CO and reduced TPR. This study also suggested that the conditioning period is attended by regional blood flow adjustments consisting of increased flow to the muscles and diminished flow to the kidney and viscera.

Randall and Smith (1974) compared the cardiovascular responses that accompany the CR and exercise in the monkey. The exercise task consisted of spinning a wheel for one minute when a discriminative stimulus was shown in order to receive food reinforcement. The CS was elicited by tone-shock pairings.

The conditioned stimulus (CS) was found to be accompanied by very profound and sustained cardiovascular responses which included increased systolic and diastolic pressure, myocardial contractility, left ventricular pressure, and left and right ventricular contractile force. There was also a significant but transient tachycardia associated with the onset of the CS.

The responses to exercise were very similar to those observed during classical conditioning. Most notably, these included increased systolic pressure, heart rate, and left ventricular contractility. The involvement of the sympathetic nervous system was demonstrated by the ability of propranolol, a beta-adrenoceptor antagonist, to block both heart rate and contractility increases.

Whereas classical conditioning elicits responses that are reflexive and involuntary in nature, operant conditioning paradigms allow the organism some control over the environment. Mastery of a task, for example, can be associated with a delay, removal, or elimination of an aversive stimulus.

A frequently cited study illustrative of this approach is that by Forsyth (1971). This study examined blood pressure and regional hemodynamic changes in the conscious monkey at various times during stressful Sidman avoidance. This particular paradigm required the subjects to learn to press a lever at least once during a 20 second period in order to reset a timer and avoid a tail shock. Thus, the animals had to master a temporal discrimination task in order to avoid punishment.

Following 3-5 days of conditioning, arterial blood pressure, heart rate, CO, TPR and regional blood flow data were collected during rest and following 20 minutes, and 4, 24, and 72 hours of Sidman avoidance. There were several noteworthy observations in this study. First, blood pressure was significantly elevated during the entire avoidance period. An elevated CO during the first 4 hour period suggested this was the mechanism responsible for the pressure rise. A shift in the hemodynamic pattern was observed following 24 hours of avoidance: TPR was elevated in addition to CO. Finally, after 72 hours on the schedule, CO had returned to baseline values and the increased pressure was solely attributable to the elevated TPR. This hemodynamic picture is reminiscent of that reported by Julius (1977) as human borderline

essential hypertension progresses to established hypertension.

Regional blood flow changes were also observed. Renal blood flow was significantly decreased, while blood flow was increased to the liver, skeletal muscle and heart during the first 20 minutes of avoidance. By 72 hours, renal, skeletal muscle and gastrointestinal blood flow were all significantly reduced.

Signalled avoidance, using various schedules of reinforcement, has also been used to produce increases in blood pressure. Such findings have been reported in squirrel monkeys conditioned to avoid electric shock on either a fixed interval or fixed ratio schedule (Herd, Morse, Kelleher, & Jones, 1969). In this study, the fixed ratio schedule involved training the subjects to press a key 30 times to turn off a green light (the discriminitive stimulus) which had been associated with electric shock. This response turned out the light and resulted in the avoidance of a shock during that 1 minute trial. The light reappeared following each 1 minute period. On the fixed interval schedule, the first key press made by the subject after a white light had been on for either 2 or 4 minutes eliminated the delivery of an electric shock during that trial.

Herd et. al. (1969) observed that both schedules produced elevations in blood pressure, but only the fixed interval schedule resulted in pressure increases which were somewhat sustained following discontinuation of the avoidance sessions. Furthermore, the subjects which showed the most persistent elevations were the most efficient responders, suggesting that these observations are linked to the

characteristics of the paradigm rather than merely to the receipt of shock.

Using another approach to operant conditioning, Anderson and Yingling (1979) have shown that physiological responses can be manipulated with a behavioral paradigm. Dogs were trained on a signalled avoidance schedule to press a response panel to avoid a shock. When this behavior was established, a second tone was presented if the subject's TPR fell below a pre-determined level. If the TPR remained below this value for the 30 second duration of the trial, the animal received an electric shock. On the other hand, an increase in TPR above this criterion level was rewarded with no shock being administered. Although these investigators were successful in producing a rise in TPR over the course of the session, there was no evidence of a permanent change in blood pressure or cardiovascular functioning.

The studies cited above show that psychological events and certain behavioral situations can exert an influence over blood pressure. There are several caveats that must be considered when trying to relate these observations to the development of hypertension. First, excursions in pressure, when they do occur, are relatively modest and transient. Second, most of the studies have been unable to demonstrate sustained pressure elevations outside of the experimental situation. Also, there is little evidence from the majority of these studies which suggests that the psychological paradigms employed produce any pathological changes in the subjects. Finally, the animals

traditionally used to study the interaction of stress and blood pressure, such as SHR and WKY rats, dogs, and primates, may be inappropriate models due to their genetic constituency. Data will be presented shortly which suggest that environmental stressors exert their most potent effect on blood pressure when the animal is genetically predisposed, by virtue of at least one hypertensive parent, to hypertension. The lack of this genetic component in animals used in the past may be responsible in part for the inability of some of the paradigms to produce sustained increases in blood pressure.

The amount of sodium in the diet is another environmental manipulation that has received widespread attention, largely due to its relevance to human hypertension. It is to this area of inquiry that attention is now turned.

Dietary Sodium and Blood Pressure

Sodium chloride (salt) was a commodity of strong social and religious significance long before it was implicated as a contributing factor in the devlopment of hypertension. Indeed, the wide ranging importance of salt through history is highlighted by the observation that the Oxford English Dictionary devotes three and one half pages to the 31 different meanings of salt. It is therefore appropriate to review briefly some of the history surrounding salt.

The longevity of several proverbs and customs involving salt illustrates its prominent and sometimes changing role. For example,

one can be the "salt of the earth." While the present day connotation of this phrase tends to imply simplicity and unpretentious behavior, the original intent was less than complimentary since it was a high salt content in some agricultural areas that made the fields infertile and thus worthless. It is also possible for a person to be "not worth his salt." This reference undoubtedly stems from the Roman custom of paying soldiers with salt (salarium); hence, the word salary. Finally, an old English proverb warns of bad luck when salt is spilled: "If the salt thou chance to spill, token sure of coming ill." It is, of course, possible to circumvent the impending misfortune by tossing a pinch of salt over one's shoulder.

The use of salt in religious ceremonies through the ages has also been notable. Jews, for example, sealed covenants by exchanging salt. Hebrews, Greeks, and Romans reportedly salted sacrifices to their gods. A gift of salt to Slavic newlyweds was thought to encourage their happiness and health. The Roman Catholic sacrament of baptism involves placing salt in the infant's mouth to symbolize purity and incorruptibility. Viewing salt differently, Egyptian priests advocated avoiding its use because of their belief that salt led to depression.

The accounts cited above are but a smattering of the lore surrounding salt. More than any other foodstuff, condiment, or aliment, salt has been both blessed and blamed for innumerable reasons. Given the changing views and beliefs about salt, one can only speculate

about the message Leonardo da Vinci was conveying by depicting a turned over salt shaker on the table in The Last Supper.

There is really no way to state definitively when salt was first used by humans. There has been, however, speculation on why it came to be used in the first place. One of the widely cited theories, although incorrect according to Meneely (1982), is that the addition of salt to the diet became necessary as man began to consume more and more vegetables and grains. This error has been propagated for over a hundred years by many respected authorities including Meneely (1954). In a later statement on the problem, Meneely (1982) corrects his position and explains the generation of this fallacy.

The problem has its origin in the simple and correct observation that herbivores will travel great distances to salt licks while carnivores do not. It was reasoned that the food chain evolved such that the herbivores got their salt from the licks and the carnivores got theirs from eating herbivores. While intuitively appealing, it was belied by the finding in the mid-1800's that the salt content of the herbivorous diet without salt licks is no different than that of the carnivorous diet. The potassium content, however, was strikingly different. The herbivorous diet contained approximately 15 times as much potassium as sodium. The carnivorous diet, on the other hand, had only three to five times as much potassium as sodium.

At the end of the 19th century, a German scientist named von Bunge became interested in this problem and began making anthropological observations to gain further insight. Specifically, he wanted to

identify groups of people who subsisted on an herbivorous diet and used salt and carnivorous groups that used no salt. When von Bunge found populations that lived on these diets, he formulated his hypothesis stating that the addition of dietary salt is needed by vegetarian populations to offset somehow the high potassium intake. This excess salt was supposedly useful in the efficient excretion of potassium.

There is no compelling scientific data to support this theory and much to argue against it. One cogent argument against this belief is found in the observation that some African tribes, who are vegetarian and according to von Bunge ought to ingest large amounts of salt, use a plant ash that has nearly pure potassium chloride as a condiment. This as well as some of the cross-cultural studies to be discussed shortly should have called this hypothesis into question long ago. Its longevity is due primarily to the frequency with which von Bunge published his views rather than to widespread scientific support.

Epidemiological Research

Much of the impetus for the study of the relationship between salt intake and blood pressure came from the observation that populations that ingest very low amounts of salt have a relatively low incidence of hypertension, while the converse is true for groups of people subsisting on a diet high in sodium. Dahl and Love (1961) presented some of the first data supporting this thesis. They published a diagram which depicts a direct correlation between salt intake and

incidence of hypertension in different geographic and racial groups. For example, the highest percentage of hypertension was noted in the inhabitants of northern Japan where the salt intake averages 26 grams/day. As a point of reference, most Westerners ingest approximately 7-12 grams of sodium/day. The other end of the spectrum indicates the near nonexistence of hypertension among Eskimos who live on a diet containing about 3-5 grams of sodium/day. While this diagram paved the way for much further exploration of the problem, it can be faulted on several points. First, the authors published none of the raw data, only percentages. Also, the criterion for what blood pressure levels constituted hypertension was not indicated. Finally, no data regarding the age, weight, or health status of the subjects were given.

It could be argued that low salt diet is merely coincidental and the absence of hypertension is explainable in terms of genetic resistance, lifestyle/environment, or low body weight. There are, however, compelling reasons to believe that this is not the case. The first observation is that in some of these populations, such as the Melanesians and Brazilian Indians, there is generally not an age related rise in pressure such as that commonly seen in Western cultures (Maddocks, 1967; Lowenstein, 1961). However, when these natives and other groups such as Polynesians and Solomon Islanders move to more Westernized areas, or when acculturation reaches their habitats, they too begin to show an age related rise in blood pressure (Prior, Evans, Harvey, Davidson, & Lindsey, 1968). This alone, of course, does not

necessarily implicate a role for salt in the development of hypertension, but it does illustrate that these individuals are not genetically protected from increases in blood pressure when their environment is manipulated.

The study by Prior et al. (1968) documented some findings that go further in suggesting a causal role for dietary salt in the development of hypertension. The populations studied in this investigation were two Polynesian tribes living on different parts of the same island. The two groups were of essentially the same genetic background (i.e., no inter-tribal marriages or unions) and engaged in the same social customs and lifestyles. The major difference between the two groups was that because of living location one group cooked all of its food in seawater while the other group used relatively fresh spring water. The researchers reported a 10% incidence of hypertension in the former group. The "fresh-water" group had a virtual absence of hypertension. The inherent controls in this study make it important because the data suggest that neither genetic nor psychological factors are the critical variables in accounting for the absence of hypertension among peoples on a low salt diet.

Another issue to be dealt with in the epidemiological literature is that of body weight. Specifically, the populations that have the lowest ingestion of salt happen to have the lowest body weights. It may be reasoned, therefore, that the low body weights protect individuals from hypertension. While lower body weight is associated with lower blood pressure, there is evidence which suggests that this

does not necessarily protect individuals from developing hypertension. A striking example is that of the Iranian nomads. These individuals are as lean and unacculturated as low salt populations such as the Yanomano Indians (Oliver, Cohen, & Neel, 1975) and Easter Islanders (Cruz-Coke, Etcheverry, & Nagel, 1964). These nomads, however, maintain a daily diet that contains about 10-15 grams of salt and have a rate of hypertension similar to that of Caucasians in the United States (Tobian, 1979).

The confounding of variables in some studies makes it clear that statements that propose a relationship between salt and blood pressure based solely on epidemiological data must be guarded. For example, it has been observed that the bushmen of the Kalahari desert who are recruited into the Kenyan army show a dramatic rise in blood pressure (Kaminer & Lutz, 1960). Since army rations contain an extremely high amount of salt for preservative purposes, it could be argued that this is evidence for a salt-blood pressure relationship. However, such assumptions must be scrutinized. Induction into the army, which entails an abrupt change of lifestyle and, in some cases, imminent death, is certainly a stressful situation. Such a circumstance may well raise blood pressure in individuals on a diet containing practically no salt.

When dealing with large groups of people, it is obviously very difficult to control for all factors that may influence blood pressure (e.g., psychological, genetic, etc.). Some studies, such as that by Prior et al. (1968), were reasonably successful in controlling for most

factors that might cloud the detection of a sodium-blood pressure relationship. Studies which have not taken these precautions, but do show an increase in blood pressure with higher amounts of dietary sodium certainly do not negate or weaken the efficacy of such a relationship. More than anything, they demonstrate that increasing levels of dietary sodium may interact with genetics, acculturation, and daily stress to produce hypertension in certain individuals.

Another area pertinent to the epidemiological literature is the apparent inconsistency of a demonstration of a positive correlation between sodium excretion and blood pressure levels or the occurrence of hypertension. Cross cultural studies typically show such a correlation, which has helped promote the view that there is a causal relationship between salt and hypertension, while within population studies do not show such a relationship (Miall, 1959; Ljungman, Aurell, Hartford, Wikstrand, & Wilhelmsen, 1981).

One way to view this issue has been called the saturation effect by some investigators (Meneely & Batarbee, 1976). This hypothesis suggests that in a large population there are subsets of individuals who are salt-sensitive and some who are salt-resistant. The high salt diets that are common in Western cultures might trigger the elaboration of hypertension in the salt-sensitive individuals while the salt-resistant group would remain normotensive. Since both normotensives and hypertensives would be exposed to the high sodium inherent in their diets, one would not expect a significant correlation between sodium excretion and blood pressure in a within-population

study. Based on this view, then, the conclusion that there is no salt-blood pressure relationship might be drawn.

Omvik (1984) has put forth a different view. This position maintains that the discrepancy between the within and between population studies is due to the comparison of pools of data which are incomparable rather than due to the lack of a causal relationship between salt and blood pressure levels. To understand this concept, the nature of the sampling procedures for urine must be considered. Most within populations studies rely on a relatively small number of urine samples for the determination of individual sodium excretion rates (Miall, 1959; Ljungman et. al., 1981). Due to the dependence of sodium excretion on several factors including blood pressure, sodium intake, and hormonal activity, an individual's sodium excretion can vary greatly between measurements. For example, it has been reported that it takes approximately fourteen 24-hour urine samples from a given subject to achieve a within subject variation less than the between subject variability (Liu, Cooper, McKeever, McKeever, Byington, Soltero, Stamler, Gooch, Stevens, & Stamler, 1979). This, then, allows the actual relationship between sodium excretion and blood pressure to become clear.

On the other hand, cross-cultural studies measure sodium excretion from large numbers of samples. This practice greatly reduces the within subject variability of sodium excretion that clouds other studies using fewer samples. Omvik's basic conclusion from all of this is that the alleged discrepancy between sodium excretions in the within

and between population studies is an artifact of the analyses of the data and should not be interpreted as evidence against the validity of the relationship between sodium intake and blood pressure levels.

Animal Research

There is a multitude of evidence from animal experimentation suggesting that dietary sodium (NaCl) can influence blood pressure. Much of this evidence focuses on the Dahl salt sensitive rat, but research on this issue has also been conducted using the WKY, SHR, and borderline hypertensive (BHR) rats, as well as dogs. The following discussion will focus on research using these animals with emphasis placed upon the various physiological systems that have been studied in an effort to understand the mechanisms by which sodium may elevate blood pressure.

One of the most widely used animals in this line of inquiry is the Dahl rat. By selectively inbreeding Sprague-Dawley rats, Dahl (Dahl, Heine, & Tassinari, 1962) developed a strain of animal which is highly sensitive to sodium (DS) and a strain that is equally resistant to dietary sodium (DR). A plethora of studies have been carried out to delineate the mechanisms involved in the elaboration of the hypertension produced in the DS rat when placed on a high sodium (8% NaCl) diet. The following discussion addresses some of the specific systems that have been studied in this model as well as other types of rats.

Renal mechanisms. One of the first studies suggesting renal involvement in sodium-induced hypertension was performed by Dahl, Heine, and Thompson (1974). Using a cross transplantation paradigm, these researchers demonstrated that a kidney from a DR rat transplanted into a DS rat will ameliorate the hypertensive effects of a high sodium diet. Conversely, a DS kidney transplanted into a DR rat will cause the latter to become hypertensive in the face of a sodium challenge. These early findings led the way to further exploration of the role of the kidney in NaCl hypertension in the Dahl strain.

Since retention of sodium may be involved in the genesis of this type of hypertension, Tobian and his colleagues have tried to elucidate the Dahl kidney's ability to handle a sodium load. In one study, this group infused isolated DR and DS kidneys at inflow pressures of 100, 130, and 160 mmHg (Tobian, Lange, Azar, Iwai, Koop, Coffee, & Johnson, 1978). All kidneys were from animals which had been maintained on a normal sodium diet. Sodium excretion by the DS kidney was significantly less that the DR kidney at all three pressures. This defect of the DS kidney could be overcome, however, with increasing pressure, such that at 160 mmHg the DS kidney excreted about 50% more sodium that the DR kidney at 130 mmHg. Since the pressure-natriuresis curve was shifted to the right for the DS kidneys, such that higher inflow pressures were required to achieve a given sodium excretion, it was hypothesized that a delay in natriuresis was related to the rise in pressure.

Iwai, Ohanian, and Dahl (1977) pursued this possibility in another

experiment employing diuretics. This design involved placing both DS and DR rats on thiazide diuretics at the beginning of the study. For 14 weeks all rats were maintained on a normal sodium diet while pressures were monitored. During the following 14 week period, all rats were given an 8% NaCl diet. Only the DS rats not being given thiazide diuretics developed hypertension. That the thiazides prevented the DS rats from developing hypertension gave credence to the claim that a renal defect affecting sodium excretion is an important component of this hypertension.

If there does indeed exist an exaggerated pressure-natriuresis curve in the DS rat, it is important to understand the responsible mechanisms. Ganguli, Tobian, and Dahl (1976) have provided one possible scenario. They have shown that plasma flow to the renal papilla is reduced by 25%-32% in the DS rat on any level of dietary sodium. This may be an important factor in the reduced natriuresis since the normal physiological response to a sodium challenge is to increase plasma flow to the papilla. Another pertinent observation was that even though papillary flow was reduced in the DS rats, plasma flow to the kidney as a whole was not different between DS and DR rats. This indicates a disproportionate elevation in vascular resistance in the vessels supplying the renal papilla. Furthermore, even when the DS rat is not hypertensive, this resistance to papillary flow is still observed, suggesting that the vessels in the DS rats are very sensitive to the hypertensive process.

In an extension of this observation, Tobian, Goto, Ganguli,

Johnson, and Iwai (1980) have demonstrated the possible involvement of prostaglandins in this phenomenon. Prostaglandins are chemical substances released into local tissue fluids and have a role in circulatory regulation. Specifically, when the DS rat is on a normal sodium diet, the prostaglandin E_2 (PGE_2) level in the renal papilla is 60% lower than in the DR rat. In addition, when both strains are placed on a high sodium diet for 4 or 11 weeks, PGE_2 concentrations double in each strain. This means, however, that the DS rat has a PGE_2 concentration equivalent to that of the DR rat on a normal sodium diet. This increase in PGE_2 concentration is a normal response to a sodium challenge and represents a mechanism that allows the animal to excrete the sodium load. The DS rat, however, cannot increase PGE_2 levels enough to meet the demand.

The parallels between papillary flow and PGE_2 concentrations are notable. At all moderate levels of sodium intake, DS rats have reduced papillary flow and PGE_2 levels, while a high sodium diet results in an increase in both variables. Thus, although PGE_2 may not exert a vasodilatory effect on the whole kidney, these data suggest that it has a pronounced effect on the papillary circulation.

Nervous system mechanisms. The involvement of the nervous system in sodium-induced hypertension has been studied extensively by many investigators including de Champlain and his colleagues. The goal of their continuing research is to understand the relationship between the sympathetic nervous system (SNS) and variations in blood pressure that

result from various levels of sodium intake in combination with deoxycorticosterone acetate (DOCA).

In an early study (de Champlain, Krakoff, & Axelrod, 1967) Sprague-Dawley rats were made hypertensive by weekly injections of DOCA along with ingestion of 1% sodium chloride drinking solution. Using tritiated norepinephrine (NE), several functions of the sympathetic nerve endings (e.g., uptake, storage, retention) were studied at various times up to 10 weeks after the beginning of treatment. Five minutes after administration of tritiated NE, the neuronal uptake and uptake into the storage vesicles were normal in several sympathetically innervated organs of DOCA hypertensive animals. It was also found, however, that these animals displayed a deficit in retention and storage of NE. For example, one hour following injection of labeled NE, the amount found in the heart, intestine, skeletal muscle, kidney and spleen was reduced as was endogenous NE levels. Finally, organs that had parasympathetic innervation (e.g., salivary gland and vas deferens) had normal NE content.

Systolic blood pressure and capacity of the heart to retain NE were inversly related in these animals: with increasing blood pressure the heart was less able to retain NE. Since this finding was noted in the hearts of normotensive rats (with endogenous NE), it was reasoned that the inability of the hypertensive heart to retain NE was related to the same mechanism involved in blood pressure regulation in normotensive animals.

In order to elucidate the mechanism responsible for the reduced retention and storage of NE, Krakoff, de Champlain, and Axelrod (1967) used tracer doses of lableled NE to chart the activity of NE in several organs. It was found that the activity of NE fell more rapidly in the hearts of hypertensive animals. Furthermore, after inhibiting tyrosine hydroxylase, which is the rate limiting step in the biosynthesis of NE, the rate of decline of NE content was markedly accelerated in the spleen and normal in the salivary glands of hypertensive rats. These observations led to the conclusion that the reduced retention and storage of NE is due to increased turnover of NE in the peripheral sympathetic fibers. Furthermore, it was found in a subsequent study (de Champlain, Mueller, & Axelrod, 1968) that this increase in turnover rate preceded the development of hypertension, thus implicating this phenomenon in the pathogenesis of this type of hypertension.

Data from Takeshita and Mark (1978) have shown the existence of a powerful neurogenic component in sodium-induced hypertension in the Dahl rat. Employing a hindquarters perfusion preparation, because this is a vascular bed which can be manipulated and studied relatively easily, it was shown that DS rats on a high sodium diet for 4 weeks had a dramatic rise in pressure that was accounted for, in part, by increased vascular resistance. In this design the hindlimbs were perfused at various flow rates, viz., 3.2, 4.2, and 5.2 ml/min. The pressure in the hindquarters at each of these rates was taken as an index of vascular resistance. When the sympathetic nerves were sectioned at the lumbar region, resistance dropped significantly in the

DS rats on a high sodium diet as compared to DS rats on a normal diet. It was concluded that this neurogenic component was responsible for approximately 50% of the observed vascular resistance. These results were not likely due to changes in vessel geometry since resistance during maximal vasodilation with papaverine was similar between groups.

A subsequent study by Takeshita, Mark, and Brody (1979) demonstrated the importance of the sympathetic nervous system (SNS) in this type of hypertension. In this experiment, DS and DR rats were injected with either 6-hydroxydopamine (6-OHDA), which destroys peripheral sympathetic nerve endings, or an inert vehicle twice weekly for 8 weeks. One week following the commencement of the injections, all subjects were started on a diet containing 8% NaCl. It was found that impairment of the SNS prevented the DS rats from developing hypertension, while the DS rats receiving the vehicle injections showed the expected SBP increase to a high sodium diet. Further, pre-treatment with 6-OHDA also lowered hindquarter vascular resistance and reduced responses to both sympathetic nerve stimulation and tyramine. Thus, an intact SNS is required for the DS to display a hypertensive response to a high sodium diet.

Sympathetic nervous system involvement has also been implicated in the salt sensitivity of the SHR by Winternitz and Oparil (1982). This group showed a positive correlation between plasma norepinephrine levels and SBP in SHR on a high salt diet, suggesting that an increase in peripheral sympathetic nerve activity is involved in the hypertensive response. The role of the peripheral sympathetic system

was implicated further by the finding that ganglionic blockade with hexamethonium bromide produced a greater percent decrease in blood pressure in the high salt group than in the groups maintained on lower amounts of dietary sodium. Finally, an increase in the severity of the hypertension was noted as a result of this dietary regimen. Taken together, these findings point to an overactive sympathetic system in SHR on a high sodium diet.

This group has also investigated brain catecholamine levels in SHR on high and normal sodium diets. After approximately one month on the respective diets, an increase in norepinephrine was noted in the anterior and dorsomedial hypothalamic nuclei (Winternitz, Wyss, Meadows, & Oparil, 1982). Since stimulation of the anterior hypothalamic nuclei produces hypotension (Struyker- Boudier, Smeets, Brouwter, & van Rossum, 1974) and since both of these areas project to the nucleus of the solitary tract (Palkovits & Zaborsky, 1977), it is tempting to speculate that this finding represents an attempt to down regulate the system as a compensatory measure. Also, introduction of norepinephrine into this area produces a dose-dependent fall in blood pressure (Day & Roach, 1974).

The involvement of the central nervous system (CNS) in sodium-induced hypertension in the Dahl rat has also been investigated. Goto, Ikeda, Tobian, Iwai, and Johnson (1981) examined the effects of electrolytic lesions placed in the anteroventral portion of the third ventricle (AV3V) on the development of hypertension in the DS rat. The AV3V lesion has been shown to prevent several forms of experimental

hypertension including Grollman (Buggy, Fink, Johnson, & Brody, 1977), Goldblatt (Haywood, Fink, Buggy, Boutelle, & Brody, 1978) and deoxycorticosteroid acetate hypertension (Fink, Buggy, Johnson, & Brody, 1977).

After placement of the AV3V lesions, DS rats received daily injections of either vasopressin (since this lesion attenuates vasopressin release) or a vehicle for 10 weeks while being maintained on a high sodium diet. Lesioned rats given the vehicle showed a significantly smaller increase in pressure than sham controls. Also, lesioned animals given vasopressin showed a smaller increase in pressure than sham controls, but a significantly larger pressure rise than lesioned rats given the vehicle. Finally, in a finding that may be related to the Ganguli et al. study (1976), the AV3V lesions increased renal papillary blood flow in the DS rats by 14%. These findings illustrate the importance of the AV3V region and vasopressin release for the full expression of sodium-induced hypertension in the DS strain.

Hemodynamic and metabolic systems. Ganguli, Tobian, and Iwai (1979) have explored the hemodynamic alterations that attend a high sodium diet in DS and DR rats. Both DS and DR rats were fed diets normal (.3%) or high (8%) in NaCl for 3 or 7 days. After 3 days on the high sodium diet, DR rats remained normotensive while the DS rats displayed an elevated MAP. This rise in pressure was attributed to an increase in CO and TPR. Conversely, DR rats on a high sodium diet had an increased CO, but TPR was decreased and MAP did not change.

Following 7 days on a high sodium diet the CO of the DS rats returned to normal values while both TPR and MAP remained elevated. Normal sodium diets had no influence on any of the hemodynamic variables measured in either strain.

Since the SHR develops hypertension in the absence of any experimental manipulation, it has been of little utility in assessing the role of environmental events, such as sodium levels in the diet, in the genesis of essential hypertension. This animal has been helpful, though, in elucidating the degree to which external events can influence the course of development of and mechanisms underlying the hypertension. For example, Chrysant, Walsh, Kem, and Frohlich (1979) have compared the hemodynamic and metabolic changes in the SHR and WKY after 3 months on a high or normal NaCl diet. In this study 8 rats of each strain were given tap water to drink and another 8 rats of each strain received 1% NaCl in tap water. The results indicate that SHR on a high NaCl diet show an increased MAP attributable to an elevated TPR. Heart rate and CO were not affected by the treatment. WKY given a high NaCl diet, on the other hand, evidenced an increased CO which was attended by a compensatory fall in TPR resulting in no change in MAP.

With respect to the metabolic data, both strains showed an increased urinary sodium excretion in response to the sodium challenge and sodium balance was maintained. The mechanisms underlying the normal sodium balance may be different in the two strains. Evidence for this contention comes from studies in the Dahl rat which suggest that when DR rats are given a high NaCl diet they respond with a

significant increase in renal papillary flow, while DS and SHR strains are unable to increase flow sufficiently to excrete the sodium load (Ganguli et. al., 1976). The implication for the study by Chrysant et. al. (1976) is that the WKY rats (since they, like the DR rat, are resistant to the hypertensive effects of sodium) could excrete the sodium load by increasing papillary flow without altering systemic hemodynamics. The SHR, on the other hand, maintain sodium balance by increasing MAP which produces a pressure diuresis. Thus, although the methods by which the strains cope with a sodium challenge are different, both are equally effective in maintaining sodium balance.

The hemodynamic data from Ganguli et. al. (1979) and Chrysant et. al. (1979) are strikingly similar to those observed in the human literature, thus giving support to the use of animals to study the human condition. Normotensives tend to respond to a sodium challenge like WKY, with an increased CO, reduced TPR and no blood pressure change. Hypertensives tend to respond like SHR, with an increased CO and TPR and an attendant pressure rise. Other hypertensives, though, do show a decrease in TPR, but it is disproportionate given the level of CO (Sullivan, Ratts, Taylor, Kraus, Barton, Patrick, and Reed,1980).

Clinical Research

Kawasaki, Delea, Bartter, and Smith (1978) studied 19 essential hypertensives and classified them as salt sensitive (SS) or non-salt sensitive (NSS) on the basis of their blood pressure responses to

sodium intake ranging from 9 to 246 mEq/day (1 gram of sodium chloride=17 mEq). If a patient showed a 10% increase in blood pressure after being on the high salt diet for six days, he was classified as SS. Of the 19 subjects, 10 were classified as NSS and 9 as SS. The NSS patients seemed to be able to handle the sodium load efficiently since they excreted more sodium and gained less weight that the SS patients. This observation suggests that the retention of sodium by the SS subjects is involved in their pressor response. It does not, however, explain the responsible mechanisms.

Analysis of plasma renin activity and aldosterone levels of these patients may provide some answers. After seven days of being on the lowest amount of sodium intake, both of these variables were signficantly lower in the SS patients. This suggests that these subjects were unable to control efficiently hormonal mechanisms responsible for maintaining sodium balance. Sodium deprivation should raise these levels dramatically in an attempt to conserve sodium. Furthermore , following the high salt period both variables decreased to the same level in both the NSS and SS patients. Since they started at different initial values, there was a greater drop in the NSS patients. It is possible that the differences in the handling of sodium in these two groups of subjects are related to these hormonal systems. Unfortunately, none of the variables essential to determining the cause of the pressure elevation, such as cardiac output or total peripheral resistance, was measured in this study.

Fujita, Henry, Bartter, Lake, and Delea (1980) carried out a study

which extended the observations of Kawasaki et. al. (1978). Again, essential hypertensives were studied while on low (9 mEq/day) and high (249 mEq/day) sodium intake and were classified as SS or NSS using the aforementioned criteria. In addition, though, all subjects were given the diuretic furosemide immediately following salt loading. The loss of sodium and reduction in blood pressure in the SS patients were greater after furosemide than those of the NSS patients. This finding gave support to the hypothesis that the sodium retained with loading and that lost with furosemide is related to the observed pressure responses. To elucidate the mechanisms involved, the authors used echocardiography to measure CO and calculate TPR.

Whereas increased CO was noted in all subjects as a result of salt loading, the increase was significantly greater in the SS subjects. In addition, TPR decreased significantly with salt loading for all subjects. There was, however, no significant difference between TPR in SS and NSS subjects. This is suprising since CO was significantly increased in the SS subjects; the normal physiological response would be to adjust TPR in the face of an elevated CO. This suggests that the TPR of the SS group remained inappropriately elevated and may indicate a heightened autonomic drive.

To pursue further the importance of the adrenergic system, Fujita et. al. (1980) examined norepinephrine (NE) levels at various points in time during the experiment. Several observations are pertinent. First, there was no difference between SS and NSS subjects following the low sodium period. From the first to third days of sodium loading

there were similar decreases in NE levels in both groups. This reduction in autonomic activity may represent a physiological attempt to compensate for the increased pressure induced by the sodium challenge. Finally, by the fourth day, when blood pressure was highest in the SS subjects, the NE levels of these subjects were significantly greater than in the NSS subjects. Thus, the relatively greater retention of sodium by the SS patients could depend in part on a heightened functioning of the autonomic nervous system. This accelerated activity is reflected in the catecholamine level and may have prevented appropriate decreases in TPR.

The hemodynamic adjustments that attend a high sodium diet have received much attention because of the importance of these variables in understanding the development of essential hypertension. One important study in this regard compared mean blood pressure (MBP), DBP, TPR, and cardiac index (CI) responses to a high and low sodium diet in normotensives and borderline hypertensives (Sullivan et. al., 1980). In normotensive subjects a low sodium diet produced a drop in MBP and CI with no change in TPR. After being on a diet containing 12 grams of sodium per day these subjects displayed an increase in CI and a fall in both TPR and MBP. Finally, a 24 gram sodium diet elevated MBP by 16% and CI by 15% while TPR dropped 11.5%.

The borderline hypertensives responded to the low sodium diet with a fall in MBP and CI and an increase in TPR. Their responses to sodium loading varied, however. Fourteen of 19 subjects showed an increase in DBP which was attended by a 20.9% rise in CI and 24.5% fall in TPR in

nine of the 14. This is the same hemodynamic pattern displayed by the normal subjects. However, the increase in CI was significantly greater in the hypertensive patients and the fall in TPR was not sufficient to prevent pressure from rising. The other five subjects displayed a 21.9% rise in TPR and 15% fall in CI as a result of sodium loading. The authors suggest that these subjects were in a more advanced stage of the disease and responded with a more robust vascular reactivity to the sodium challenge. Finally, a third subset of five borderline hypertensives showed no pressure response to a high sodium diet.

The hemodynamic data from the Sullivan et. al. (1980) study are similar to those found in much of the literature and suggest a general pattern of response among normo- and hypertensives when challenged with a high salt diet: normotensives tend to vasodilate and maintain pressure homeostasis while hypertensives respond with vasoconstriction and elevated blood pressure.

While this dichotomous response has been noted in several studies, an understanding of the precise mechanisms involved remains elusive. One relevant experiment was designed to evaluate the effects of varying sodium intake on arterial pressure and forearm vascular resistance in borderline hypertensives (Mark, Lawton, Abboud, Fitz, Connor, & Heistad, 1975). Forearm vascular resistance and blood flow was assessed in six subjects after ten days of high (410 mEq/24 hr) and low (10 mEq/24 hr) sodium intake. The high sodium diet increased vascular resistance and blood pressure and decreased forearm blood flow in five of the subjects. Further, the vasoconstrictor response to

lower body negative pressure, which is a stimulus to neurogenic vasoconstriction, was enhanced in subjects on a high sodium diet. The authors contrasted these results with those of an earlier study using normotensives in which a high sodium diet produced forearm vasodilation and no changes in blood pressure (Abboud, 1974). Mark et al. (1975) conclude that a high sodium diet in borderline hypertensives produces increases in blood pressure, neurogenic vasoconstriction, and forearm vascular resistance and that these responses are vastly different from those displayed by normotensives.

In an attempt to elucidate the mechanisms involved in this response, Takeshita, Imaizumi, Ashihara, and Nakamura (1982) tested the hypothesis that an altered wall to lumen ratio of the resistance vessels may contribute to the increased vascular resistance in hypertensive patients on a high sodium diet. Using the same criteria for salt sensitivity as Fujita et al. (1980) and Kawasaki et al. (1978), nine subjects were classified as salt sensitive and eight were not salt sensitive. The extent of any structural vascular changes of the forearm resistance vessels due to salt loading was examined by assessing forearm vascular resistance during reactive hyperemia following 10 minutes of arterial occlusion. The high sodium diet increased vascular resistance and decreased maximal vasodilator capacity in salt sensitive subjects. These variables were not altered in the non-salt sensitive subjects. Also, the high sodium diet produced augmentation of forearm vascular responses to ice on the forehead and intravenous phentolamine in both groups, but the effect

was more pronounced in the salt sensitive group. These results tend to support the view that salt loading produces structural changes in the forearm resistance vessels and that these changes are responsible, in part, for the increase in vascular resistance. These findings compliment the animal work carried out by Limas, Westrum, Limas, and Cohn (1980). These researchers showed that salt loading produced a significant thickening of the vessel wall in the intrarenal arteries and aorta in SHR. Since the vascular hypertrophy preceded the onset of the salt-induced acceleration in blood pressure, it appeared that the vascular response was not secondary to a worsening of the hypertension.

Another approach in the clinical literature has addressed the role of sodium in hypertension at the cellular level. Specifically, the area of increased intracellular sodium content ($[Na^+]_i$) due to abnormal cation transport has been vigorously researched in recent years. The first clue that this system might be involved in the pathogenesis of essential hypertension came in 1952 when Tobian and Binion noted increased sodium content in the arterial walls of hypertensive patients. This area of inquiry has flourished in the last decade with sophisticated studies of red and white blood cell and smooth muscle cell sodium content in hypertensives and their relatives, as well as in normotensives.

Numerous observations of the sodium content of red and white blood cells from hypertensive patients and normotensive controls have been published. The white blood cell studies and many, but not all, of the red blood cell studies indicate that on the average $[Na^+]_i$ is

significantly higher in patients with essential hypertension than in normotensive individuals. Some hypertensive patients were found to have an unusually high red blood cell sodium level, even in studies in which the mean $[Na^+]_i$ values for hypertensive patients and normotensive subjects were not significantly different (Wiley, Clarke, Bonacquisto, Scarlett, Harrap, & Doyle, 1984). While many hypertensive patients have red blood cell $[Na^+]_i$ levels within the normal range, the red blood cell $[Na^+]_i$ distribution curve for the hypertensive patients appeared to be skewed toward higher $[Na^+]_i$ levels (Adermonmu & Salako, 1979).

Some normotensive first-degree relatives of hypertensive patients also have high $[Na^+]_i$ levels as compared to normotensive individuals with a negative family history (Ambrosioni, Costa, Montebugnoli, Tartagni, & Magnani, 1981). This raises the possibility that some changes in sodium metabolism may be detectable in persons who may be prehypertensive.

The possibilty that $[Na^+]_i$ is elevated in various cell types in hypertensive patients is particularly intriguing since such a defect in vascular smooth muscle cells could promote calcium entry and thereby increase vascular tone (Blaustein, 1977). Thus, it is a logical undertaking to try to elucidate the various sodium transport mechanisms and identify any defects that may exist in patients displaying increased $[Na^+]_i$. Although there are several methods by which sodium is transported across membranes, the active transport of sodium via Na^+-K^+ ATPase activity has received considerable experimental

attention. Suppression of the Na^+-K^+ pump, which is largely responsible for maintaining the proper ionic distribution in the intra- and extracellular fluids by inhibiting Na^+-K^+ ATPase, represents a crucial aspect of a hypothesis that attempts to link increased $[Na^+]_i$ to hypertension.

The first step in validating this hypothesis, which has been promoted primarily by Blaustein (1977; 1983), requires the ability to measure sodium pump activity and to demonstrate its deficiency in patients with essential hypertension and in experimental animals. One of the most frequently used methods for assessing pump activity measures what is known as ouabain sensitive rubidium uptake of blood vessels. Rubidium can substitute for potassium in active transport across cell membranes. It is used in the study of pump activity because its radioactive form has a longer half life and lower energy emission than radioactive forms of potassium. The ouabain sensitive rubidium uptake is that fraction of the uptake related to active transport because ouabain inhibits the Na^+-K^+ pump which is responsible for active transport. This value is calculated as the difference between rubidium uptake without and with ouabain. Rubidium uptake in the presence of ouabain reflects passive penetration into the cells since the pump is inhibited.

The particular procedure for executing all of this is as follows. First, the vessels are incubated at 0° C in K^+ free solution to depress the pump and load the cells with sodium. Next, to stimulate the pump the vessels are incubated at 37° C in a K^+ free solution containing

non-radioactive rubidium. One half of the vessel is placed in a fluid without ouabain and the other half is placed in a fluid with ouabain. Rubidium uptake is measured approximately 20 minutes later.

Another technique used in this area of study is the examination of cardiac microsomal Na^+-K^+ ATPase activity. Like Na^+-K^+ pump activity in intact cells, the ability of a cell membrane to split adenosine triphosphate, i.e., total ATPase activity, is partially inhibited by oubain. The residual activity is due to Mg^+-ATPase. Sodium-potassium ATPase activity, then, is the difference between the total ATPase activity and the Mg^+-ATPase activity.

The investigation of a cellular abnormality in cation transport is an integral aspect of the hypothesis that the development of hypertension in man is due to an inherited defect in the ability of the kidney to excrete sodium. The hypothesis develops as follows. As sodium intake increases, there is an increase in extracellular volume which will preserve an ionic balance required for normal cellular functioning. The sodium retention gives rise to the increased secretion of a circulating sodium transport inhibitor (or natriuretic hormone). This agent would suppress Na^+-K^+ ATPase and hence Na^+-K^+ pump activity. It is interesting to note that this substance is sometimes referred to as a oubain-like substance because of oubain's similar action on pump activity.

The question at hand, though, concerns how the depression of Na^+-K^+ pump activity gets translated into increased blood pressure. In the case of the kidney, Na^+-K^+ pump underactivity would favor sodium

and water excretion and would help compensate for the reduced ability of the kidney for excretion. The link between this and hypertension, Blaustein (1983) hypothesizes, is the belief that this defect, viz., a cellular abnormality in cation transport, can be generalized to other cells. Blaustein has postulated a way in which a rise in $[Na^+]_i$ could lead to increased vascular resistance. In this scenario, inhibition of sodium pumps in sympathetic neurons would tend to increase intracellular calcium because sodium and calcium are coupled by a counterflow transport mechanism (i.e., sodium ions enter in exchange for exiting calcium ions). Then, since neurotransmitter release is triggered by the rise in intracellular calcium, there would be both a tonic or spontaneous as well as depolarization-induced catecholamine release. This increase in transmitter release could be expected to activate the smooth muscle contraction to a greater extent than normal.

Psychological Stress, Dietary Sodium and Hypertension

The above discussion suggests that both psychological stress and increased dietary sodium intake can influence blood pressure. It would seem that a logical extension of these observations would be the investigation of the interaction of these two environmental manipulations. Suprisingly though, few studies have addressed this issue.

Ely and Weigand (1983) have studied this relationship in the SHR. These experiments were designed to determine whether dietary sodium,

territorial stress or the combination of both stressors altered the rate of development of hypertension in the SHR and catecholamine levels in selected brain areas. Their data suggest that territorial stress for 8 weeks or a combination of high dietary sodium and stress for 18 weeks significantly elevates the SBP of the SHR. Increased dietary sodium alone did not significantly elevate pressure. Further, territorial stress elevated norepinephrine levels in the nucleus of the solitary tract and amygdala and dopamine levels in the hippocampus and amygdala. Stress plus high sodium resulted in elevated dopamine and norepinephrine levels in the amygdala and area postrema, respectively. These data provide support for the contention that neurochemical changes in the limbic and brainstem regions are associated with hypertensive responses to environmental manipulations in some types of animals.

Other efforts along these lines have utilized dogs. In one study, the effect of free-avoidance conditioning, constant saline infusion, or the combination of both treatments upon blood pressure levels was examined (Anderson, Kearns, & Better, 1983). It was reported that only the saline infusion plus conditioning for 15 days produced any increase in blood pressure, with final SBP values barely reaching the 140 mmHg range during the avoidance session.

Freidman and Iwai (1976) studied the blood pressure responses displayed by the Dahl rat maintained on a normal sodium diet when placed on a conflict schedule. This experiment involved food depriving the animals and making them bar press for food reinforcement. Some

responses, however, were followed by a cutaneous electric shock. Chronic exposure (6 hours/day for 26 weeks) produced SBP values of approximately 160-170 mmHg in the DS rats. These values were significantly higher than free-feeding control subjects. Dahl salt-resistant rats showed no hypertensive response when placed on the same schedule. Interestingly though, Dahl salt-sensitive rats not placed on the conflict schedule became hypertensive over time. This is due, in part, to the fact that these animals are inbred for their sensitivity to sodium and they will become hypertensive with time from even the very low amount of sodium found in normal rat chow (Rapp, 1982). It thus appears that the DS rat is sensitive to the hypertensive effects of a sodium challenge, but not to a psychological stressor. These researchers have also shown that conflict plus a 2% NaCl diet will exacerbate the hypertension in the DS rat. The pressures observed, though, were still lower than those observed when the DS rat is placed on a high sodium diet.

Another approach to the study of the interaction of stressful environmental stimuli and sodium has involved the assessment of renal functioning. The increase in sympathetic nerve activity that accompanies stressful environmental stimulation has long been appreciated. The effects of SNS outflow on the renal handling of sodium and water has been the subject of recent research which attempts to link environmental stress with the development of hypertension.

Several experiments by Barajas and his colleagues have demonstrated the rich noradrenergic innervation of the mammalian kidney

(Barajas, 1978; Barajas, 1979). Postganglionic sympathetic fibers project to the ascending limb of the loop of Henle, distal and proximal convoluted tubules, and the collecting duct (Barajas, Powers, & Wang, 1984). This extensive innervation plays a role in a variety of renal functions including renal tubular sodium reabsorption and renin secretion (DiBona, 1982).

Of primary importance for hypertension is the observation that increases in efferent renal sympathetic nerve activity (ERSNA) produce similar changes in tubular reabsorption of sodium and decreases in urinary sodium excretion (Koepke & DiBona, 1985b). These variables are, of course, influenced by the amount and rate of fluid filtration by the kidney. It is therefore equally important to realize that these changes in the renal handling of sodium have been documented in cases where glomerular filtration rate (GFR) and renal plasma flow (RPF) do not change. The most likely explanation for the changes in sodium excretion is an increase in sodium reabsorption along the nephron that is directly related to an increase in ERSNA. Specific studies will be discussed shortly.

Based on experiments conducted in rats using low frequency renal nerve stimulation and pharmacological blockade of adrenoceptors, the antinatriuretic response to ERSNA appears to be mediated by alpha-one adrenoceptors. The abolition of the antinatriuresis with phenoxybenzamine (non-selective alpha blockade) and guanethidine indicate the involvement of alpha receptors. Furthermore, the antinatriuresis is unaltered by nonselective alpha-two blockade with

yohimbine and rauwolscine, nonselective beta blockade with propranolol, or selective beta-one blockade with atenolol (Koepke, DiBona, 1985a). Thus, by process of elimination with various antagonists, alpha-one receptors seem to mediate the antinatriuretic response to renal nerve stimulation.

Several studies in dogs and rats illustrate the ability of environmental and behavioral manipulations to produce the physiological alterations described above. In one experiment dogs were subjected to avoidance conditioning in which they learned to press a panel prior to or during a tone in order to avoid receiving a cutaneous electrical shock to a hind leg (Koepke, Light, & Obrist, 1983). This stressful paradigm produced an antinatriuretic and antidiuretic effect in 21 of 30 dogs studied. Individual differences were cited as accounting for the failure of nine dogs to show any change in these renal variables. The physiological mechanisms mediating the renal responses in the 21 dogs were also studied. In approximately half of these dogs, there was no change in GFR or RPF, thus suggesting increased tubular reabsorption of sodium. In 10 dogs, however, the avoidance paradigm decreased GFR and RPF. The antinatriuretic and antidiuretic responses noted in these dogs were due to renal vasoconstriction and decreased filtrate.

Renal sympathetic nerve activity has been measured in conscious SHR during control and stress sessions (Koepke & DiBona, 1985a). The major goals of this study were to assess the central actions of beta receptor antagonists on ERSNA and sodium excretion. The results indicate that SHR show increased ERSNA to bouts of air stress and that

intracerebroventricular (i.c.v.) administration of beta antagonists are capable of blocking this effect. Specifically, following i.c.v. injection of saline, ERSNA of SHR still increased in response to air stress. When injected with either propranolol or timolol, however, conscious SHR showed no ERSNA response to stress. The increase in ERSNA to air stress was associated with decreased sodium excretion. Since GFR and RPF did not change, increased tubular reabsorption of sodium was postulated as the responsible mechanism. Furthermore, this antinatriuretic response was abolished by i.c.v. injections of both propranolol and timolol. Since these responses did not occur when the same doses of these drugs were given intravenously, it appears that the ERSNA response to stress is mediated via central beta adrenoceptor mechanisms.

The aforementioned studies were conducted using animals maintained on a normal sodium diet. Given the evidence of the relationship between sodium intake and blood pressure, the effects of altering sodium intake on renal responses to air stress in WKY and SHR were studied (Koepke & DiBona, 1985b). SHR on a high sodium diet displayed greater sodium retention to air stress that SHR on a normal diet. Also, renal denervation abolished the antinatriuresis in SHR on either diet. It was also determined that the antinatriuretic responses to stress in SHR are associated with increased ERSNA. SHR on a high sodium diet had greater increases in ERSNA than SHR on a normal diet. In contrast, this type of diet had no influence on the renal response of WKY to the same stress.

Lundin and Thoren (1982) have reported similar findings. These researchers showed that exposure to mental stress, in the form of controlled blasts of air to the face, results in a significant antinatriuresis in SHR compared to WKY. This is particularly interesting in light of the fact that the blood pressure of SHR increased dramatically during stress. Furthermore, although renal sympathetic nerve activity was higher at rest in SHR than WKY, the magnitude of change in this variable was significantly greater in SHR. Also, the antinatriuretic response to stress was abolished by surgical renal denervation.

Finally, renal responses to stress have been investigated in humans. Light, Koepke, Obrist, & Willis (1983) have shown that family history of hypertension is related to sodium and water retention during mental stress. Specifically, young men with one or two hypertensive parents displayed an antinatriuretic and antidiuretic response to competitive mental tasks compared to age matched subjects with no family history for hypertension. Further, there was a direct relationship between the degree of sodium and water retention and the magnitude of heart rate increase. This observation is suggestive of heightened sympathetic nerve activity and can be related to the experiemtnal findings previously discussed.

In summary, the above discussion suggests that one way in which environmental stress and sodium may be involved in the hypertensive process is at the level of renal functioning. Specifically, the sympathetic outflow that accompanies stress contributes to increased

renal nerve activity. By virtue of the extensive innervation of the nephron by postganglionic sympathetic fibers, this enhanced nerve activity results in increased tubular reabsorption of sodium. That is, more sodium is retained during stress in certain animals. A critical question, and one that will be addressed shortly, is whether this phenomenon of increased sodium retention is found in borderline hypertensive animals. This has important implications concerning the role of this system in the development of hypertension since these animals become frankly hypertensive when subjected to at least two environmental stressors, viz., increased dietary sodium and psychological stress administered in the form of a shock-shock conflict paradigm.

The Role of Potassium and Calcium

The discussion thus far has centered on the role of sodium in hypertension, but it is not the only electrolyte to be considered. Potassium has been recently cited as having therapeutic value in the treatment of essential hypertension (Caggiula, Wing, & Jacob, 1983). One reason for the current interest in potassium can be found by re-examining the epidemiological studies previously referenced. As was mentioned, the tribes that subsisted on diets low in sodium were primarily herbivorous. These diets consisted of large quantities of fruit and grains which are high in potassium content. A more recent interpretation of the data suggesting a lack of hypertension in these

populations puts forth the idea that the high potassium intake, rather than the low sodium diet, is responsible for the lower blood pressures (Langford & Watson, 1975).

The hypotensive effect of increased potassium intake has been studied more closely in several clinical trials. In one such study, 23 patients with mild essential hypertension were given either a one month treatment with potassium tablets or placebo pills (MacGregor, Markandu, Smith, Banks, & Sagnella, 1982). By the end of the one month trial, the average blood pressure had dropped by 4% in the group given potassium. Furthermore, this fall in blood pressure was independent of urinary sodium excretion thus excluding the possibility that the depressor effect was the result of a loss of sodium. These data suggested to the authors that dietary potassium supplementation in conjunction with reduced sodium intake may be a method of treatment for some patients with essential hypertension.

Further evidence for the therapeutic use of potassium is found in another study of 16 hypertensives and eight normotensives (Parfrey, Wright, Goodman, Vandenburg, Holly, Evans, & Ledingham, 1981). This experiment employed a randomized crossover design in which all subjects were given a diet supplemented with 100 mMol sodium for 12 weeks and then switched to a dietary regimen with no added sodium and 100 mMol potassium for another 12 weeks. The results of this study were later reanalyzed and published in a subsequent paper (Holly, Evans, Goodwin, Vandenburg, & Ledingham, 1981). The results reported here are from this latter source.

After 12 weeks on an increased sodium intake diet both groups showed an increase in blood pressure. Also, plasma renin activity and aldosterone levels fell significantly in the normotensive, but not in the hypertensive, group. Moreover, norepinephrine was significantly elevated in the hypertensive group compared to controls. These data, which are reminiscent of Kawasaki et al. (1978) and Fujita et al. (1980), suggest that these subjects are not able to suppress these hormonal systems appropriately in the face of a sodium challenge.

Following 12 weeks on the high potassium diet systolic and diastolic pressure fell in the hypertensive group only. Furthermore, the change in blood pressure was significantly correlated with the fall in plasma norepinephrine. The authors concluded that decreased sodium intake and potassium supplements lower blood pressure in mild hypertensives and that the mechanisms of action for the sodium-induced pressor response may be related to abnormal responses of plasma renin and norepinephrine.

In a related study, Parfrey, Wright, Holly, Evans, Condon, Vandenburg, Goodwin, and Ledingham (1981) studied the effects of modified dietary electrolyte intake on the blood pressure of young adults with and without a familial history of hypertension. Both groups were studied during three different dietary regimens: low sodium, low sodium/high potassium, and high sodium. Baseline data indicated that the subjects with a positive family history for hypertension had higher blood pressures and plasma norepinephrine levels, though only the latter difference was statistically significant.

After four weeks on the high sodium diet, the blood pressure of both groups was higher than while on the low sodium diet. Furthermore, the change in diastolic pressure correlated negatively with the change in plasma renin activity.

The low sodium/high potassium diet produced modest decreases in the blood pressure of the positive family history group. These changes were correlated with changes in plasma norepinephrine. This diet, however, produced a small rise in systolic pressure in the subjects with normotensive parents.

These data suggest that the young men with and without a familial predisposition to hypertension display a modest hypertensive response to increased dietary sodium. It also suggests, however, that these groups respond differently to increased dietary potassium. Specifically, only the subjects with hypertensive parents demonstrated a fall in blood pressure which was correlated with the change in plasma norepinephrine.

A number of animal studies have shown similar hypotensive actions to potassium. For example, Dahl, Leitt, and Heine (1972) demonstrated that increases in potassium intake produced a fall in blood pressure in Dahl S rats. In this experiment, rats were fed a diet containing 4.5% NaCl and varying amounts of potassium supplements. As the level of potassium increased from normal to high, systolic blood pressure dropped dramatically in these animals.

In another study, potassium supplementation seemed to protect rats from two kidney/one clip hypertension (Suzuki, Kondo, & Saruta, 1981).

This surgical manipulation elevated systolic pressures in rats drinking normal water to approximately 180-200 mmHg. Rats drinking .5% potassium chloride, however, displayed pressures only in the 140-150 mmHg range. These animals also had increased urinary sodium excretion and decreased plasma renin activity, both of which are typically observed in animals with this surgery.

Despite the evidence that potassium exerts a hypotensive effect in animals and man, the mechanisms of this action are not clear. It is unknown, for example, whether the decrease in blood pressure is caused by a fall in cardiac output, total peripheral resistance, or both. Furthermore, the hypotensive effects of potassium may be linked with the attendant changes in sodium balance. Young, McCaa, Pan, & Guyton (1976) studied this possibility in normal dogs and adrenalectomized dogs maintained on fixed levels of mineralocorticoids. Increasing potassium intake produced negative sodium balance (i.e., losing more Na that is taken in) in both groups. The adrenalectomized dogs became hypotensive due to greater sodium loss and contraction of extracellular fluid volume as a result of not being able to increase aldosterone secretion in order to conserve sodium. Thus, a loss of sodium and water leading to a reduction in fluid volume and cardiac output may be one method by which potassium can lower blood pressure.

There is also evidence for a local, rather than systemic, site of action for potassium. Overbeck and Clark (1975) infused potassium chloride into the hindlimbs of normotensive, renal hypertensive, and genetically hypertensive rats and measured decreases in vascular

resistance. The data indicated that the drop in resistance in normotensive and SHR rats was greater than in the renal hypertensive subjects. The demonstration of a vasodilatory action by potassium on isolated preparations suggests that one mechanism of action is related to a direct effect on arterial smooth muscle cells. Finally, the importance of calcium must not be overlooked. This cation has long been recognized as critical in the control of muscle contractility and blood pressure regulation at both the molecular and whole organism level. The evidence suggestive of a role for calcium in hypertension can be broken down into two large divisions: calcium deficiency/excess and the inverse relationship between calcium intake and blood pressure.

Many researchers have documented increases in blood pressure in both normotensive and hypertensive rats attendant with long term dietary calcium deprivation (Itokawa, Tanaka, & Fujiwara, 1974; Schleiffer, Pernot, Berthelot, & Gairand, 1984). Unfortunately, clear cut conclusions are precluded due to confounding variables. For example, calcium restriction is accompanied by severe weight loss, growth retardation, and secondary hyperthyroidism. The hyperthyroidism is a result of overactivity of the parathyroid glands in an effort to increase blood calcium levels by increasing bone resorption and renal reabsorption of calcium. The increased level of parathyroid hormone confounds the relationship between calcium and blood pressure because this hormone has been shown to influence vascular resistance and arterial pressure (Rosenthal & Roy, 1972; Blum, Kirsten, & Worth, 1977).

Epidemiological studies have also reported higher blood pressures with calcium deficiency. For example, many researchers have reported that reduction of dairy products increases blood pressure (Reed, McGee, & Yano, 1982; Stanton, Braitman, Rilry, Khoo, & Smith, 1982; McCarron, Morris, & Cole, 1982). However, the strength of the relationship is questioned by the fact that pressures in these studies increased only slightly and never reached hypertensive levels. Unfortunately, as with the epidemiological studies regarding sodium, these experiments have inherent flaws that make the precise nature of the calcium-blood pressure relationship unclear. For example, some studies do not control for the intake of other electrolytes such as sodium and potassium that may influence blood pressure independently of calcium. Also, in some cases restriction of dairy products was part of the treatment for obesity which is correlated with higher blood pressures.

Perhaps more convincing evidence for the hypothesis that calcium intake can influence blood pressure is found in studies showing reductions in blood pressure with increases in dietary calcium. These findings have been noted in both animal and human studies.

One such experiment examined the effect of calcium supplementation in the development of hypertension in SHR (Ayachi, 1979). Results indicate that increased calcium intake significantly attenuated the development of hypertension in this model. It is equally important to note, though, that the final values did not differ between groups. Thus, it appears that calcium merely slows the inevitable development

of severe hypertension in SHR. Also, high dietary calcium lowered blood pressures in WKY.

Similar findings have been reported in humans (Belizan, Villar, Pinella, Gonzalez, Sainz, Garerra, & Sibrian, 1983). In this study normotensive men and women were given dietary calcium supplementation for 22 weeks. The authors reported that blood pressure decreased significantly from baseline values in both sexes. Unfortunately, the data are reported in terms of percentage changes so it is unclear what the actual values were.

While the preceding discussion suggests that calcium supplementation may be a useful antihypertensive treatment, it must be weighed against the evidence that calcium excess can be just as detrimental as calcium deficiency. An understanding of this phenomenon requires an appreciation for the role that calcium plays in the contractile process of muscle tissue. Contraction of muscle, including vascular smooth muscle, is acheived by the interaction of the contractile proteins, actin and myosin. In the relaxed state, these two proteins are separated by a tropin-tropomyosin complex located on the actin. In the presence of calcium, which is released from the sarcoplamsic reticulum of the muscle during a nerve impulse, the tropin-tropomyosin complex shifts positions and creates a binding site for actin and myosin. This binding causes ATP to be hydrolyzed which produces the energy enabling the filaments to slide together, thus creating a contraction. Some researchers have pointed out that even though the factors involved in the pathogenesis of hypertension

(humoral, neural, cardiac, etc.) may vary, the calcium concentration in the muscle represents a common denominator at the cellular level (Johannson, 1981; Webb & Bohr, 1978).

As would be predicted, then, increased extracellular fluid calcium concentrations have been associated with increased peripheral resistance and hypertension (Weidmann, Massry, Coburn, & Maxwell, 1980). The assumption is that the increased calcium increases the contractility of vascular smooth muscle. Giving support to this is the observation that some antihypertensive and vasodilatory agents, such as hydralazine and diazoxide, work in part by reducing cytoplasmic calcium concentrations.

The exact sequence of events involving calcium that would lead to hypertension remains unclear. One theory holds that the key to understanding the problem is related to a defect in membrane calcium regulation that is not secondary to hypertension, but is genetically transmitted (Postnov, 1981). Using this as a cornerstone, a hypothetical model can be constructed incorporating concepts from many researchers (de Wardener & MacGregor, 1983; Haddy & Pamnani, 1984; Bianchi, Cusi, & Guidi, 1983; Blaustein & Hamlyn, 1983). This approach would begin with the suggestion that genetic hypertension is characterized in the prehypertensive state by a renal defect in the ability to excrete sodium. Since water is retained with sodium, intrathoracic blood volume would expand and cellular sodium content would also increase. The next event would be an increase in circulating Na^{+}-K^{+}-ATPase inhibitors which would inhibit the sodium

pump and serve to offset partially the renal defect in sodium excretion by reducing sodium transport. In this way, more sodium would remain in the tubules and be excreted. The relation between this scenario and the development of hypertension has been stated earlier. To recapitulate briefly, Blaustein (1983) believes that the inhibition of sodium pumps is generalizable to cells other than those in the renal system. Thus, sodium pumps in sympathetic neurons would be inhibited resulting in increased intracelluar calcium levels. Since transmitter release is triggered by a rise in intracellular calcium, the increase in circulating catecholamines would be expected to affect vascular smooth muscle contractility. While the intricacies of this hypothesis have yet to be proved, it represents one way that calcium concentrations might be related to elevated blood pressure.

The Borderline Hypertensive Rat

It is clear by now that environmental influences such as psychological stress and dietary manipulations can perturb blood pressure and, in some cases, lead to hypertension. Unfortunately, until recently an animal model has not existed which develops hypertension when challenged with a variety of stressors. For example, the Dahl S rat becomes hypertensive when placed on a diet high in sodium, but psychological stress has little lasting effect on blood pressure. The SHR is an inbred strain of rat that becomes hypertensive regardless of

what is done to it. It is, therefore, not useful in studying the role of environmental factors in the genesis of hypertension. Finally, animals such as the primate, dog, and the normotensive rat do not seem to have the proper genetic predisposition for environmental events to serve as a trigger for the elaboration of high blood pressure.

The borderline hypertensive rat (BHR) is a backcross of WKY and SHR and has a resting SBP in the range of 140-160 mmHg. This mixed genetic history of the BHR appears to provide an appropriate predisposition for the elaboration of a hypertensive response to environmental stressors. One challenge that has been shown to produce hypertension and vascular pathology in the BHR is chronic exposure to a conflict paradigm (Lawler, Barker, Hubbard, & Schaub, 1980). In this study, animals were trained to turn a wheel at the presentation of a tone to avoid five tail shocks. Following this three week signaled avoidance training period, the rules were changed such that failing to respond still resulted in five tail shocks, but turning the wheel resulted in one tail shock. Thus, the subject must "choose" whether to receive one shock or five. Such a paradigm produced elevated SBP (188 mmHg) after 12 weeks and was maintained for a 10 week shock-free recovery period.

It was reasoned that the viability of the BHR as a model of essential hypertension would be enhanced if it could be shown to be sensitive to other environmental stressors that influence the human condition. A logical next step would be to challenge the BHR with a diet high in sodium. These studies have shown that when the BHR is

given a diet containing 8% NaCl for 2-3 weeks, SBP rises to values comparable to those observed during conflict stress (Sanders, Cox, & Lawler, 1985; Lawler, Sanders, Chen, Nagahama, & Oparil, 1985). It was also observed that a high NaCl diet appears to blunt the pressor response to acute stress in the BHR (Sanders et. al., 1985). That is, BHR on a high NaCl diet were significantly less reactive to acute stress, as measured by change scores from home cage rest, than BHR on a normal diet. Interestingly, Lawler et. al. (1985) have shown that a diet high in sodium reduces plasma and urinary epinephrine and dopamine levels in the BHR. This suggestion of reduced sympathetic activity may be responsible, in part, for the reactivity data described above and may represent a physiological attempt to downregulate the system as a compensatory measure.

Rationale

The above discussion indicates that the BHR develops hypertension in response to at least two environmental factors that have been identified as being important in understanding human essential hypertension, viz., psychological stress and increased dietary sodium. The experiment outlined on the following pages proposes to explore these relationships further using the BHR and WKY. Since stressful paradigms can lead to sodium retention in the SHR, a parent of the BHR, and since sodium retention has been associated with the development of hypertension, the comparison of renal excretory responses of the BHR during rest and stress is of special interest. Furthermore, since the BHR develops hypertension when exposed to either increased dietary sodium or stress, the analysis of blood pressure and renal responses to these stressors may provide information regarding possible mechanisms involved in the hypertensive response. The purpose, then, of the present study is to examine and compare the renal and blood pressure responses during control and aversive classical conditioning sessions in the BHR and WKY while on either a normal or high sodium diet.

The primary cardiovascular dependent variables to be studied are systolic, diastolic, and mean blood pressure, and heart rate. With respect to the renal system, urinary volume, sodium excretion, glomerular filtration rate (GFR) and renal plasma flow (RPF) are of interest. Urinary sodium excretion is important because of the recent evidence suggesting that stressful paradigms result in sodium retention

in some animals and humans. GFR and RPF are valuable measurements because the amount of sodium excreted depends, to a large extent, on the amount of filtrate formed by the kidneys. This, in turn, is contingent upon the amount of plasma filtered and the rate at which it is filtered. It can be seen that alterations in blood pressure and sympathetic nervous system activity, by virtue of their ability to constrict or dilate arterioles, will have important implications for the blood flow to the kidneys and rate of filtration. Finally, it is important to recognize that the kidneys have the ability to autoregulate blood flow within normal physiological limits. Thus, blood pressure and nerve activity, must change somewhat dramatically before it will have a substantive effect on GFR and RPF. For the present study, it can be seen that in order for any statements to be made regarding sodium retention and hypertension, it is important to be able to place it in the context of observed changes in GFR and RPF.

The questions to be addressed are enumerated below and are designed not only to learn more about the interactions between a genetic predisposition to hypertension, stress, and sodium intake, but also to add to the body of basic physiological knowledge concerning the BHR. The specific points to be addressed by this study are:

1. To what extent does a high sodium diet influence the blood pressure of WKY and BHR? Does this diet affect their reactivity to acute stress?

2. Is there an alteration in the morphology of the conditioned response of the BHR and WKY while on a high sodium diet as compared to a normal sodium diet?
3. What is the glomerular filtration rate (GFR), renal plasma flow (RPF), and urinary sodium excretion of the BHR at rest?
4. What influence does a high sodium diet have on GFR and RPF in the BHR as compared to the WKY?
5. What happens to GFR, RPF, and urinary sodium excretion during behavioral stress? Is there a difference between dietary groups? Between strains?
6. To what extent, if any, does a sodium challenge alter catecholamine levels in the BHR and WKY?

CHAPTER II

METHODS AND MATERIALS

Subjects

The subjects in this study, 20 male borderline hypertensive (BHR) and 20 male Wistar-Kyoto (WKY) rats, were bred in the animal facility from female spontaneously hypertensive (SHR) and WKY male and female rats purchased from Taconic Farms (Germantown, NY). Tail cuff systolic blood pressures (Narco Bio-systems) were used to verify that SHR and WKY parent rats had the appropriate blood pressures for their respective strains. Females were group housed according to strain for two weeks to synchronize estrous. Following this period, one male was placed with two females, one from each strain, for two weeks. Females were then housed individually and males were housed 2 per cage.

All pups were weaned at 4 weeks of age, and males were housed in triplets according to strain until they were 7 weeks old, at which time they were housed individually for the duration of the study.

Apparatus

Home Cages

All animals were individually housed in standard plastic

laboratory cages measuring 16 cm x 6 cm x 9 cm. They were housed in a climate controlled animal facility and were on a 12 hour light/dark cycle.

Metabolic Cages

The stainless steel metabolic cages used in this study measured 27 cm x 23 cm x 17 cm. A small wire screen located in the urine funnel underneath each cage prevented food and fecal matter from falling into a collection vial. Food and water were available to the animal through a small metal attachment at the back of the cage.

Conditioning Cages

Classical conditioning and recording sessions were carried out while the animal was placed in a plexiglass conditioning box measuring 7.0 cm x 28.0 cm x 8.5 cm. The subjects were secured in the boxes by slipping a wooden disc (.9 cm i.d. x 1.9 cm o.d.) over the tail and taping it to the box. Sound attenuating chambers, containing a house light, speaker, and ventilation fan, will accommodate 2 conditioning boxes. A 2 m x 3 m x 2.5 m room in the laboratory is large enough to house 4 of these chambers.

Computer Systems

The onset and duration of tones (CS) and shocks (UCS) used in the classical conditioning paradigm were presented by a KIM-1 microprocessor (MOS Technology) with custom interfacing (Murray & Lawler, 1977) which was controlled by a SYM-1 microprocessor (Synertek Systems Corp.). Computer software and hardware involved in data acquisition and analysis will be described in a subsequent section.

Blood Pressure Monitoring Equipment

Tail plethysmography was used to determine indirect systolic blood pressures for all parent and experimental subjects. The tail artery was dilated by placing the subject in a plexiglass restraint (Narco Bio-Systems) which forced the animal to lie on a heating plate which was maintained at 37° C. The pressure signal was detected by placing the tail through an inflatable cuff which, when expanded, occluded blood flow. As the cuff was deflated, a Narco pneumatic pulse transducer, also attached to the tail, detected the pulse wave. The signal was then sent into a Narco Bio-Systems electrophysiograph coupler (Model 7211) and amplifier (Model 7070) housed in a 4 channel Narco physiograph (Model PMP-4A).

All animals had femoral artery catheters implanted for the direct measurement of systolic and diastolic blood pressure and heart rate. The catheter was attached to a Narco Bio-Systems RP 1500 pressure

transducer which was connected to a Grass low-level D.C. preamplifier (Model 7P1F) and a driver amplifier (Model 7DAF) in a Grass 8 channel physiograph (Model 7D).

The blood pressure signal was collected and stored by the SYM-1 microprocessor. After the recording session the data were transferred to an IBM PC, stored on a floppy diskette, and printed on a dot matrix printer (Epson FX-80). Computer software developed in the laboratory was used to analyze specific time intervals of interest.

Renal Function Assessment Equipment

Glomerular filtration rate and renal blood flow were determined from urine and plasma concentrations of inulin and para-aminohippuric acid (PAH), respectively. The inulin and PAH solution was infused into the subject via a femoral venous catheter using a Harvard Apparatus infusion/withdrawal pump (Model 940). Urine was collected into small vials located below the cage through a chronically implanted bladder catheter. Inulin and PAH concentrations were determined using a spectrophotometer (Bausch and Lomb, Spectronic 20). Urinary electrolyte analyses were done with an ion-specific analyzer (Orion Research, Model 1020) and urine volume was measured gravimetrically. Detailed procedures concerning the surgical techniques and measurement protocols are discussed in later sections and in Appendices B and C.

Procedure

At 7 and 8 weeks of age systolic blood pressures were determined for all rats in each strain using tail plethysmography. This insured that values of all animals were in an acceptable range for that strain and that no group differences, other than strain, existed prior to treatment. The subjects were also placed in metabolic cages for a 48 hour period for baseline urine collections which were used for catecholamine and electrolyte analyses. The first 24 hour period was an adaptation period for the animals and the second 24 hours was the collection period. The protocol for this procedure is detailed in Appendix A.

Following the metabolic studies, one group in each strain began receiving a specially prepared rat chow containing 8% sodium chloride (NaCl) purchased from ICN Nutritional Biochemicals (Cleveland, OH). The other group in each strain was maintained on normal Purina rat chow containing .3% NaCl. This yielded a total of 4 groups in the study: BHR-8% NaCl, WKY-8% NaCl, BHR-.3% NaCl, WKY-.3% NaCl. All animals remained on their respective diets for approximately 7-8 weeks during which time they had free access to food and water. Indirect weekly systolic blood pressures were monitored for all subjects during this period.

Classical Conditioning

The last week of the dietary regimen was also the period during which the subjects were conditioned on a classical aversive conditioning paradigm. All animals were exposed to 40 trials, each 90 seconds in duration, for 5 consecutive days. For the conditioning session, the animals were placed in the plexiglass boxes previously described. These boxes were placed in the sound attenuating room for the session.

The presentation of the CS and UCS was controlled by a KIM-1 microprocessor which was housed in a small room adjacent to the conditioning room. The CS was a 2000 Hz, 75 db tone and was presented 14 seconds before the delivery of a .3-.4 mA shock (UCS), 1 second in duration, to the subject through two cutaneous tail shock electrodes, each 2 cm in area. The CS was on during the presentation of the UCS and both ended simultaneously after the fifteenth second following CS onset. A fixed 90 second interstimulus interval was used.

During the last 2 days of the conditioning period, metabolic studies were again conducted. The protocol employed here was the same as that referred to earlier and is described in Appendix A.

Surgical Procedure

Following the last conditioning session, all subjects were anesthetized with anhydrous ether and the femoral artery and vein and

the bladder were catheterized. The arterial catheter was made out of a 20 cm piece of PE-60 attached to a 2 cm section of teflon tubing (.012 mm, i.d.). The teflon tubing was slipped into the PE-60 and household cement was placed around the junction to seal the catheters together. The venous catheter was made out of a 22 cm piece of PE-50.

The procedure for cannulation of the artery and vein is virtually identical. Each vessel was visualized and isolated from any adjoining vessels and nerves through an incision made in the ventral surface of the left hindlimb. It was then tied off distally (3-0 silk) and a loosely looped ligature was placed around the vessel to serve as an occluder during placement of the catheter and to tie the latter down once it was in place. Once the catheter was introduced into the vessel and secured, it was anchored to adjacent muscle (5-0 silk), tunneled up the back of the animal, and exited out the nape of the neck. All sutures utilized 3-0 silk. The catheter was protected by placing the animal in a custom-made rat jacket. Finally, the catheter was plugged with a steel obturator and flushed daily with .3-.5 ml of heparinized saline (330 units/cc) to maintain patency.

The bladder catheter was modified from the design of Gellai and Valtin (1979). The catheter was 4 cm long and made out of Silastic medical tubing (.078 mm i.d.x.125 mm o.d) attached to a 12 gauge stainless steel hypodermic needle. The bladder was visualized through a suprapubic incision and any urine was aspirated out with a syringe. A small incision was then made in the bladder and a purse string suture with 5-0 silk was made. The phlanged end of the catheter was

introduced into the bladder. The bladder tissue was secured around the catheter by pulling the purse string. Another ligature around the catheter was used to secure it to the muscle and skin. The stainless steel portion of the catheter was plugged with an obturator to prevent clogging and infection. All animals were treated with a 60,000 units of procaine penicillin (300,000 units/cc). This was administered intramuscularly one time following surgery and as needed at the sign of any infection.

Cardiovascular Recording

Control period. Following a 3 day recovery period, direct blood pressure measurements were obtained in conscious subjects. This recording was done in the plexiglass conditioning boxes after the subject had habituated to the environment. It was necessary to use this apparatus rather than to have the animals freely moving because urine collections for renal function tests were done at the same time. All animals were placed in the plexiglass box for 30 minutes on each of the five days prior to the recording session. The infusion pump was running during this time so that the animals became acclimated to both the box and the noise of the pump. Finally, a 30 to 60 minute habituation period preceded each recording session.

Prior to placement of the rat in the recording box, body weight and a hematocrit were determined in order to verify the health of the subject. Hematocrit levels of 30-45 and body weights within 10-20

grams of pre-surgical levels were considered acceptable.

Direct measurements of systolic and diastolic blood pressure and heart rate were obtained by attaching the arterial catheter to a pressure transducer connected to a Grass physiograph (see Blood Pressure Recording Equipment). Data were collected on line for 60 minutes by an IBM PC.

At the end of the recording session, a .5 ml blood sample was obtained from the arterial catheter and collected in a cold microfuge tube containing 2.7 mg of EGTA and 1.7 mg of glutathione to prevent coagulation. After the sample was spun down at 4° C, an aliquot of plasma was extracted and frozen for future radioenzymatic analysis of catecholamines.

Stress period. Data collection during this session was executed by placing the animal in the plexiglass box described previously. The arterial catheter was exited through a hole in the top of the box and attached to the pressure transducer mounted on the side of the box. The CS and UCS were presented by the KIM-1 microprocessor as previously described (see Classical conditioning). A .5 ml blood sample was obtained at the end of the 60 minute session using the procedure described above.

Renal Function Assessment

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined using inulin and para-aminohippuric acid (PAH),

respectively. Inulin is a polysaccharide that has the property of not being reabsorbed by the tubules of the nephron. However, since it has a molecular weight of only 5400, it is small enough to pass through the glomerular membrane. Furthermore, inulin is not actively secreted by the tubules. These attributes of inulin mean that the glomerular filtrate contains the same concentration of inulin as does the plasma, and as the filtrate flows down the tubules all of the inulin ends up in the urine. That is, all of the glomerular filtrate formed is cleared of inulin, and this is equal to the amount of plasma cleared. Therefore, plasma clearance per minute of inulin is equal to the GFR.

PAH is similar to inulin in that it passes freely through the glomerular membrane. An important difference, however, is that most of the PAH is secreted by the tubular cells into the tubules. This characteristic allows the clearance of PAH to be used as an estimate of renal plasma flow.

GFR is estimated by the equation $C_{in} = V \times U_{in}/P_{pah}$, and RPF by the equation $C_{pah} = V \times U_{pah}/ P_{pah}$ where C=clearance period, V=volume, U=urine, P=plasma, in=inulin, pah=para-aminohippuric acid. Both are expressed in ml/min.

These data were collected concurrently with the cardiovascular data during the rest and stress periods. The stainless steel portion of the bladder catheter was extended through an opening in the bottom of the plexiglass box and a small piece of Silastic tubing was attached which channeled the urine into the collection vial. A solution of 10% inulin and 1% PAH was prepared by dissolving the substances in warm

deionized water and stirring. At the beginning of the sessions the rats were given a priming infusion via the venous catheter of .2 ml/minute for 10 minutes. A sustaining infusion of 10 ul/minute/100 grams of body weight was then begun. After allowing 30-60 minutes for stabilization of urine flow rate as well as plasma inulin and PAH concentrations, the clearance periods were initated. Each clearance period was 20 minutes long, so three samples were taken in the hour session. A blood sample (.2-.3 ml) was drawn from the arterial catheter at the midpoint of each clearance period and urine samples were collected in small vials placed under the conditioning box at the end of the clearance periods. Inulin and PAH concentrations were determined using a spectrophotometer and followed the protocols described in Appendices B and C.

Assay Procedures

The technique which was utilized for the determination of plasma catecholamine levels employed a single isotope radioenzymatic-thin layer chromatographic procedure (Cryer, Santiago, & Shah, 1974) and a modification by Upjohn diagnostics (Kalamazoo, MI). The catecholamines were converted to their O-methylated derivatives by incubating the samples at 37°C for 60 minutes with [^{3}H]-S-adenosyl-L-methionine (New England Nuclear, Boston, MA) and catechol-O-methyl transferase, extracted from rat liver. The reaction was stopped at the end of this period by placing the samples in ice water and adding 50 ul of 1 M

borate buffer, pH 11.1, containing .2 umole of normetanephrine and metanephrine, to each sample tube. The O-methylated tritium labeled products were extracted with 2 ml of toluene-isoamyl alcohol (3:2, v/v) taken up into 100 ul of .1 N acetic acid, and extracted a second time with 1 ml of toluene-isoamyl alcohol (3:2, v/v). The tritiated metanephrines and normetanephrines, corresponding to epinephrine and norepinephrine, respectively, were separated by thin layer chromatography. The solvent system for this procedure consisted of tert-amyl alcohol, benzene, and methylamine (6:2:3, v/v). The areas of the silica plate corresponding to [^{3}H]-metanephrine and [^{3}H]-normetanephrine were visualized under ultraviolet light, scraped in to 20 ml scintillation vials, and oxidized to [^{3}]-vanillin by the addition of $NaIO_4$. The samples were then radioassayed using liquid scintillation spectrometry. Appendix D outlines this protocol.

Inulin and PAH concentrations were determined using a colormetric assay. In the case of inulin, .5 ml of deproteinated plasma or diluted urine was mixed with 2.5 ml of anthrone-acid reagent. The tubes were vortexed and placed in a water bath (75° C) for five minutes and then placed in an ice water bath for five minutes. The tubes were read at 620 um after reaching room temperature. The PAH concentration was determined by mixing 175 ul of deproteinated plasma or 2 ml of diluted urine with the reagents. The tubes were read at 520 um after five minutes. Complete protocols for these assay techniques are found in Appendices B and C.

Termination of Subjects

At the end of data collection, all animals were decapitated and their brains were removed for analysis of central catecholamines. This was not part of the present study, but these data will be used as pilot data for another study.

CHAPTER III

RESULTS

Data are discussed in two major sections: cardiovascular (systolic blood pressure, diastolic blood pressure, mean pressure, and heart rate) and renal (urinary sodium excretion, urinary volume, GFR, and RPF). Cardiovascular data were collected for one hour during both the control period and stress session. The data from the one hour control period were collapsed into means for the first, second, and third 20 minutes. The lowest value of these three means was used as that subject's control value. Data from control and stress sessions were analyzed using both raw data and change scores. Statistical analysis of cardiovascular data utilized repeated measures analyses of variance and Tukey HSD post-hoc tests. There were two between factors (strain and diet) and two within factors (seconds and trial blocks) in the analysis. Strain consisted of BHR and WKY, diet contained data for normal and high sodium animals, seconds consisted of 15 values (the first of which was the average for the tone off period and the remainder of which utilized the 14 seconds of the tone on period) and trial blocks contained eight levels (each representing the response of five consecutive trials).

A second set of analyses utilized change scores which were calculated two ways. In the first instance, the raw values from each animal for each second utilized in the above analyses were subtracted

from a control period baseline, which was defined as the lowest 20 minute value in a 60 minute recording session. The second change score was calculated by substracting each second of the tone on period from the pre-CS mean for each animal. These change scores were subjected to the same repeated measures analyses of variance as above.

The renal data were acquired from a 20 minute collection period in the control and stress sessions. Although urine and plasma were collected every 20 minutes during the one hour session, there was typically only one clearance period for which a complete analysis could be done. Thus, the renal fucntion data represent only one 20 minute period for each subject. Statistical analyses of these variables employed two way analyses of variance and Tukey HSD tests. In these analyses, there were three between factors: strain, diet, and condition. Strain consisted of WKY and BHR subjects, diet contained data for normal and high sodium animals, and condition consisted of data collected during the control and stress periods.

The data presented below were collected from four experimental groups: WKY, normal sodium (n=7); WKY, high sodium (n=6); BHR, normal sodium (n=6); and BHR, high sodium (n=6). Unless otherwise indicated, these sample sizes apply to all analyses. The level of significance for all analyses, unless otherwise stated, was $p < .05$.

Cardiovascular Data

Control Period

Systolic blood pressure. Table 1 depicts the cardiovascular data collected during the control period for BHR and WKY animals on the two diets. It is apparent that the sodium diet had a profound effect on the BHR but little or no effect on the WKY. This was confirmed by a two way analysis of variance on each dependent variable.

Figure 1 shows control systolic and diastolic blood pressure for all groups. A two way analysis of variance on the systolic blood pressure data revealed a significant main effect of strain [$F(1/19) = 24.72$], diet [$F(1/19) = 3.39$], and a strain by diet interaction [$F(1/19) = 4.42$]. Subsequent Tukey HSD tests, with a critical difference (CD) of 24 indicated that BHR on a high sodium diet had significantly higher (187 mmHg vs 162 mmHg) systolic blood pressure than BHR on a normal diet. There was no difference between dietary groups in the WKY strain.

Diastolic blood pressure. These data were subjected to a two way analysis of variance which revealed a significant main effect of strain [$F(1/19) = 10.28$] and diet [$F(1/19) = 4.72$] and a marginally significant (p .10) interaction [$F(1/19) = 2.90$]. Tukey HSD comparisons (CD = 23) indicated that BHR on a high sodium diet had significantly higher (129 mmHg vs 105 mmHg) diastolic pressure than BHR

Table 1. Control cardiovascular data for BHR and WKY on normal and high sodium diets. Values are means ± SEM.

	BHR (n=12)		WKY (n=11)	
	Normal Na+	High Na+	Normal Na+	High Na+
SBP(mmHg)	161±2.5	186±2.6	144±2.3	143±1.6
DBP(mmHg)	105±2.4	129±2.1	96±2.5	99±1.6
MBP(mmHg)	124±2.4	147±2.2	112±2.4	114±1.6
HR(bpm)	437±5.2	390±4.5	423±5.1	458±7.2

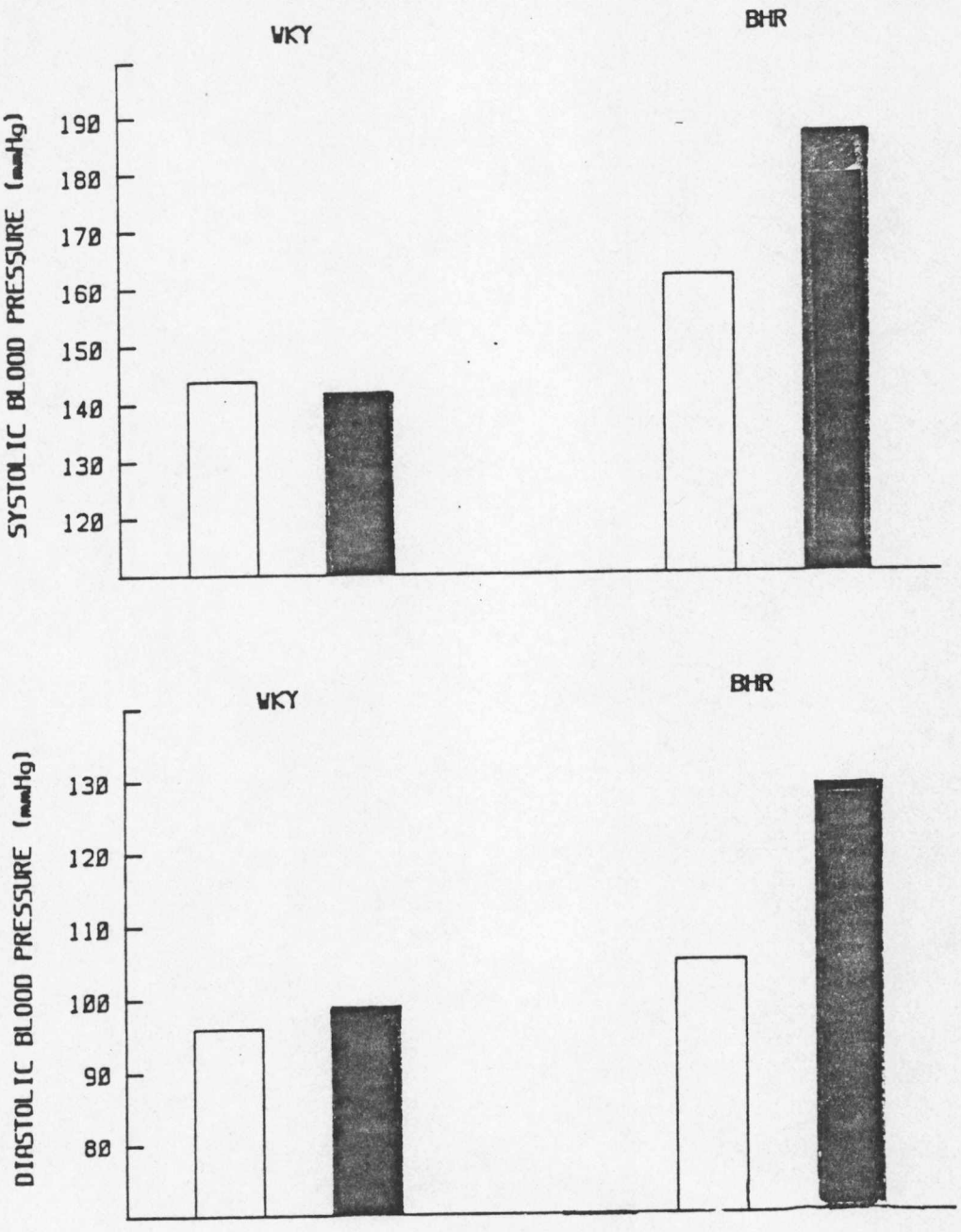

Figure 1. Control systolic (top) and diastolic (bottom) blood pressure for BHR and WKY on normal (open bars) high (shaded bars) sodium diets.

on a normal diet. Again, no differences were found with respect to WKY subjects.

Mean pressure. As shown in Figure 2, the high sodium diet also had a dramatic effect on the mean pressure in the BHR strain. A two way analysis of variance on these data revealed a significant main effect of strain [F(1/19) = 14.48] and diet [F(1/19) = 4.32], and a marginally significant (p .08) strain by diet interaction [F(1/19) = 3.22]. Tukey comparisons (CD = 23) indicated that BHR on a high sodium diet had significantly higher (147 mmHg vs 124 mmHg) mean pressure than BHR on a normal diet.

Heart rate. These data from the control period are also shown in Figure 2. A two way analysis of variance on this variable revealed no significant or marginal main effects or interactions.

In summary, the results presented above indicate that a high sodium diet significantly increases systolic, diastolic, and mean pressure in the BHR strain, while the same diet has no significant effect on these variables in the WKY subjects.

Stress Period

Systolic blood pressure. Although the main analysis of variance for systolic blood pressure did not reveal a quadruple interaction of strain by diet by trial blocks by seconds, this analysis indicated a significant main effect of strain [F(1/20) = 11.84] and trial blocks

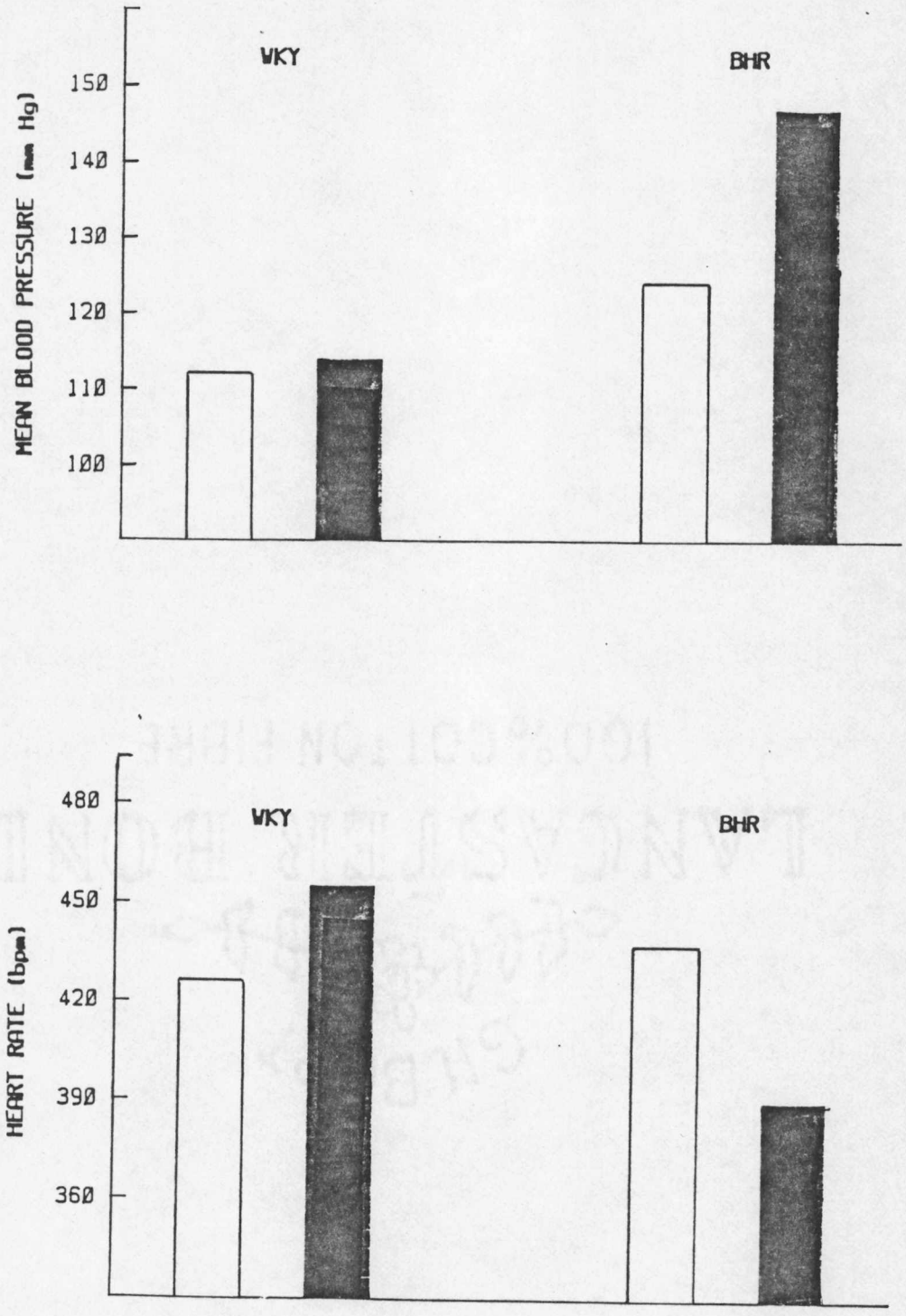

Figure 2. Control mean blood pressure (top) and heart rate (bottom) for BHR and WKY on normal (open bars) and high (shaded bars) sodium diets.

[F(7/140) = 3.00] and a significant triple interaction of trial blocks by strain by diet [F(7/140) = 2.15].

This interaction is depicted in Figure 3 and suggests a divergent response pattern of systolic blood pressure across trial blocks in the WKY strain. An analysis of variance on the WKY data (excluding the control period) revealed a trial blocks by diet interaction [F(7/77) = 1.58]; no interaction was seen in the BHR data. The source of the interaction is further clarified by Tukey HSD tests (CD = 5.2) which revealed that WKY subjects on a high sodium diet had significantly higher systolic blood pressures at trial blocks five through eight compared to WKY on a normal sodium diet. In addition, a one way analysis of variance on the WKY normal sodium group revealed a significant main effect of trial blocks [F(7/42 = 4.18]. Subsequent Tukey comparisons (CD = 5.6) indicated that trial blocks three through eight were significantly lower than trial block one. Thus, it appears that WKY on a normal sodium diet adapted to the stress session across trial blocks while WKY on a high sodium diet maintained the same pressure throughout the measurement period. Both groups of BHR, on the other hand, responded similarly across trial blocks.

The adaptation of systolic blood pressure across trial blocks was investigated in another analysis. A two way analysis of variance with repeated measures across trial blocks was performed on trial blocks one and eight. The analysis of these two trial blocks was carried out on systolic and diastolic blood pressure and heart rate in light of recent studies in our laboratory which suggested that SHR and WKY differ in

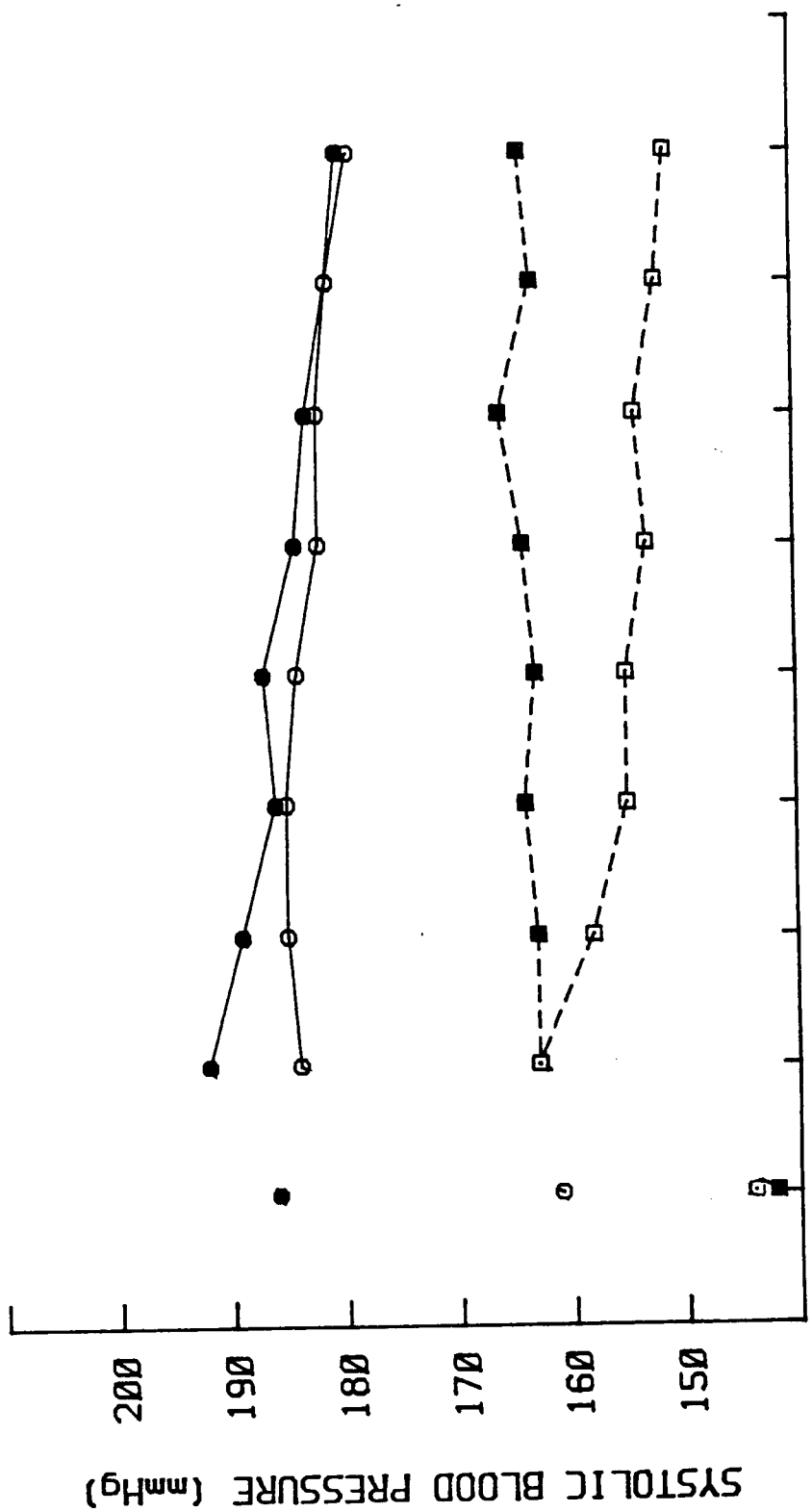

Figure 3. Systolic blood pressure for the control period (C) and each trial block of stress (1-8) for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (shaded symbols) sodium diets.

their blood pressure responses when the early and late phases of the stress session are compared. This analysis revealed a significant main effect of strain [F(1/20) = 4.26] and trial blocks [F(1/20) = 6.18], and a significant triple interaction of trial blocks by strain by diet [F(1/20) = 4.26]. Referring again to Figure 3, it can be seen that the dietary groups in the WKY strain differ in their systolic blood pressure responses at trial block eight compared to trial block one. This was confirmed by an analysis of variance which indicated a significant interaction of trial blocks and diet [F(1/11) = 7.10] in the WKY strain. Follow-up Tukey HSD tests (CD = 7.8) revealed that while there is no difference between WKY groups at trial block one, WKY on a normal sodium diet had significantly lower systolic blood pressure at trial block eight (151 mmHg vs 163 mmHg) compared to the high sodium group.

Figure 4 illustrates the second by second systolic blood pressure data for the pre-CS mean and the 14 seconds collapsed across all trial blocks of the tone on period. That the BHR are consistently higher than WKY subjects throughout the 15 second period was verified by a two way analysis of variance with repeated measures across seconds which revealed a significant main effect of strain [F(1/21) = 12.55]. There were no other significant main effects or interactions. Although BHR are higher than WKY throughout the CS period, it can be seen that diet had no measurable influence on the magnitude or duration of the conditioned response. The degree of change from the pre-CS period will be discussed shortly.

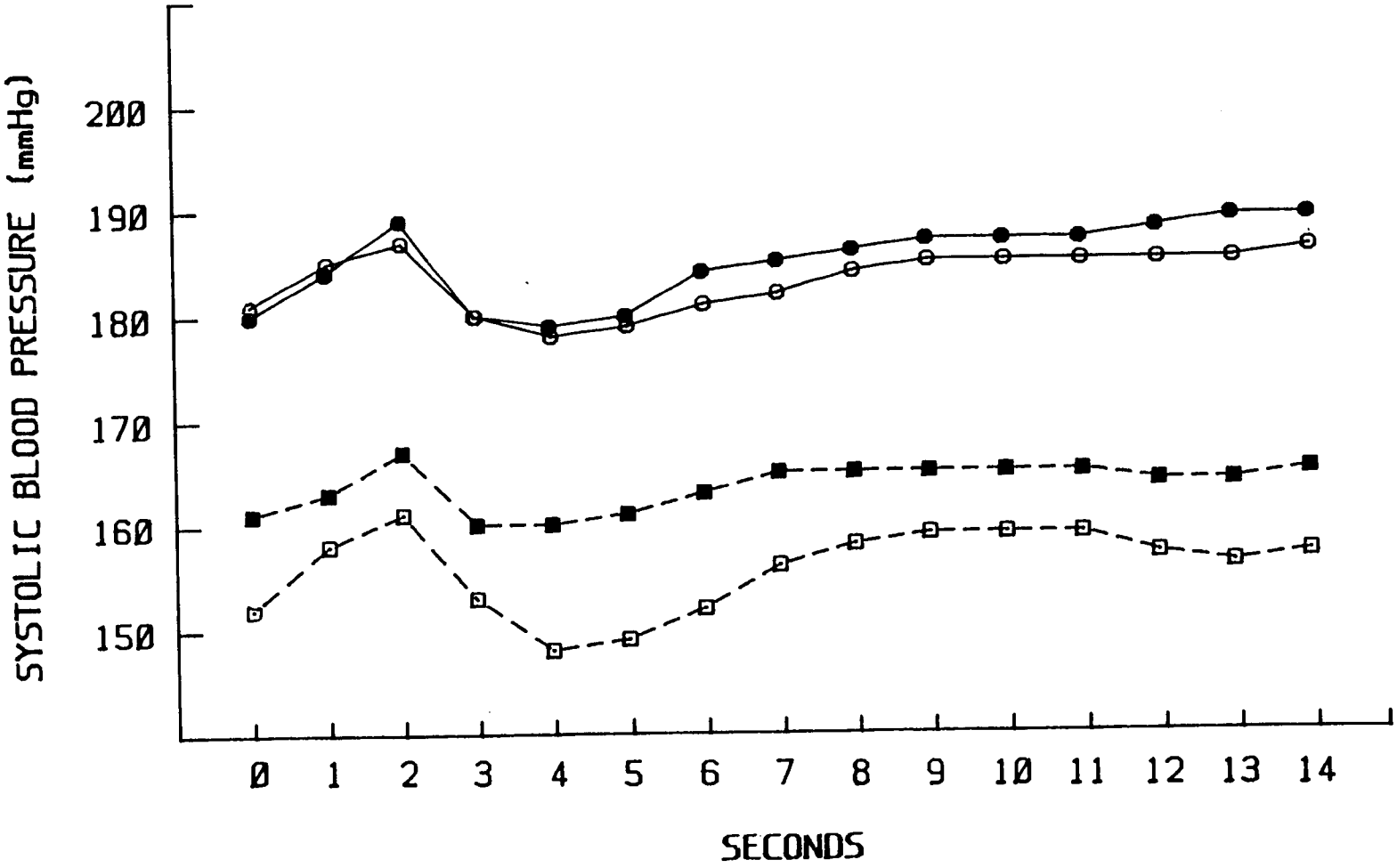

Figure 4. Second by second systolic blood pressure for BHR (solid lines) and WKY (dashed lines) on normal (filled symbols) and high (open symbols) sodium diets. Second 0 is the pre-CS mean; seconds 1-14 represent the CS period.

The reactivity of systolic blood pressure to stress was assessed using change scores which were calculated by subtracting the mean of the stress session from the control value for each subject which had complete data. For those subjects that had missing data, the mean of that subject's group was used to fill in the missing cell. These scores, which are shown in Figure 5, clearly indicate a difference in the responsiveness between dietary groups in the BHR strain. A two way analysis of variance (between-subjects) on these data revealed a significant strain by diet interaction [$F(1/21) = 5.62$]. Tukey HSD tests (CD = 19) showed that BHR on a high sodium diet had significantly lower change scores compared to both BHR on a normal diet (-2 mmHg vs 18 mmHg) and WKY on a high sodium diet (-2 mmHg vs 19 mmHg). Thus, a high sodium diet makes the BHR much less reactive to stress, while the same diet does not decrease the reactivity of WKY. In fact, there is a tendency for a high sodium diet to increase the reactivity of WKY.

Since a few of the subjects utilized in the control period were unable to be used in the stress session, due to technical problems, these subjects were replaced in order to keep the population of the groups at an acceptable level. Although an analysis of variance with repeated measures was precluded by this substitution of some subjects, the effect of diet on reactivity between groups across trial blocks is clearly seen in Figure 6. Of particular interest are the curves showing the differential effect of a high sodium diet on the reactivity of the BHR and WKY strains. That is, it appears that a high sodium

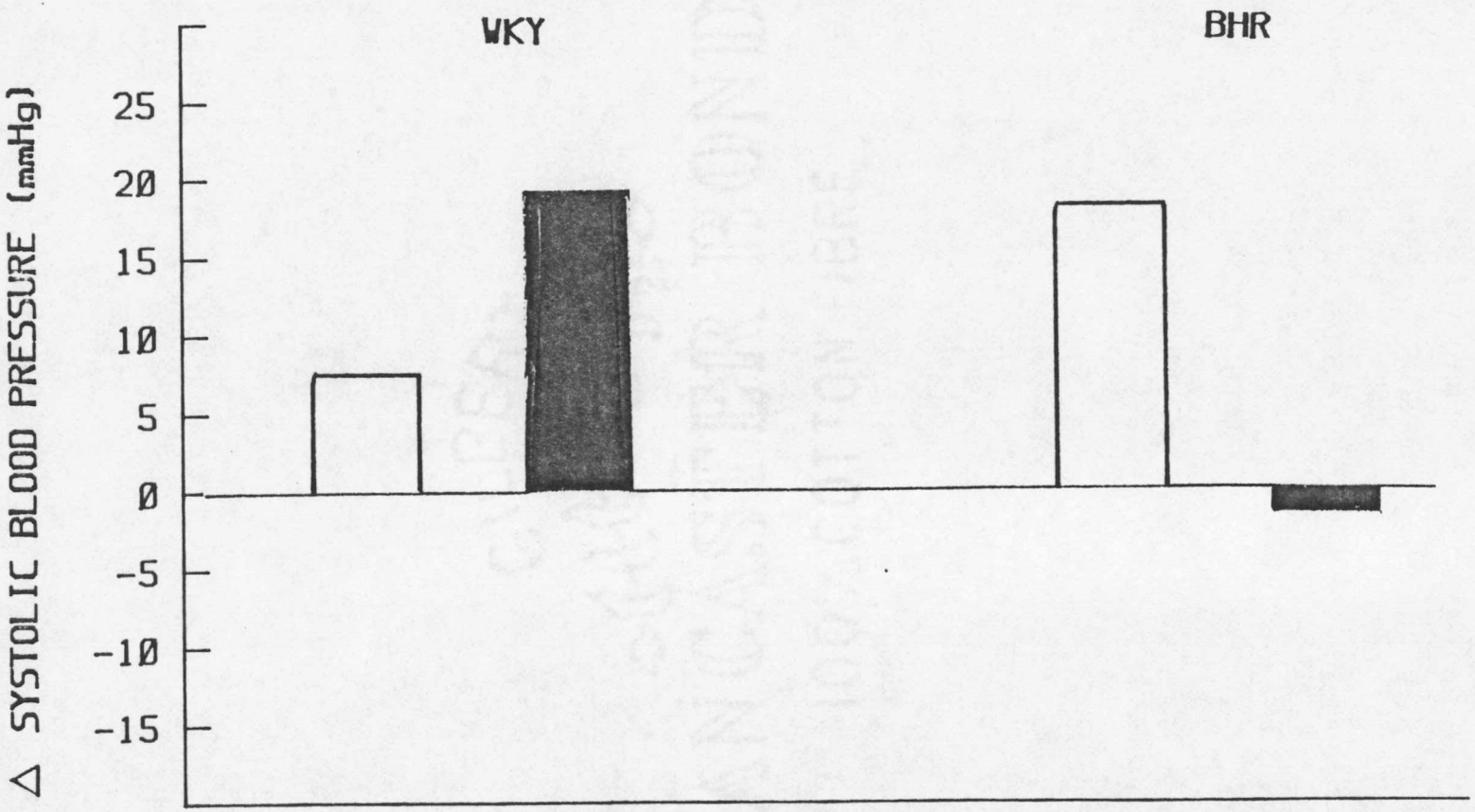

Figure 5. Systolic blood pressure change scores (stress session-control period) for BHR and WKY on normal (open bars) and high (shaded bars) sodium diets.

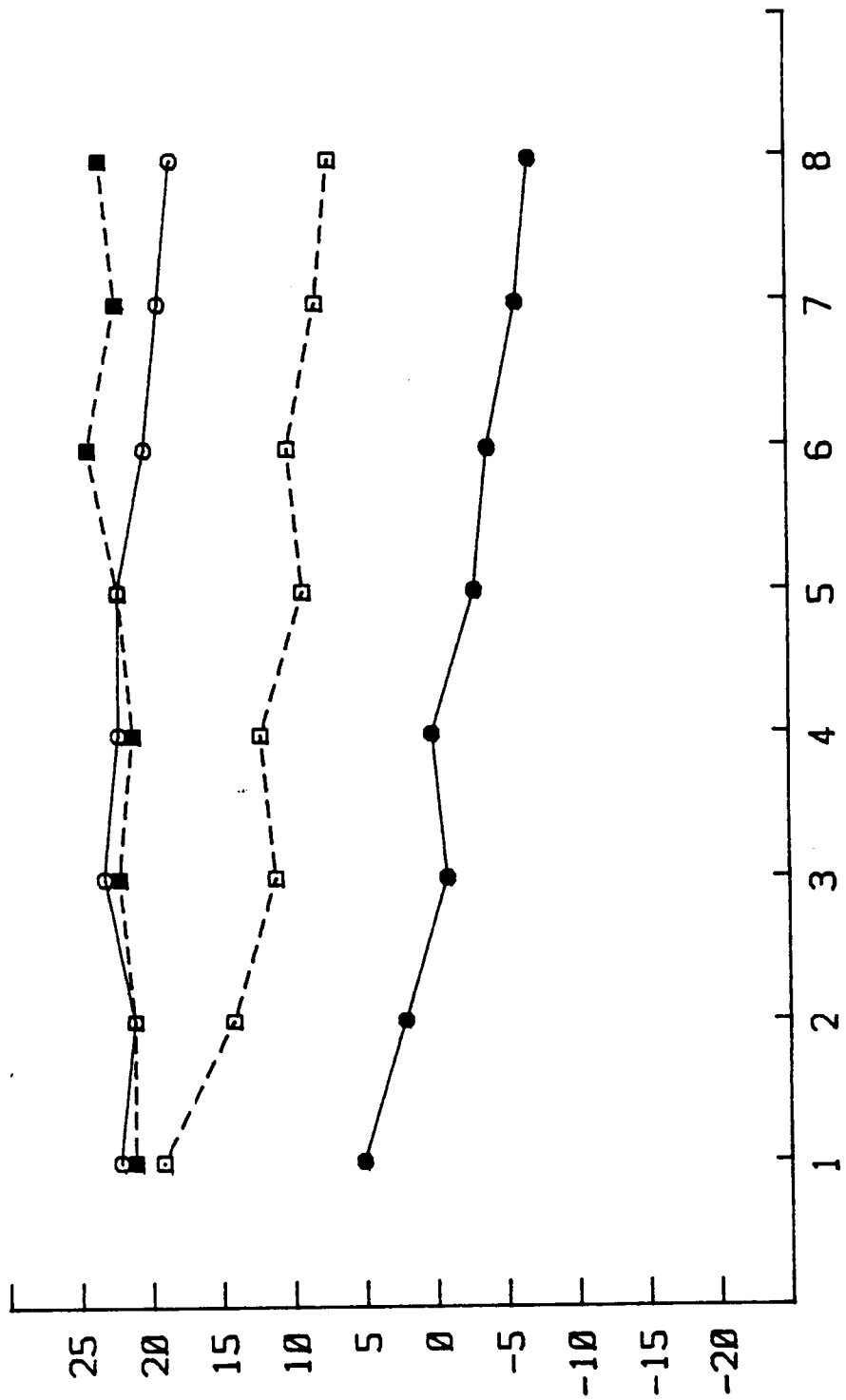

Figure 6. Systolic blood pressure change scores (each trial block - control period) across trial blocks for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets.

diet dampens the reactivity of BHR throughout the stress session, while WKY on this diet are as reactive as BHR on a normal diet. Finally, Figure 7 depicts the magnitude of change from the pre-CS mean to each second of the CS period. While the morphology of the curves is very similar and not significantly different, it appears that there is a tendency for WKY on a normal sodium diet to be the most reactive while a high sodium diet makes this strain least reactive. These data suggest that while a high sodium diet influences the reactivity of WKY and BHR to the stress session compared to the control period, this treatment has little effect on the systolic blood pressure response to the CS during the stress session.

Diastolic blood pressure. With respect to diastolic blood pressure, a two way analysis of variance with repeated measures revealed a main effect of seconds [$F(14/266) = 15.02$] and a quadruple interaction of strain by diet by trial blocks by seconds [$F(98/1862) = 1.32$]. Since a quadruple interaction in which two of the factors are dimensions of time cannot be depicted with one graph, the clearest and most meaningful presentation of these complex relationships is seen in two figures, each of which illustrates a significant triple interaction.

Figure 8 shows diastolic blood pressure for all groups during the control period and across trial blocks. An analysis of variance on the trial block data revealed a significant triple interaction of strain by diet by trial blocks [$F(7/133) = 2.65$]. Inspection of this figure suggests two possible ways this interaction may occur. The first

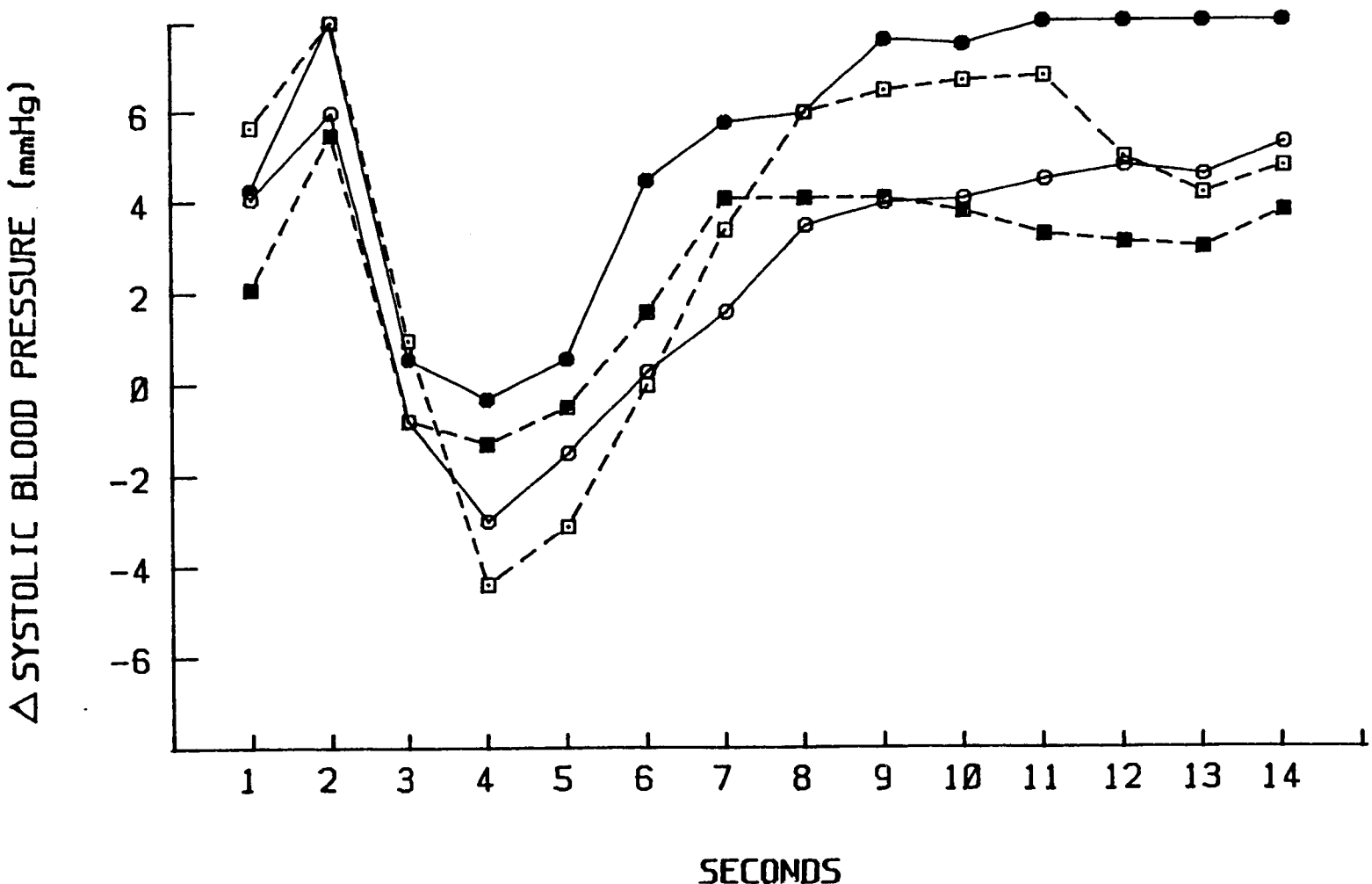

Figure 7. Second by second change scores (each second - pre CS mean) during CS period for systolic blood pressure for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets.

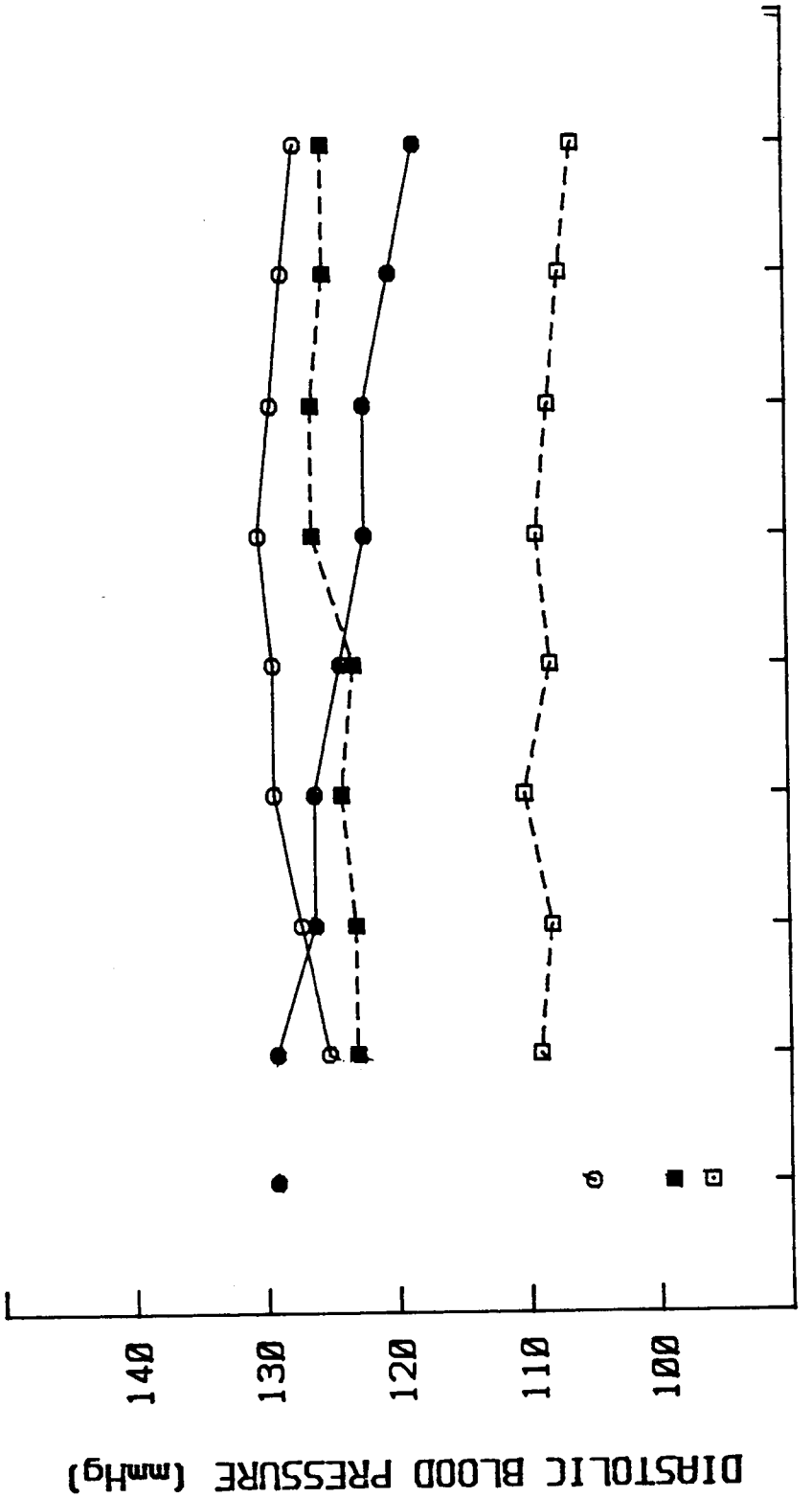

Figure 8. Diastolic blood pressure for the control period (C) and each trial block of stress (1-8) for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets.

concerns the observation that while the diastolic pressure of the WKY groups are relatively stable across trial blocks, the BHR groups appear to show a divergent response. This was confirmed with a two way analysis of variance on the BHR data which revealed a significant interaction of diet by trial blocks [F(7/70) = 2.84], an effect absent in the WKY data. Tukey post-hoc comparisons (CD = 5.4) indicated that BHR on a high sodium diet had significantly lower diastolic blood pressure at trial blocks five through eight compared to BHR on a normal diet. Also, a one way analysis of variance performed on the data for the BHR subjects in the high sodium group revealed a marginally significant (p .10) main effect of trial blocks [F(7/28) = 1.91]. This finding suggests a trend for the diastolic blood pressure of this group to adapt across the stress session.

This trend toward adaptation in BHR on a high sodium diet is related to the second contributing factor in the triple interaction. The lines representing the high sodium groups (filled symbols) in Figure 8 cross approximately half way through the stress session, thus creating a significant interaction of trial blocks by strain [F(7/70) = 2.92] when these data were subjected to a repeated measures analysis of variance. Subsequent Tukey HSD tests (CD = 5.5) revealed that BHR on a high sodium diet are significantly higher than their WKY counterparts at trial block one (129 mmHg vs 122 mmHg), but significantly lower at trial blocks seven (119 mmHg vs 125 mmHg) and eight (118 mmHg vs 125 mmHg). These findings suggest that BHR on a high sodium diet tend to adapt across the stress session compared not only to BHR on normal

adapt across the stress session compared not only to BHR on normal sodium diet, but also compared to WKY on a high sodium diet.

The other striking feature of Figure 8 is the observation that a high sodium diet seems to elevate the diastolic pressure of the WKY subjects to levels comparable to BHR in either dietary group. This was verified with several follow-up analyses. A two way analysis of variance with repeated measures across trial blocks performed on the WKY data revealed a significant main of effect of diet [$F(1/11) = 7.36$], an effect absent in the BHR data. Also, the consistently higher diastolic pressure of BHR on a normal diet compared to WKY on this diet was confirmed with a two way analysis of variance which revealed a significant main effect of strain [$F(1/11) = 4.04$]. Taken together, these findings support the suggestion that a high sodium diet elevates the diastolic blood pressure of WKY subjects relative to those WKY on a normal diet and that this increase is to the level observed in BHR subjects on both diets.

As with systolic pressure, a comparison of diastolic pressure was made between trial blocks one and eight. An analysis of variance with repeated measures revealed a significant triple interaction of strain by diet by trial blocks [$F(1/20) = 5.23$]. Visual examination of trial blocks one and eight in Figure 8 and the preceding analyses suggest that the trend for BHR on a high sodium diet to have lower diastolic pressure as the session progresses may be responsible for this interaction. This possibility was investigated using a two way analysis of variance with repeated measures which indicated a

significant interaction of diet by trial blocks [F(1/10) = 4.77]. Follow-up Tukey HSD comparisons (CD = 9) showed that BHR on a high sodium diet had significantly lower diastolic blood pressure at trial block eight compared to trial block one (118 mmHg vs 129 mmHg). Furthermore, this group was significantly lower than BHR on a normal diet at trial block eight (118 mmHg vs 127 mmHg). No differences were found with respect to either group of WKY subjects at trial blocks one and eight.

Figure 9 is the second figure to be used in clarifying the quadruple interaction found with the overall analysis of diastolic pressure. This figure shows the second by second diastolic blood pressure for the pre-CS mean and the 14 seconds of the tone on period. The overall analysis of diastolic pressure revealed a significant triple interaction of strain by diet by seconds [F(14/266) = 2.32)]. Figure 9 suggests a pattern reminiscent of the trial block data (Figure 8) in that it appears that the diastolic pressure of the four groups respond, in a general way, on a second by second basis very much as they do when the data are viewed in terms of trial blocks. Closer scrutiny of this figure, though, reveals an interesting difference. It appears that the morphology of the curve representing WKY on a high sodium diet is somewhat less pronounced (in the early seconds of the tone on period) when compared to the other three curves in general, and the BHR on a high sodium diet curve in particular. This difference between strains on a high sodium diet was confirmed using a two way analysis of variance with repeated measures which revealed a

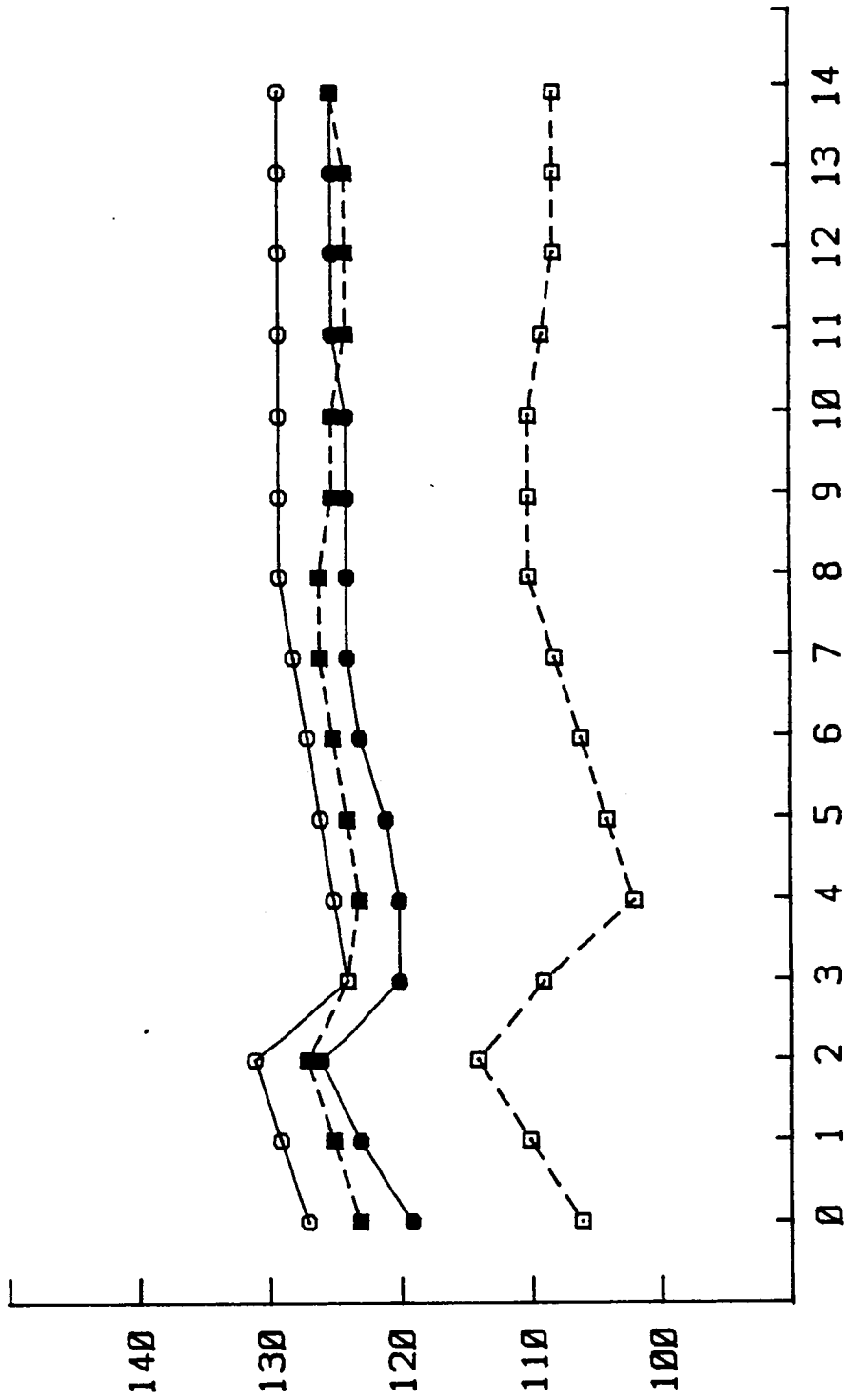

Figure 9. Second by second diastolic blood pressure for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets. Second 0 is the pre-CS mean; seconds 1-14 represent the CS period.

significant interaction of strain by seconds [F(14/140) = 2.04]. Tukey HSD tests (CD = 2.2) indicated that WKY on a high sodium diet had significantly higher diastolic pressure at seconds zero, three, four, and five compared to BHR on this diet.

The triple interaction depicted in this figure is also attributable to differences in the response of the WKY groups during the tone on period. A two way analysis of variance with repeated measures performed on the WKY data revealed a significant main effect of diet [F(1/11) = 7.50] and a significant diet by seconds interaction [F(14/154) = 2.12]. It can be seen in Figure 9 that although WKY on a high sodium diet respond differently to the tone compared to the other groups, as discussed above, this group is consistently higher across all seconds compared to its normal sodium counterpart. It appears, then, that WKY on a high sodium diet do not show as exaggerated a response to the CS as the other groups. The BHR groups, on the other hand, respond similarly across the tone on period.

The differences in the response of diastolic blood pressure to the CS were investigated in another set of analyses. One way analyses of variance on each individual group revealed a significant main effect of seconds for BHR on both normal and high sodium diets [F(14/70) = 17.37 and F(14/70) = 29.48, respectively] and WKY on a normal diet [F(14/84) = 54.46]. Subsequent Tukey post-hoc analyses (CD = 2.6, 2.2, 3.2, respectively) indicated that these three groups all showed a similar pattern characterized by a significant increase in diastolic blood pressure from second zero to two, followed by a significant decrease at

second three. Noticeably absent from this pattern are WKY on a high sodium diet. The question of reactivity will be addressed shortly in terms of change scores.

Thus, although somewhat complicated, it appears that the most meaningful analysis of the quadruple interaction referred to earlier is in terms of two triple interactions. The follow-up analyses described above indicate that the differential responses of the experimental groups, particularly the WKY high sodium group, with respect to both trial blocks and seconds, provide the source for the quadruple interaction.

As with systolic pressure, the reactivity of diastolic pressure to stress was analyzed as change scores which were calculated as previously described for systolic pressure, the results of which are depicted in Figure 10. A two way analysis of variance on these data revealed a significant main effect of strain [$F(1/21) = 5.97$] and a significant strain by diet interaction [$F(1/21) = 5.35$]. Tukey post-hoc comparisons indicated that BHR on a high sodium diet had significantly lower change scores than WKY on the same diet (-6 mmHg vs 25 mmHg). The change scores from the control period for all groups across trial blocks are depicted in Figure 11. Even though an analysis of variance with repeated measures was precluded due to the reasons discussed earlier, it appears that a high sodium diet enhances the reactivity of WKY with respect to both systolic and diastolic pressure and that this increased reactivity is sustained across the session.

The reactivity of diastolic blood pressure for all groups to the

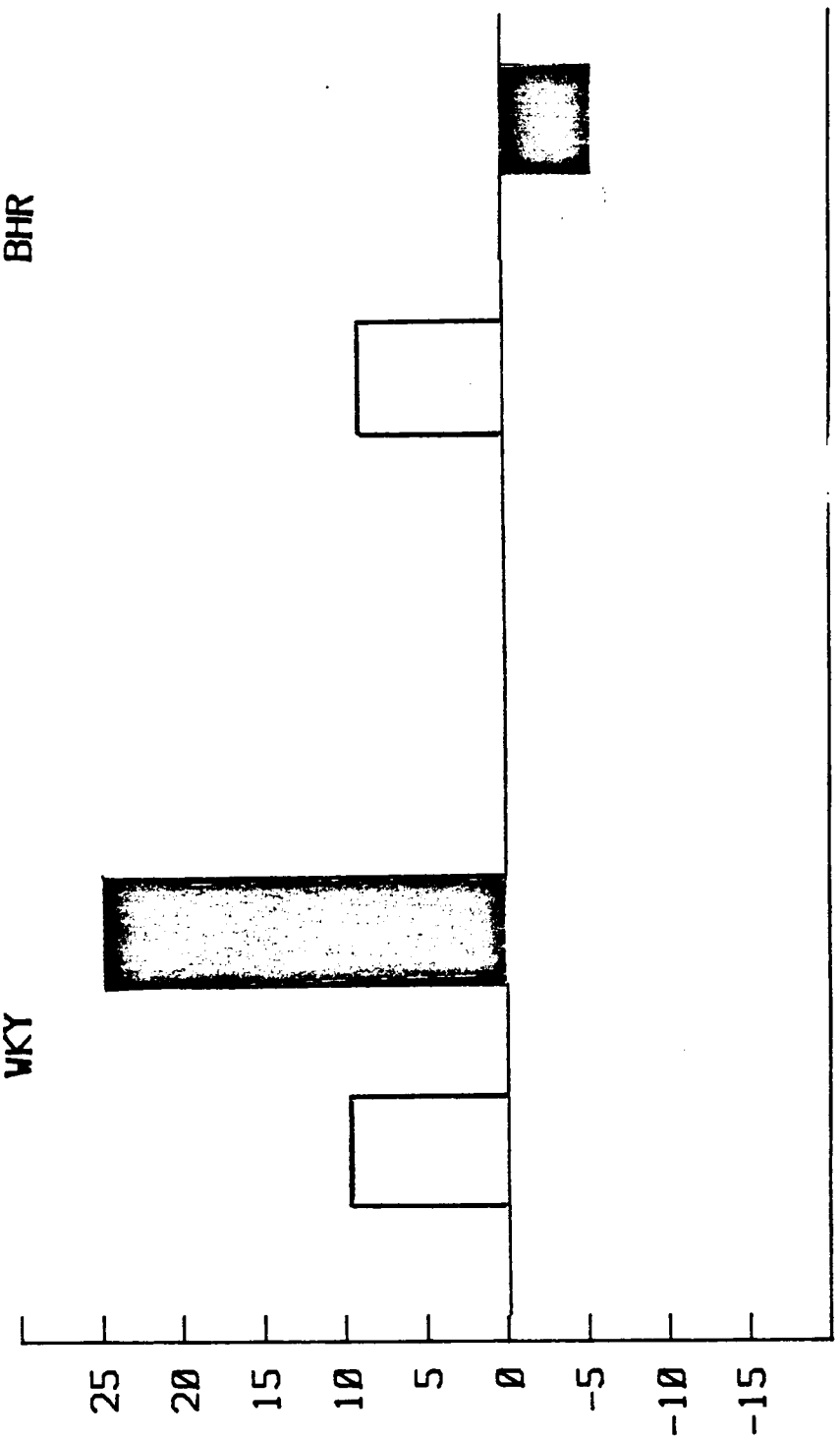

Figure 10. Diastolic blood pressure change scores (stress session - control period) for WKY and BHR on normal (open bars) and high (shaded bars) sodium diets.

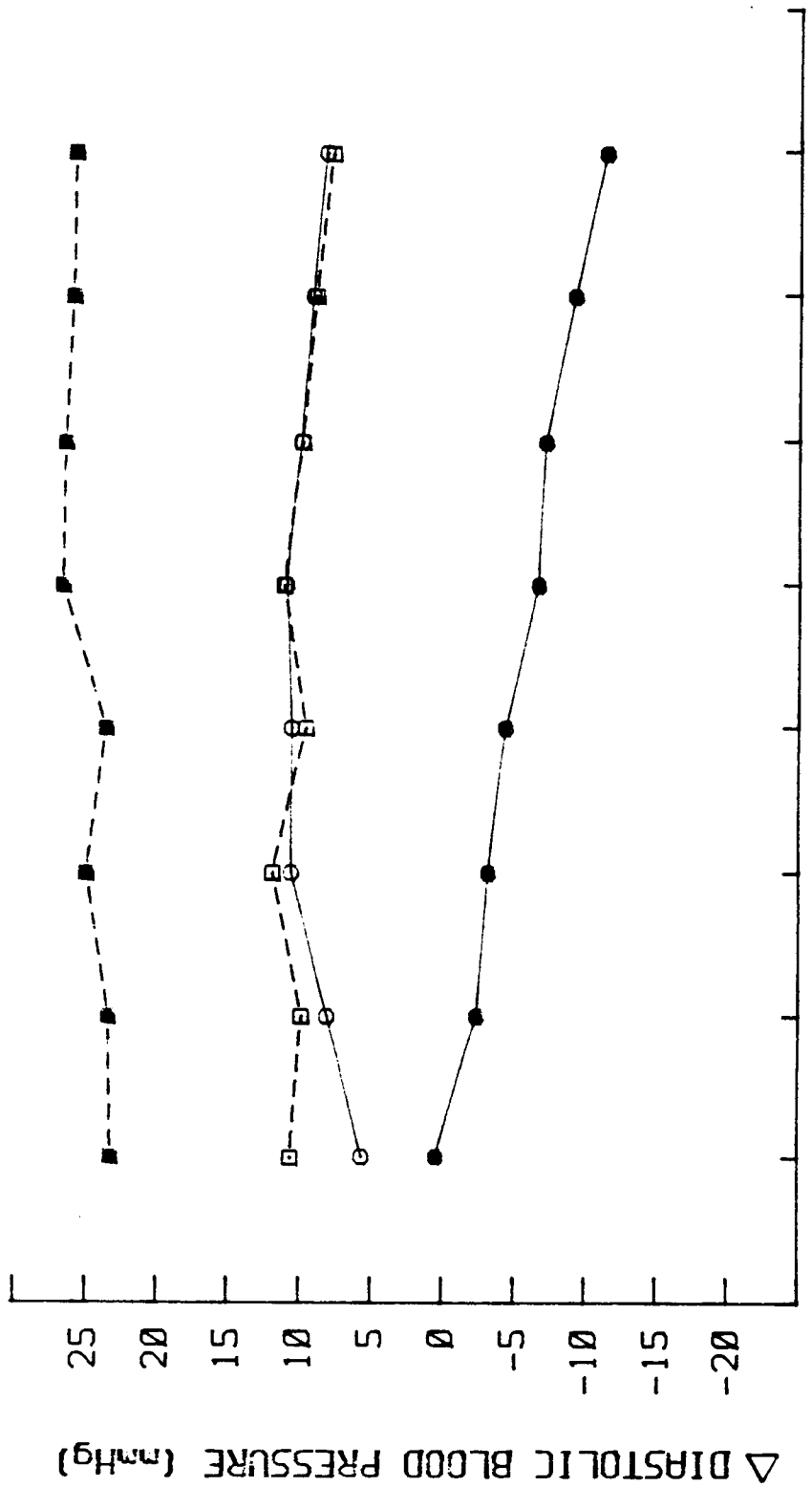

Figure 11. Diastolic blood pressure change scores (each trial block - control period) across trial blocks for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets.

CS was assessed as change scores which were calculated as the difference from the pre-CS mean to each second of the CS period. These scores for all groups across the CS period are shown in Figure 12. An analysis of variance with repeated measures across seconds performed on these data revealed a marginally significant (p .07) triple interaction of strain by diet by seconds [F(13/273) = 1.65]. Examination of this figure suggests that while the pattern of response is similar for the two BHR groups (solid lines), there is a difference in the magnitude of the response between the two WKY groups (dashed lines). Indeed, a two way analysis of variance on the WKY data indicated a significant diet by seconds interaction [F(13/143) = 2.23], an effect absent in the BHR. Subsequent Tukey HSD comparisons (CD = 2.7) between WKY groups revealed the source of the interaction: WKY on a high sodium diet had a smaller change score than WKY on a normal sodium diet at second two (4 mmHg vs 8 mmHg) but a higher change score at second four (no change vs -3 mmHg). These findings further confirm what the analyses of the raw data suggested: WKY on a high sodium diet are less reactive to the CS than WKY on a normal sodium diet.

Another source of the triple interaction is the response of the high sodium groups. A two way analysis of variance performed on BHR and WKY data on this diet revealed a significant strain by seconds interaction [F(13/130) = 2.15]. Tukey HSD post-hoc comparisons (CD = 2) between high sodium groups of each strain revealed WKY to be lower at seconds two and eight through fourteen.

The results described above, along with visual inspection of

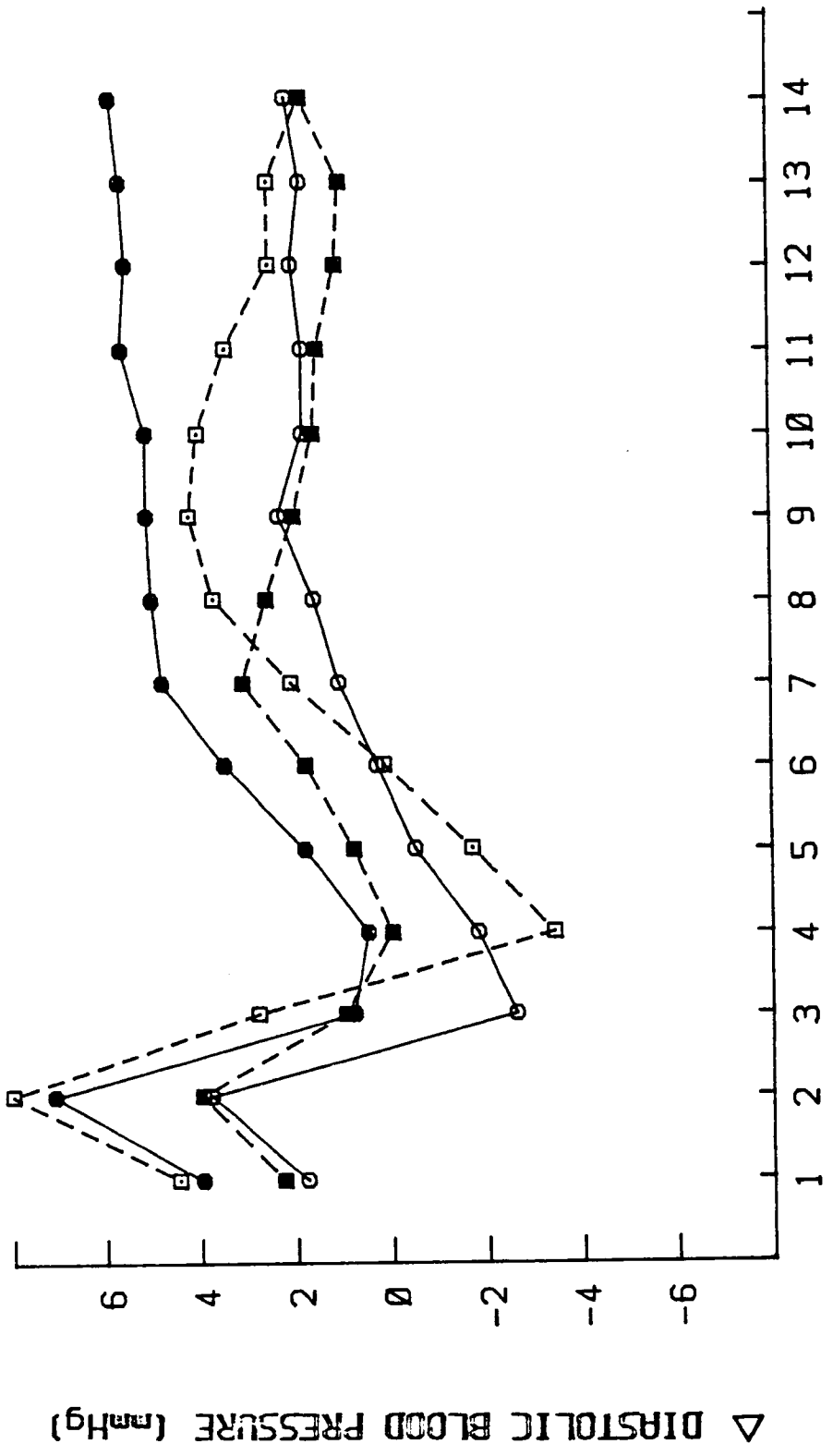

Figure 12. Second by second change scores (each second - pre CS mean) during CS period for diastolic blood pressure for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets.

Figures 9 and 12, suggest that WKY on a normal sodium diet show the most pronounced CR with respect to diastolic pressure, while a high sodium diet seems to dampen the response of this strain. Finally, although there is no significant difference between groups, BHR on a high sodium diet tend to have somewhat higher change scores than their normal sodium counterparts even though the morphology of the curves suggests that the pattern of reactivity is similar.

Heart rate. The main analysis of variance performed on the heart rate data revealed a significant main effect of seconds [F(14/266) = 1.95] and a significant quadruple interaction [F(18/1862) = 1.38]. As with diastolic pressure, the quadruple interaction found in this analysis is shown in two figures, each depicting a significant triple interaction.

Figure 13 shows the heart rate data for the control period and across all trial blocks. It appears that WKY on a normal sodium diet have a decrease in heart rate as the session progresses. This was confirmed by a two way analysis of variance on trial blocks 1-8 which revealed a marginally significant interaction (p .10) of strain by diet by trial blocks [F(7/133) = 1.74]. After a two way analysis of variance performed on each strain (grouped by diet) and each diet (grouped by strain) failed to clarify the source of the interaction, each trial block was subjected to a two way analysis of variance. trial block eight revealed a significant strain by diet interaction [F(1/19) = 5.78]. Tukey HSD comparisons (CD = 24.6) indicated that WKY on a high sodium diet had significantly higher heart rate at this trial

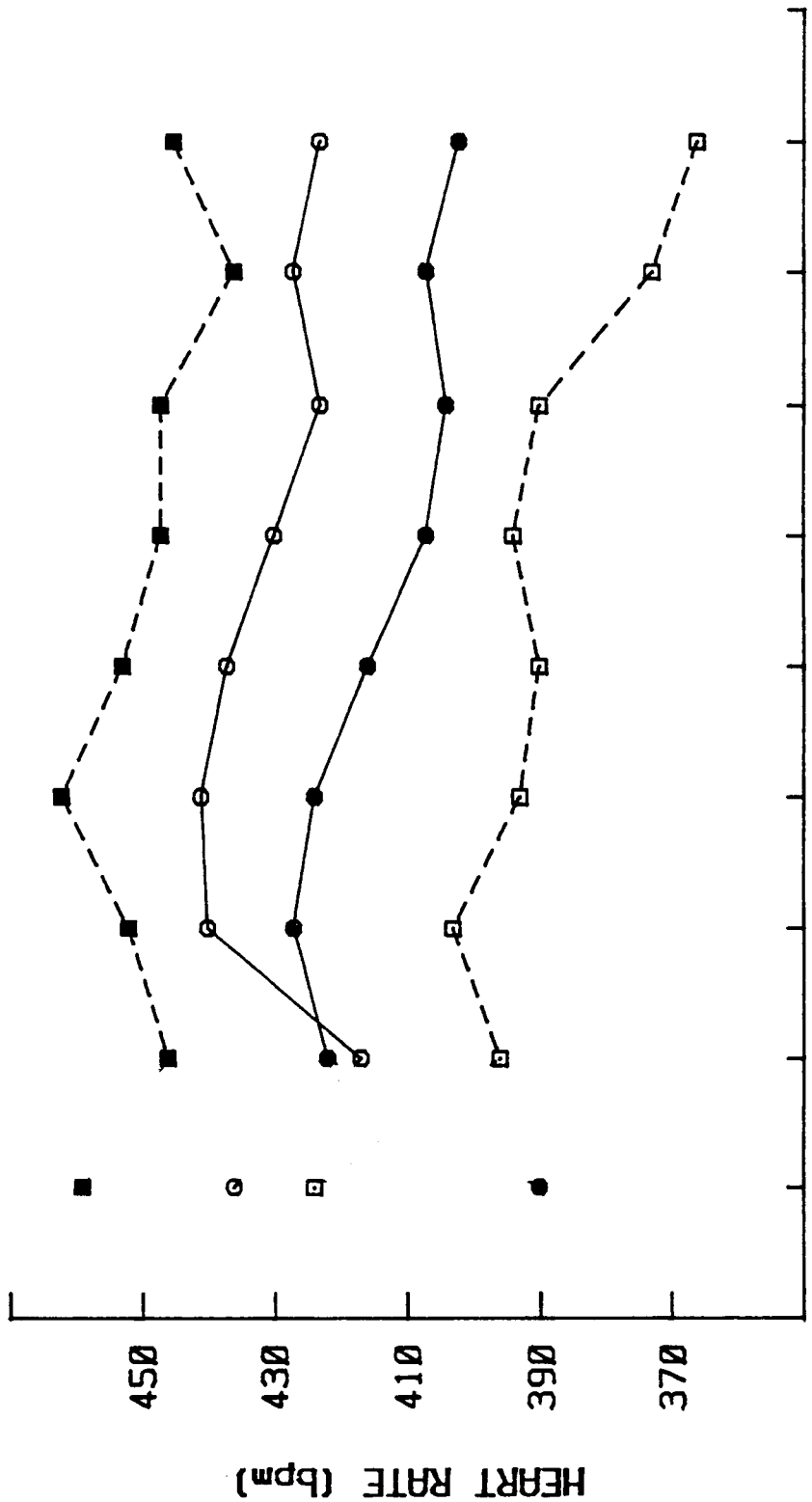

Figure 13. Heart rates for control period (C) and each trial block of stress (1-8) for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets; (WKY, high sodium n=5).

block compared to both WKY on a normal diet (445 bpm vs 366 bpm) and BHR on a high sodium diet (445 bpm vs 402 bpm).

The tendency for WKY on a normal sodium diet to adapt across the course of the stress session was also addressed using a one way analysis of variance across trial blocks which revealed a significant main effect of trial blocks [$F(7/35) = 2.84$] for WKY on a normal sodium diet, an effect absent in in the other three groups. Subsequent Tukey HSD tests (CD = 19.3) made between points on this curve indicated that WKY on a normal sodium diet had a significantly lower heart rate at trial blocks seven and eight compared to trial block one (373 and 366 bpm, respectively, vs 394 bpm). Thus, it appears that WKY on a normal sodium diet showed an adaptation of heart rate across the session that was not found in the other groups.

As with the other two variables, the adaptation of the groups was examined by an analysis of variance between trial blocks one and eight. This analysis revealed a significant triple interaction of trial blocks by strain by diet [$F(1/19) = 4.49$]. This interaction was the result of the differences between experimental groups in trial block eight and was described above.

Figure 14 depicts the second by second heart rate data for the pre-CS mean and the 14 seconds of the tone on period. The overall analysis of variance utilizing these data revealed a significant triple interaction of strain by diet by seconds [$F(14/266) = 1.95$]. Examination of this figure suggests that while the BHR groups are relatively stable across the seconds, the WKY groups respond somewhat

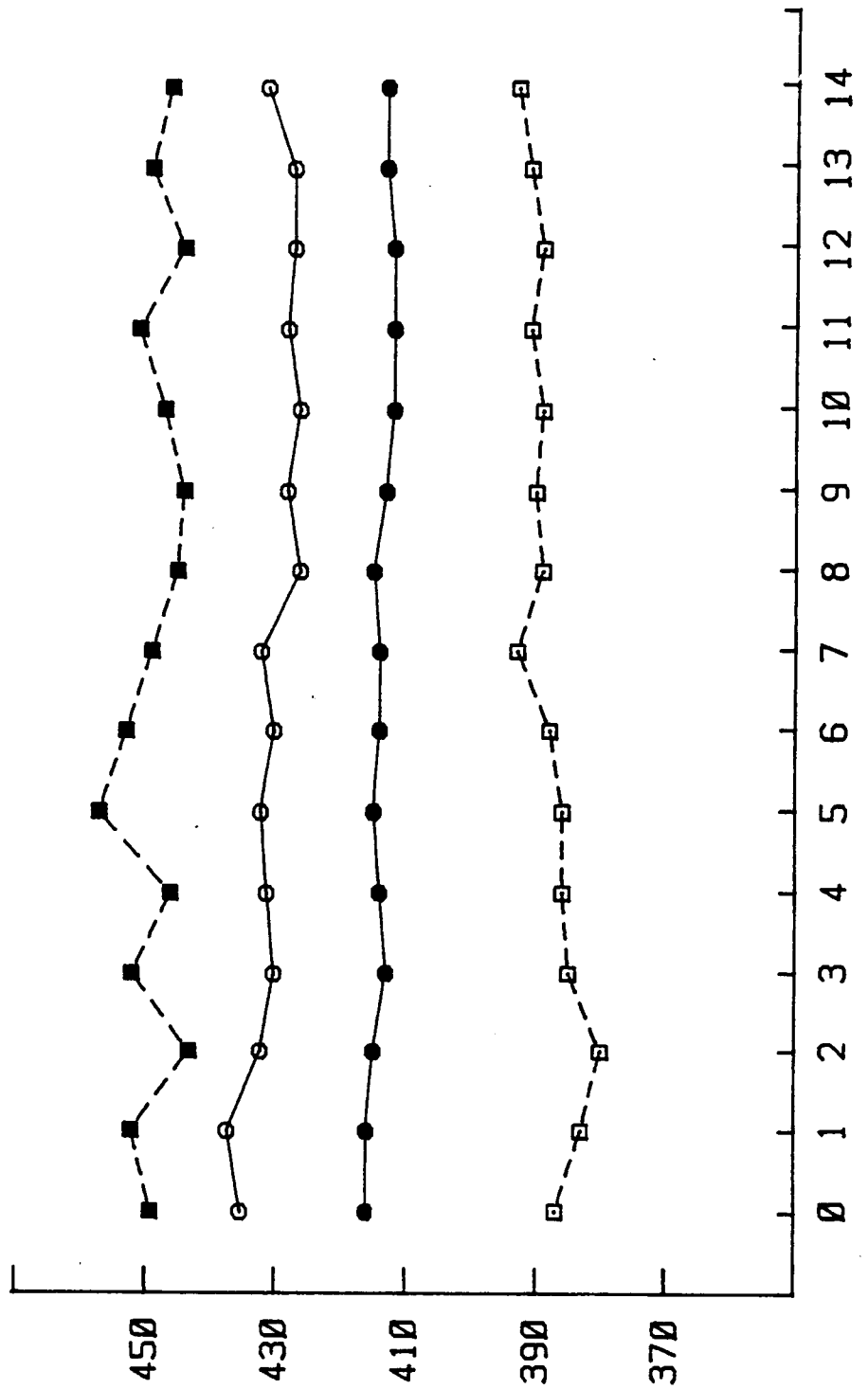

Figure 14. Second by second heart rate for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets; (WKY, high sodium n=5). Second 0 is the pre-CS mean; seconds 1-14 represent the CS period.

differently. This was verified with a two way analysis of variance on the WKY data which indicated a significant diet by seconds interaction [F(14/126) = 1.91], an effect absent in the BHR. Tukey HSD tests (CD = 8) revealed that WKY on a high sodium diet are consistently higher than their normal sodium counterparts.

Although the analysis of change scores from the control period to the stress session revealed no significant main effects or interactions, an examination of the reactivity of the experimental groups to the CS provides some interesting comparisons. The overall analysis of these data revealed a marginally significant (p .06) effect of strain [F(1/19) = 3.92] and a significant quadruple interaction of strain by diet by trial blocks by seconds [F(91/1729) = 1.43]. As with other quadruple interactions, these results will be discussed using two figures, each representing a triple interaction.

Figure 15 depicts the heart rate change scores (each second of CS period - Pre CS mean) during the CS period. The main analysis on heart rate change scores indicated a significant triple interaction of strain by diet by seconds [F(13/247) = 1.73]. There are several interesting features seen in this graph. First, it appears that the WKY on a high sodium diet are more reactive to the tone (that is, greater and more frequent fluctuations of change scores) compared to this strain on a normal diet. This was confirmed by: 1) a repeated measures analysis of variance on the WKY data which revealed a significant diet by seconds interaction [F(13/117) = 3.07] and 2) the lack of an interaction in the BHR subjects. Subsequent Tukey tests (CD

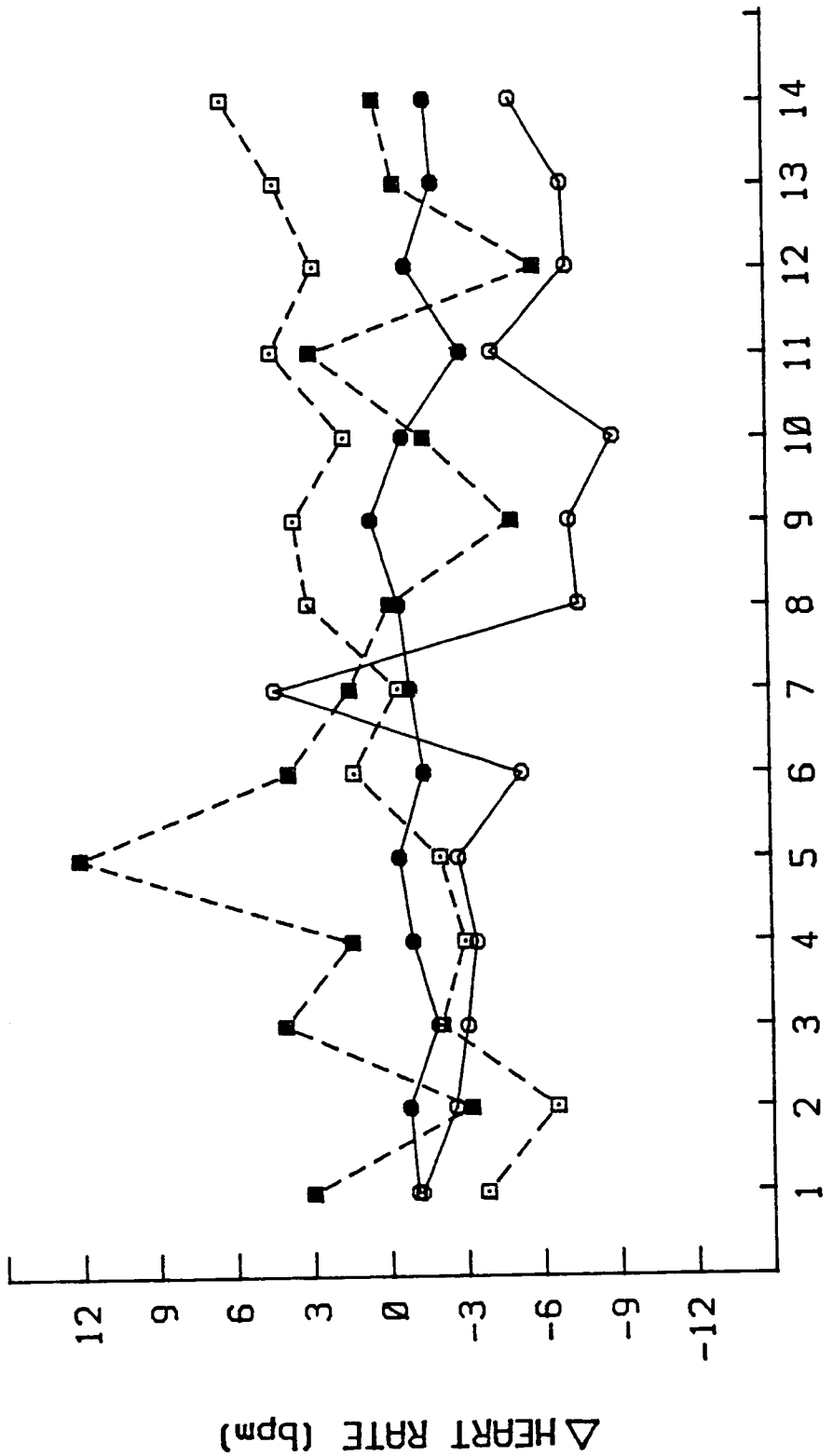

Figure 15. Second by second change scores (each second - pre CS mean) during CS period for heart rate for BHR (solid lines) and WKY (dashed lines) on normal (open sybmols) and high (filled symbols) sodium diets; (WKY, high sodium n=5).

= 7.4) indicated that, compared to WKY on a normal diet, the subjects in the high sodium group had higher change scores at seconds three and five, but lower scores at seconds nine and 12.

Another striking comparison can be made between the curves depicting the change scores for each strain on a high sodium diet. As suggested above, a high sodium diet appears to make the WKY more reactive to the tone. Inspection of Figure 15 suggests that the same diet has a much different, almost opposite, effect on BHR. This observation was supported by a repeated measures analysis of variance on the high sodium data from both strains which revealed a significant strain by seconds interaction [$F(13/117) = 2.25$]. Tukey HSD tests (CD = 6.3) indicated that, compared to BHR on a high sodium diet, WKY on this diet had greater change scores at seconds five and 11, but had a lower score at second 12.

The stability of change scores for BHR on a high sodium diet was also addressed by performing a one way analysis of variance on each group. These analyses revealed a significant main effect of seconds for WKY on both normal [$F(13/65) = 2.39$] and high sodium [$F(13/52) = 2.38$] as well as BHR on a normal sodium diet [$F(13/65) = 1.95$]. On the other hand, BHR on a high sodium diet showed no main effect of seconds. Taken together, these data suggest that a high sodium diet greatly exaggerates the heart rate reactivity of WKY, while markedly dampening the ability of BHR to respond to the CS.

A final feature of this figure deserving comment concerns the difference in change scores between WKY and BHR on a normal diet. This

is most apparent in the latter half of the tone on period. A repeated measures analysis of variance on these data revealed a significant main effect of strain [F(1/10) = 5.74] and a significant strain by seconds interaction [F(13/130) = 3.32]. Tukey HSD tests (CD = 6.7) indicated that WKY had greater change scores at seconds eight through 14.

The main heart rate change score analysis also revealed a significant triple interaction of strain by diet by trial blocks [F(7/133) = 2.06]. These data are shown in Figure 16. After a two way analysis of variance with repeated measures on each strain (grouped by diet) and each diet (grouped by strain) failed to reveal the source of the interaction, a repeated measures analysis of variance was performed on each trial block. These analyses revealed a significant strain by diet interaction [F(1/19) = 5.85] in trial block two and marginally significant (p .07) interactions in trial blocks five and six [(F(1/19) = 3.51 and F(1/19) = 3.48, respectively]. Since these interactions were not found in trial blocks one or eight and since there was not a trend in any particular direction, it was determined that the analysis concerning the triple interaction of strain by diet by seconds provided the most meaningful data in clarifying the quadruple interaction.

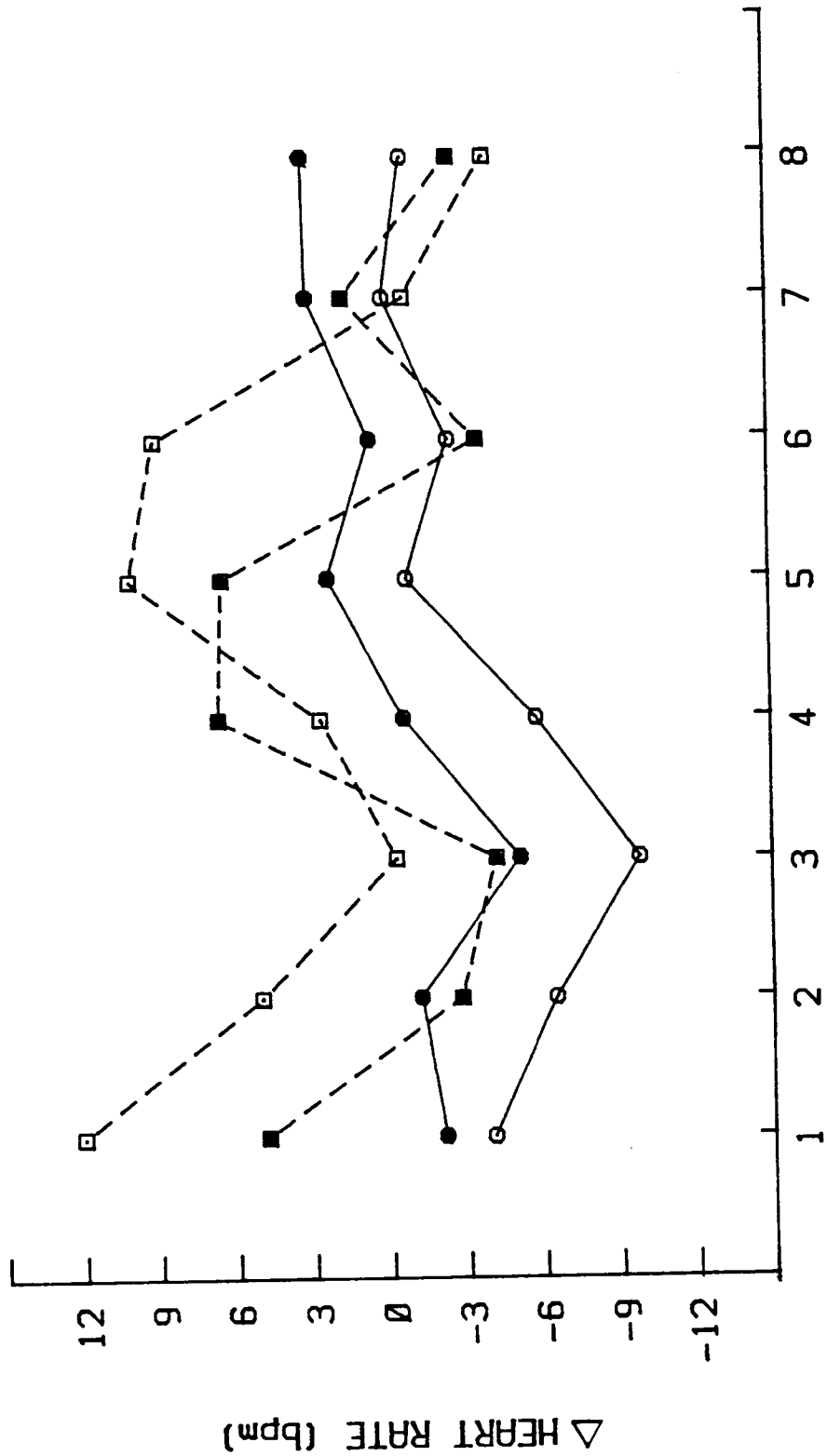

Figure 16. Heart rate change scores (each second - pre CS mean) across trial blocks for BHR (solid lines) and WKY (dashed lines) on normal (open symbols) and high (filled symbols) sodium diets; (WKY, high sodium n=5).

Renal Data

Table 2 depicts the urinary volume, sodium excretion, GFR, and RPF data for WKY and BHR subjects on normal and high sodium diets during control and stress session. Because of the questions these data are addressing, they are most clearly presented by variable, rather than by grouping factor.

Urinary Volume

Figure 17 shows the urinary volumes for WKY (top panel) and BHR (bottom panel) for each diet during control and stress sessions. Inspection of both panels of this figure suggests that a high sodium diet greatly increases the volume of urine excreted in both strains. This was confirmed by a two way analysis of variance which revealed a significant main effect of diet [$F(1/41) = 31.5$], a marginally significant (p .08) main effect of condition [$F(1/41) = 3.22$] and a marginally significant (p .07) strain by diet interaction [$F(1/41) = 3.34$]. Tukey HSD tests (CD = 5.4) indicated that WKY and BHR subjects on a high sodium diet excreted more urine during control and stess sessions. The main effect of condition is clarified by visual inpsection of Figure 17 which shows the same pattern of response for all groups to stress. That is, both WKY and BHR on a high sodium diet tend to retain somewhat more volume compared to their values during the control period.

Table 2. Renal function responses in BHR and WKY on normal and high sodium diets during control and stress sessions. Values are means±SEM.

	WKY (n=12)		BHR (n=13)	
	Normal Na+	High Na+	Normal Na+	High Na+
V ul/min/100g BW				
C	13.8±.75	21.8±1.1	14.2±.53	19.4±2.6
S	12.2±.47	18.7±1.5	14.6±.53	16.8±1.8
U_{Na} uEq/min/100g BW				
C	2.3±.07	3.9±.06	2.1±.04	4.8±.57
S	2.4±.10	4.0±.31	1.8±.04	3.8±.38
GFR ul/min/100g BW				
C	905±6.1	978±3.6	932±5.7	957±30
S	896±6.5	953±10.2	910±34.0	931±18.4
RPF ml/min/100g BW				
C	3.8±.03	4.0±.04	3.8±.03	4.0±.04
S	3.7±.03	3.9±.03	3.8±.04	3.6±.04

C=control, S=stress, V=urinary volume, U_{Na}=urinary sodium excretion, GFR=glomerular filtration rate, RPF=renal plasma flow.

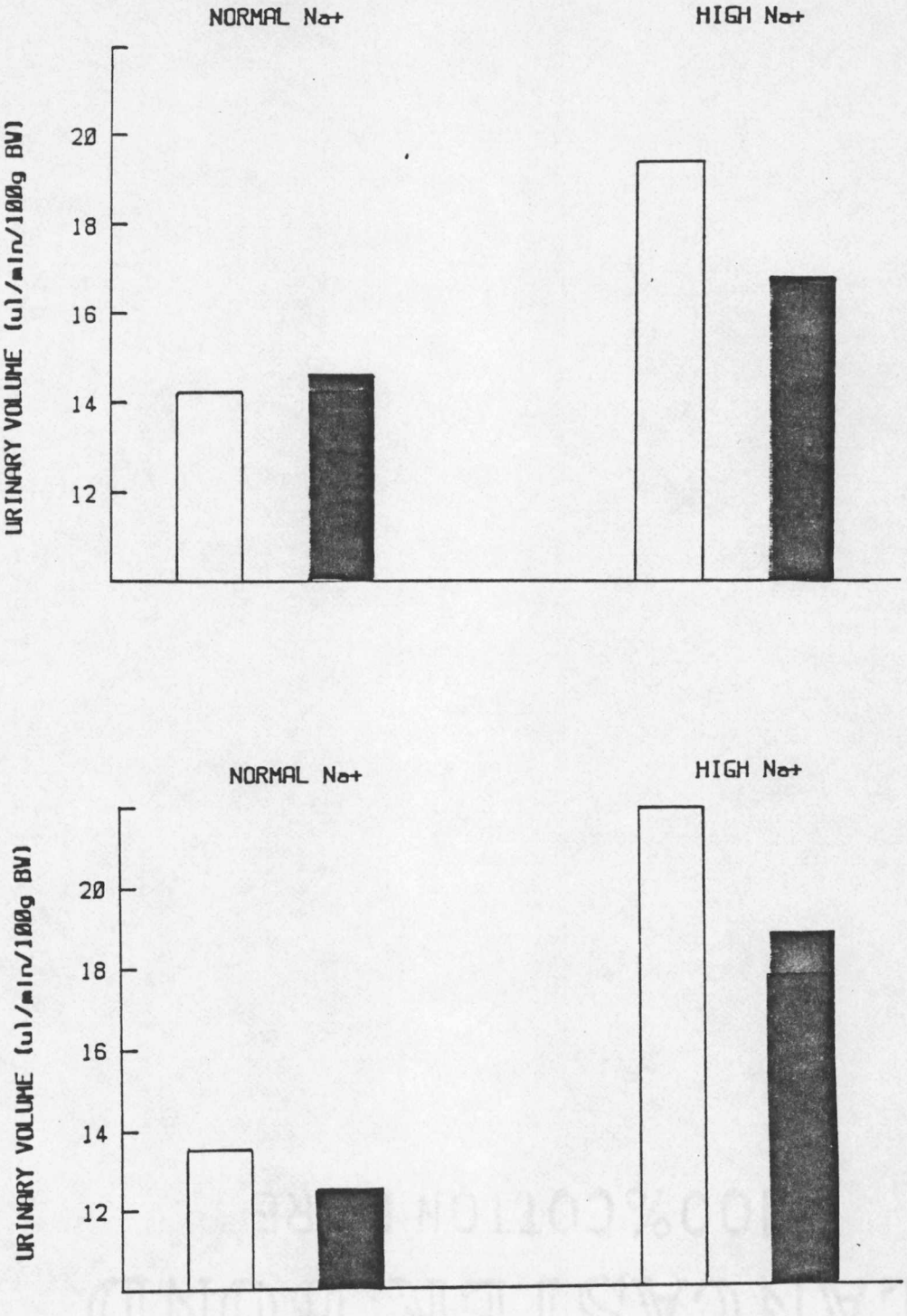

Figure 17. Urinary volumes for WKY (top panel) and BHR (bottom panel) on normal and high sodium diets during control and (open bars) and stress (shaded bars) periods.

Sodium Excretion

Figure 18 shows the urinary sodium excretion rates for WKY (top panel) and BHR (bottom panel) for each diet during control and stress sessions. There are two interesting points to be made regarding this figure. First, as with urine volume, it appears that a high sodium diet greatly increases the amount of sodium excreted in WKY and BHR during both control and stress sessions. This was confirmed with a two way analysis of variance which revealed a significant main effect of diet [F(1/41) = 101.5] and a marginally significant (p .07) strain by diet interaction [F(1/41) = 3.29]. Tukey HSD tests (CD = 1) indicated that both WKY and BHR on high sodium diet excreted significantly more sodium than their normal sodium counterparts during both control and stress sessions.

The other interesting feature of this figure concerns the apparent decrease in sodium excretion in BHR on a high sodium diet during the stress session compared to the WKY strain. A two way analysis of variance verified this observation with a significant interaction of strain by condition [F(1/41) = 4.16]. Subsequent Tukey HSD tests (CD = 1) revealed that, when compared to control values, BHR on a high sodium diet excreted significantly less sodium (3.8 ul/min/100g BW vs 4.8 ul/min/100g BW) during stress than BHR on a normal diet (2.1 ul/min/100g BW vs 1.8 ul/min/100g BW). As can be seen from the top panel of Figure 18, there were no differences in the WKY strain between control and stress session values in either diet.

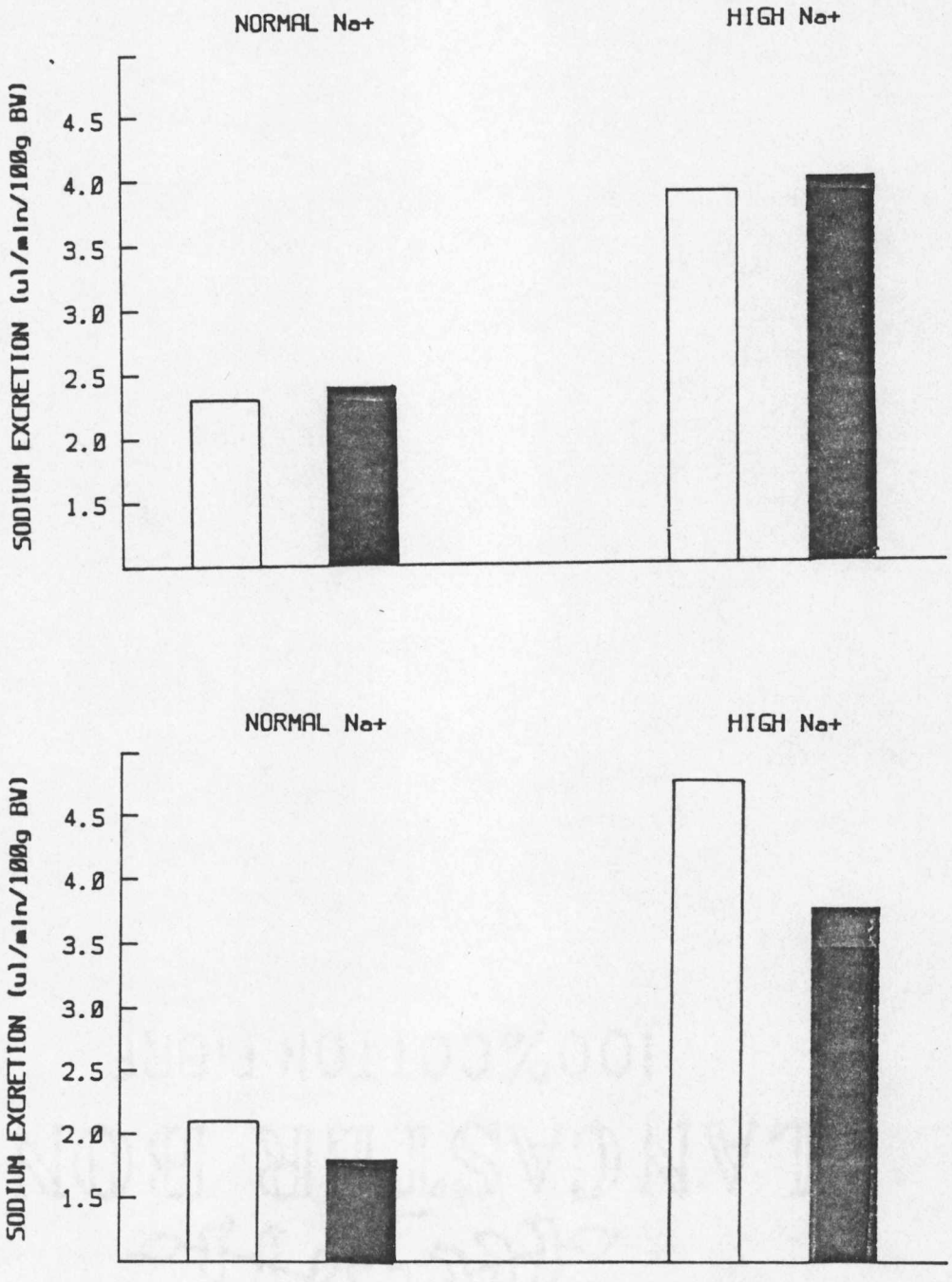

Figure 18. Urinary sodium excretion rates for WKY (top panel) and BHR (bottom panel) on on normal and high sodium diets during control (open bars) and stress (shaded bars) periods.

Glomerular Filtration Rate

Figure 19 shows GFR for WKY (top panel) and BHR (bottom panel) on high and normal sodium diets during control and stress sessions. A two way analysis of variance on these data revealed a significant main effect of diet [$F(1/41) = 17.69$] and a marginally significant (p .06) main effect of condition [$F(1/41) = 3.62$]. Thus, a high sodium diet increases GFR in both strains and there appears to be a very slight trend to decrease GFR during stress in both strains.

Renal Plasma Flow

Figure 20 shows RPF for WKY (top panel) and BHR (bottom panel) on high and normal sodium diets during control and stress sessions. A two way analysis of variance on these data revealed a significant main effect of strain [$F(1/41) = 3.78$], diet [$F(1/41) = 6.17$] and condition [$F(1/41) = 22.98$] and a significant triple interaction of strain by diet by condition [$F(1/41) = 4.91$]. Inspection of this figure suggests that the source of the interaction is the stress response of the high sodium BHR group. This was confirmed with an analysis of variance on the BHR data which revealed a significant interaction of diet by condition [$F(1/21) = 10.47$]. Subsequent Tukey HSD tests (CD = .2) revealed that during stress BHR on a high sodium diet had significant decrease in renal plasma flow compared to BHR on a normal diet (3.6 ml/min/100g BW vs 4.0 ml/min/100g BW).

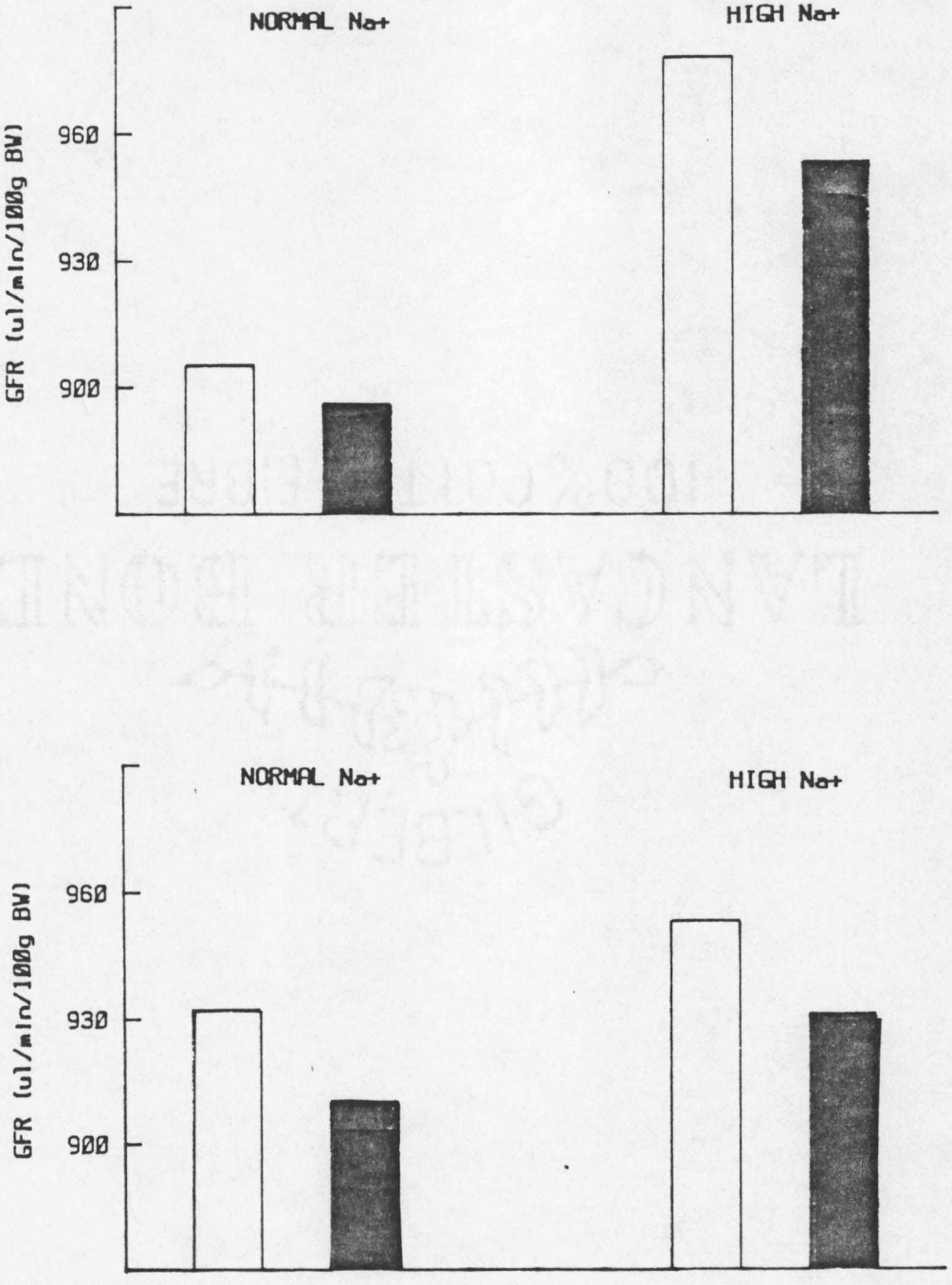

Figure 19. Glomerular filtration rates for WKY (top panel) and BHR (bottom panel) on normal and high sodium diets during control (open bars) and stress (shaded bars) periods.

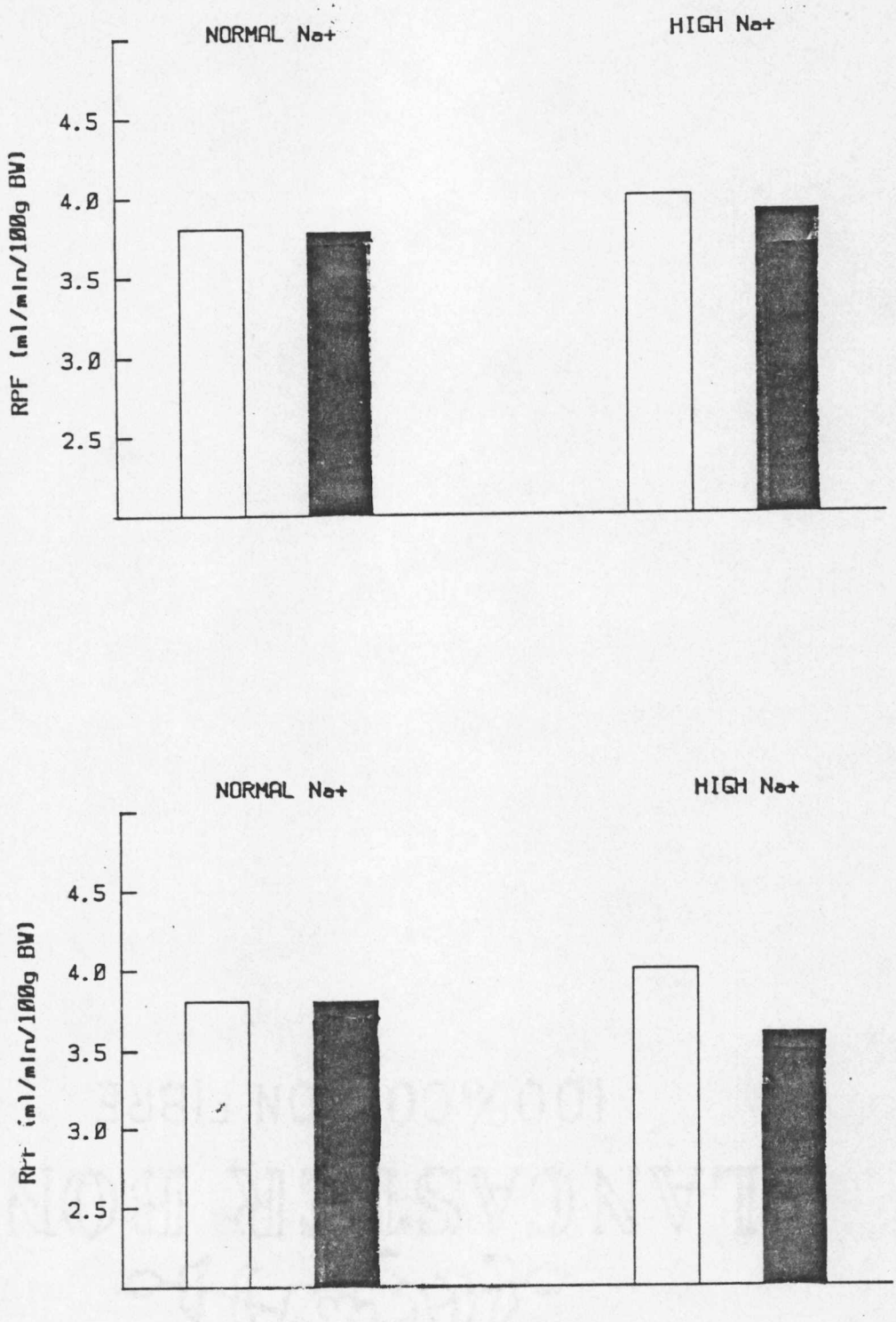

Figure 20. Renal plasma flow for WKY (top panel) and BHR (bottom panel) on normal and high sodium during control (open bars) and stress (shaded bars) periods.

Metabolic Data

Table 3 shows the body weights, urinary volumes, sodium and potassium concentrations, and 24 hour electrolyte excretion rates for all subjects during the metabolic study period. A two way analysis of variance was performed on these data which revealed a significant main effect of diet for volume [$F(1/26) = 37.26$], sodium and potassium concentrations [$F(1/26) = 92.05$, $F(1/26) = 130.57$, respectively], sodium excretion rate [$F(1/26) = 174.90$], and a marginally significant (p .10) main effect of diet for potassium excretion rate [$F(1/26) = 2.49$]. Thus, it appears that a high sodium diet increases electrolyte excretion rate similarly in both WKY and BHR.

Catecholamine Data

Figure 21 depicts the norepinephrine values for WKY (top panel) and BHR (bottom panel) on normal and high sodium diets during control and stress sessions. A two way analysis of variance on these data revealed a significant main effect of strain [$F(1/34) = 6.40$], diet [$F(1/34) = 4.33$], and condition [$F(1/34) = 4.33$], and a significant triple interaction of strain by diet by condition [$F(1/34) = 6.12$]. Inspection of Figure 21 suggests that one source of this interaction may by the differing responses of the WKY subjects. This was verified with a two way analysis of variance on these data which revealed a significant interaction of strain by diet [$F(1/17) = 5.59$]. Tukey HSD

Table 3. Body weight, urinary volume, urinary sodium and potassium concentrations, and 24 hour electrolyte excretions. Values are means±SEM.

	WKY (n=15)		BHR (n=15)	
	Normal Na+	High Na+	Normal Na+	High Na+
BW grams	387±3.9	371±8.6	353±9.5	355±4.1
Vol (ml/24h)	24±4.2	64±6.1	29±5.1	55±5.4
Na+ (mEq/L)	127±5.9	220±15.7	141±1.5	244±2.1
K+ (mEq/L)	190±8.1	89±6.6	168±3.9	104±11.0
Na+ Excre (mEq/24h)	2.9±.43	13.2±.63	4.1±.57	13.3±1.2
K+ Excre (mEq/24h)	4.4±.62	5.4±.4	4.8±.53	5.4±.45

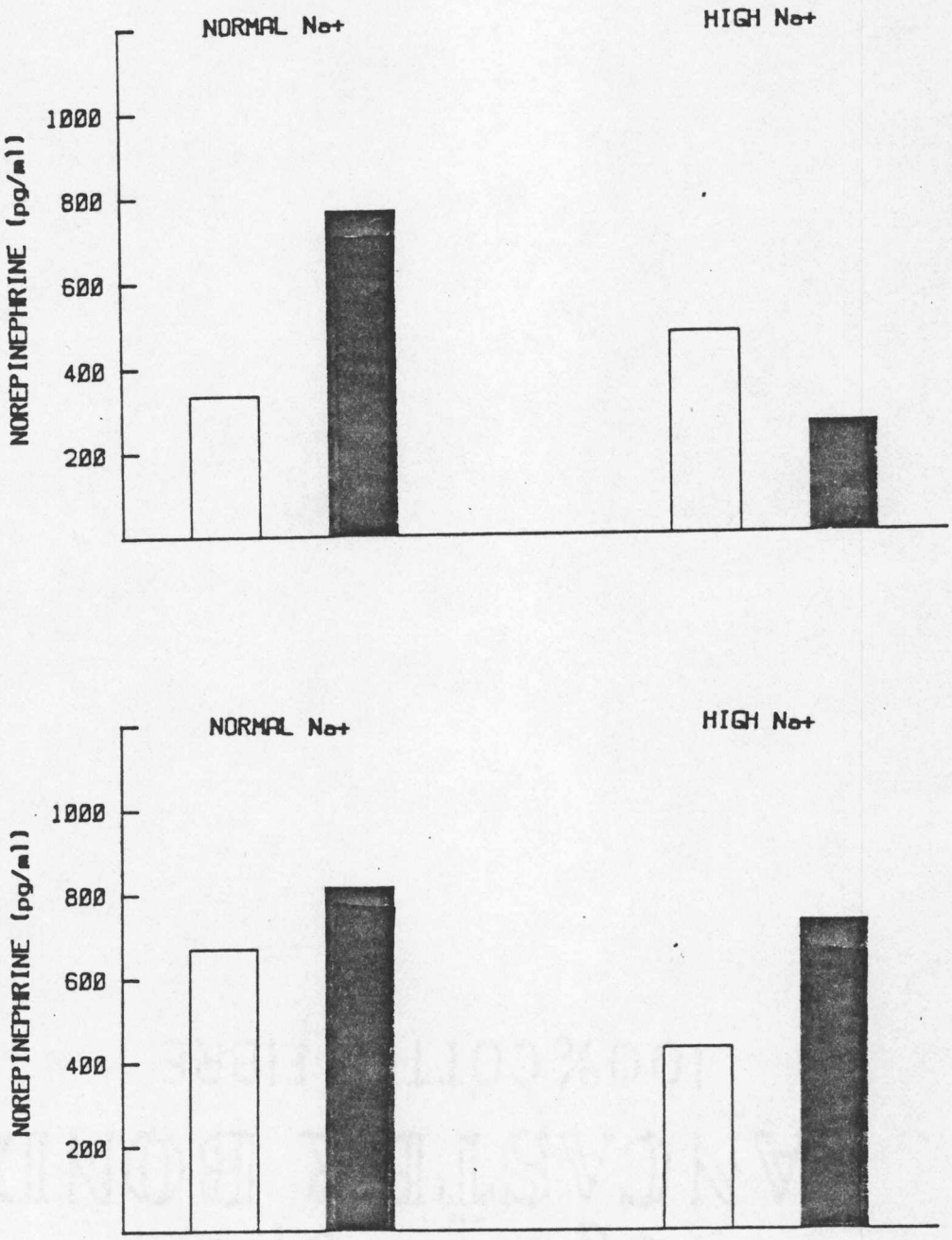

Figure 21. Plasma norepinephrine levels for WKY (top panel) and BHR (bottom panel) on normal and high sodium diets during control (open bars) and stress (shaded bars) periods.

tests (CD = 544) indicated a trend for WKY on a high sodium to have a blunted norepinephrine response to stress compared to their normal sodium counterparts during this condition (263 pg/ml vs 767 pg/ml). Another interesting feature of this figure is the apparent difference in response to stress between WKY and BHR on high sodium. A two way analysis of variance on the data for all high sodium subjects revealed a significant interaction of strain by condition [F(1/20) = 5.75]. Tukey HSD tests (CD = 420) indicated that WKY on high sodium diet exhibited a significantly diminished response to stress compared to BHR on a high sodium diet (263 pg/ml vs 736 pg/ml).

Figure 22 shows the epinephrine values for WKY subjects on normal and high sodium diets during control and stress sessions. A two way analysis of variance on these data revealed a significant main effect of diet [F(1/16) = 1.25]. Examination of this figure indicates that high sodium severely diminishes epinephrine values during both control and stress periods in WKY. Unfortunately, these data are not available for BHR. Taken together, the most striking feature of the catecholamine data is apparent ability of a high sodium diet to reduce sympathetic nervous system function in WKY.

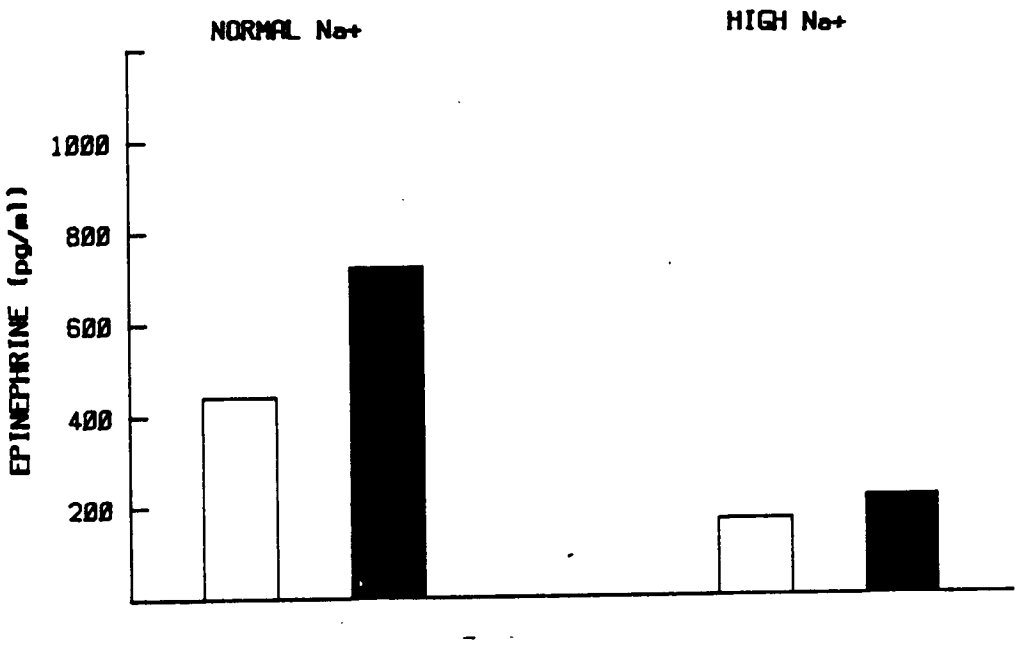

Figure 22. Plasma epinephrine levels for WKY on normal and high sodium diets during control (open bars) and stress (shaded bars) periods.

CHAPTER IV

DISCUSSION

One of the major goals of the present study was to document the effects of a high sodium diet and behavioral stress on blood pressure responses and renal functioning in the normotensive and borderline hypertensive rat. Various stress paradigms and a high sodium diet have each been studied independently in the BHR; however, a systematic study of the effects of both manipulations has not been executed. Furthermore, the influence of these stressors on renal functioning in BHR has not been documented. Data and literature reviewed from epidemiological, clinical, and animal research indicate that the study of the interaction of these variables in an animal genetically predisposed to hypertension could provide valuable insight into the ability of environmental factors to produce this disease.

The results from the control period suggest that a high sodium diet produces hypertension in BHR while not affecting blood pressure in WKY. Since the BHR has been shown to develop hypertension when chronically exposed to a shock-shock conflict paradigm (Lawler et al., 1981), this finding is important in that it demonstrates this animal's susceptibility to another environmental trigger widely thought to be involved in the development of human essential hypertension. The importance of this observation is highlighted by the fact that no other currently available animal model develops hypertension when exposed to

both of these environmental agents. That is, many animals exhibit a pressor response to behavioral stress or to a sodium challenge (e.g, SHR, WKY, Dahl) but an animal that becomes hypertensive when exposed to either manipulation was not available until the BHR was developed.

The differing cardiovascular responses to the stress session and to the CS (in terms of both raw data and change scores) in WKY and BHR on high and normal sodium diets provide some insight into the nature of the interaction of these two environmental factors. First, the trial block data presented suggest that the adaptation across trials exhibited by WKY on a normal sodium diet is prevented by a high sodium diet. Even though the blood pressure levels for WKY on a high sodium diet are not as high as BHR, their failure to adapt across the stress session is similar to the BHR data in the present study. This pattern of response is also reminiscent of the behavior of SHR (Lawler et al., 1985). These authors reported that SHR exposed to a tone-shock conditioning paradigm showed greater reactivity as well as a slower adaptation to the stress compared to WKY. Also relevant to this discussion are the observations of Hallback and Folkow (1974) who noted that SHR exhibited greater reactivity to an alerting stimulus than WKY. Thus, although neither a high sodium diet or stress produces hypertension in WKY independently, the data support the view that the combination of the two results in a pattern of response similar to animals that are frankly hypertensive (SHR) and genetically predisposed to hypertension (BHR). Also, it is relevant to suggest that with repeated exposure to stress, the failure of WKY on a high sodium diet

to return to baseline could be a potentially critical factor in the eventual elaboration of cardiovascular disease. BHR, on the other hand, responded similarly (i.e., 180-190 mmHg) on both diets and showed no trend toward adaptation.

Another interesting finding of the present study concerns the influence a high sodium diet has on the reactivity of WKY and BHR to stress. The data presented suggest that this diet has a much different effect on these two strains. If one looks at reactivity as a change score from the control period to the mean of the eight trial blocks of the stress session (as opposed to second by second changes from the pre-CS mean) it appears that a high sodium diet severely diminishes the ability of BHR to increase blood pressure during the stress session. The raw data in conjunction with these change scores suggest that either a high sodium diet or the stress paradigm can raise the blood pressure of BHR to hypertensive levels (c. 180-190 mmHg), but the combination of these two environmental stressors is not additive. That is, exposing BHR to both factors simultaneously does not appear to produce a greater degree of hypertension than exposure to either manipulation separately. With respect to WKY, a high sodium diet seems to make these subjects just as reactive to the stress session as BHR on a normal sodium diet. This observation is discussed in more detail below.

When reactivity is assessed as the magnitude of change from a pre-CS mean to each second of the CS period, an interesting picture also emerges. The data suggest that a high sodium diet has much the

same effect on the reactivity of BHR to the CS as it does on their reactivity to the stress session in general. That is, these animals have a much diminished reactivity regardless of whether it is viewed as the degree of change from the control period to the mean of the eight trial blocks or as the degree of change from the pre-CS mean to each second of the tone on period. The reactivity of WKY on a high sodium diet, however, appears to be different depending on how it is assessed. As mentioned earlier, when viewed as a change score from the control period to the mean of the entire stress session, WKY on this diet seem to be slightly more reactive than their normal sodium counterparts and just as reactive as BHR on a normal sodium diet. When the responses to the CS are assessed, however, a high sodium diet blunts the reactivity of blood pressure in WKY. These data suggest that while a high sodium diet makes WKY somewhat more reactive to the stress session, they are not very reactive to the CS within the stress session when compared to WKY on a normal sodium diet.

There is evidence from other studies suggesting that the sympathetic nervous system may be involved in the development and maintenance of sodium-induced hypertension in some animal models. For example, hypertension can be prevented in Dahl salt-sensitive rats by destruction of peripheral noradrenergic nerve endings with guanethidine (Friedman, Tassinari, Heine, and Iwai, 1979) and by injection of 6-hydroxydopamine (Takeshita and Mark, 1979). Furthermore, the sympathetic system has been implicated in the blood pressure response to increased dietary sodium in the SHR. Specifically, a high sodium

diet increases the severity of hypertension in SHR and is associated with increased plasma catecholamine levels (Winternitz and Oparil, 1982). Finally, increased sympathetic activity has been found to be associated with hypertension induced by deoxycorticosterone acetate (Reid, Zivin, and Kopin, 1979).

It is equally important to realize that not all studies have found evidence suggestive of heightened sympathetic activity with a high sodium diet. For example, it has been reported that resting catecholamine levels are similar in Dahl salt-sensitive and resistant rats regardless of dietary sodium content (McCarty and Saavedra, 1982). Interestingly, a high sodium diet blunted the catecholamine response to immobilization stress in the Dahl salt-sensitive rat, but caused a greater increase in the Dahl resistant rat. Even though plasma catecholamine levels were reduced in both salt sensitive and resistant rats on a high sodium diet when samples were taken following decapitation, these authors concluded that there is no compelling evidence suggesting a critical role for the sympathetic nervous system in sodium-induced hypertension in the Dahl rat.

In the present study there is a suggestion of reduced sympathetic activity associated with a high sodium diet in that it appears that this diet blunts the plasma catecholamine response to stress in the WKY subjects compared to their normal sodium controls. Indeed, increased dietary sodium reduced epinephrine levels in WKY during both the control and stress session. The diminished cardiovascular reactivity to the CS exhibited by this group may be a reflection of this dampened

sympathetic activity. Also, the low levels of catecholamines may represent a compensatory mechanism to reduce autonomic functioning in the face of two stressful environmental stimuli. The nature of this mechanism remains unclear, but may involve a reduction in the availability of the biosynthetic enzymes tyrosine hydroxylase and phenylethanolamine-N-methyl transferase, or an alteration in the rates of uptake and metabolism, which has been associated with a high sodium diet in Dahl salt-resistant rats (McCarty and Saavedra, 1982). With respect to BHR, increased dietary sodium appears to have little effect on the plasma catecholamine response to stress. This observation is consistent with previous work suggesting that BHR on a high sodium diet show a similar norepinephrine response to cold stress when compared with normal sodium counterparts (Lawler et al., 1985).

With respect to the renal function data, the most interesting results concern the slight antinatriuresis and decrease in RPF exhibited by BHR on a high sodium diet. In contrast, WKY on either diet showed no difference in these variables during control or stress sessions. Since these variables have never before been measured in the BHR, the most meaningful discussion of these results is in the context of what is known about the behavior of its SHR and WKY parents.

When SHR are stressed with blasts of air they retain more sodium and increase renal sympathetic nerve activity compared to similarly treated WKY (Koepke & DiBona, 1985; Lundin & Thoren, 1982). Furthermore, since glomerular filtration rate and renal plasma flow were not altered and since renal denervation abolished the

antinatriuretic response, it appears likely that the sodium retention was due to increased renal sodium reabsorption mediated by the renal nerves (Koepke & DiBona, 1985). In the present study, BHR showed no alteration in renal functioning as a result of stress. It appears therefore that the BHR respond more similarly to their WKY parent in this regard. Alternatively, the other studies mentioned utilized air stress rather than mild cutaneous electric shock. A difference in the nature of the stressors used cannot be excluded as a possible explanation for the observed results. It is also possible that a more dramatic response adapted out across the week of conditioning.

A high sodium diet in conjunction with stress has been demonstrated to produce an even greater antinatriuretic response as well as a greater increase in renal sympathetic nerve activity in conscious SHR (Koepke & DiBona, 1985). In the present study, the interaction of a high sodium diet and behavioral stress resulted in a slight sodium retention and decrease in renal plasma flow in BHR that was not observed in similarly treated WKY. Thus, it appears that a high sodium diet is required before the BHR exhibits an antinatriuretic response to behavioral stress. Even though the magnitude of the response is not as great as that observed in SHR, a potentially pathogenic role for this sodium retention in the BHR cannot be excluded. Specifically, it remains to be determined whether the combination of increased dietary sodium and behavioral stress interact at the level of renal functioning to exacerbate the development of hypertension in the BHR. The examination of this hypothesis would

ideally involve a study of renal sympathetic nerve activity in BHR during control and stress periods and the effects of renal denervation on sodium excretion. Furthermore, recent evidence suggests that beta-two adrenoceptors in the posterior hypothalamus are involved in the mediation of increased renal sympathetic nerve activity and sodium retention to environmental stress in SHR (Koepke, Jones, & DiBona, 1986). Therefore, the role of central nervous system mechanisms in the elaboration of these responses represents another avenue of study.

Though not formally pursued in this experiment, the mechanisms by which a high sodium diet may produce hypertension in the BHR are of interest. Since BHR on a high sodium diet also had relatively low heart rate, it is reasonable to suggest that the hypertension is not a result of increased cardiac output. This issue also may be addressed in terms of what is known about the nature of the responses to a sodium challenge in the parents of the BHR, the WKY and SHR. Chrysant et al. (1979) studied hemodynamic variables (cardiac output and total peripheral resistance) in WKY and SHR on a high sodium diet for three months. These investigators reported an increase in blood pressure in SHR attributable to an elevation in total peripheral resistance. WKY, on the other hand, showed an elevated cardiac output which was offset by a compensatory drop in resistance, resulting in no change in blood pressure. Furthermore, Limas et al. (1980) reported that a high sodium diet accelerates the development of hypertension in the SHR and produces a significant degree of vascular lesions not found in similarly treated WKY. These studies suggest that if the BHR is

responding similarly to its hypertensive parent, either or both of these mechanisms may be involved in the hypertensive process.

An important question, and one not addressed by the present study, is whether the sodium-induced hypertension in the BHR is sustained after removal of the diet. When made hypertensive with a conflict paradigm, the blood pressure of the BHR remains elevated for at least a 10 week follow-up period following termination of the paradigm (Lawler et al., 1981). The demonstration of permanent sodium-induced hypertension in the BHR would strengthen the case for the use of this animal as a model for human essential hypertension. Another question relevant to human essential hypertension concerns the proportion of BHR that demonstrate sodium sensitivity. It has been observed in pilot experiments and in one subject in the present study that not all BHR develop hypertension when given a sodium challenge. This bears a striking resemblance to the human condition in that only a subset of borderline hypertensives and those individuals with a family history of hypertension exhibit increases in blood pressure when placed on a high sodium diet. An important experiment in this regard would be to document the prevalence of sodium sensitivity in a large population of BHR and delineate the physiological mechanisms that are responsible for producing and preventing an increase in blood pressure.

The ability of environmental manipulations such as psychologically stressful paradigms and increased dietary sodium to influence blood pressure has been well documented. The various approaches this interest has taken were illustrated in the Introduction. Given the

somehat voluminous nature of this body of literature, it is surprising that few studies have addressed the effects on blood pressure of combining these two environmental stressors, i.e., a sodium challenge along with a stressful behavioral paradigm. The experiments that have taken this approach have either reported negligible results (Anderson et al., 1982) or have studied primarily variables other than blood pressure, e.g. brain catecholamines (Ely & Weigand, 1983). It is apparent from the studies reviewed earlier that an investigation of the interaction of these two variables was appropriate. The present experiment represents only one of many approaches that need to be investigated in untangling the complexities of essential hypertension.

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APPENDIXES

APPENDIX A

PROTOCOL FOR METABOLIC STUDIES

Metabolic studies are conducted over a 48 hour period. The first 24 hours is an adaptation run. A few 100 ml beakers should be prepared so that any bugs can be worked out before the collection run, which is the second 24 hour period. All subjects should be placed in cages during the adaptation run so that they can adjust to the metabolic cages and food.

1. Preparation of beakers (100 ml).

   a. Place 700 ul of 6NHCL in beaker using repeatable pipetter. Prepare 6NHCL by mixing 50% concentrated HCL with 50% distilled water. 30 ml should be sufficient for 16 subjects.

   b. Place 4-5 ml of mineral oil in beaker using glass 10 ml pipette.

2. Preparation of food.

   a. Crush food with hammer and then crush further in

      blender until powder. A tried and true method is to put some food in a cloth and crush it with the hammer on the floor.

   b. Mix powder with water in blender;setting is "liquefy". The ratio is two cups of food : four cups of water. Mixture should be soupy but not watery.

3. Preparation of cages.

   a. Weigh subjects and food and water containers as they are being placed on rack.

   b. Place subject labels on each cage.

   c. Place beaker under each cage so that boli will go down spout and miss beaker.

4. Collection of data.

   a. Weigh each subject and food and water container upon removal from rack.

b. Pour urine into 100 ml graduated cylinder.

c. Urine and oil will separate. Read and record volume of urine. Typical readings for subjects on a normal diet range from 30-50 ml/24hr. On a high salt diet the values range from 60-90 ml/24hr. When reading the volume, read the meniscus.

d. Aspirate mineral oil with pump and disposable Fisher pipettes.

e. Pour about 10-15 ml of urine into test tube and cover with Parafilm. Make sure subjects number is on tube.

f. Pour another aliquot into small beaker and aspirate any surface oil. Read pH. Calibrate Ph meter with pH 4 (clear) and pH 7 (yellow) buffers. pH 4=4.1 and pH 7 =7. pH electrode should have rubber sleeve pulled down to expose opening. Calibrate at beginning and in middle of run. Typical pH readings are around 1.5 to 3.5.

g. Centrifuge tubes, six at a time, in refrigerator for 20 minutes on setting of 70.

h. Use Fisher disposable pipettes to get aliquot of urine and place in microfuge tube. Put remainder of urine in vial, being careful not to stir up sediment at bottom of tube. Put all collected urine samples in freezer in surgical suite of lab.

POINTS TO KEEP IN MIND. Beakers and test tubes should have subject numbers on them. Storage tubes should have subject number, date and name of study on labels. All labels should be secured with Scotch tape. All glassware, labels blender, data sheets, mineral oil and 6NHCL can be found in cabinet in A303A. Finally, plan on at least 2 hours to get things organized for a metabolic run and another 2 hours to collect, measure and store the samples at the end of the run.

APPENDIX B

DETERMINATION OF INULIN

1. Reagent.

   a. Slowly add 250 ml concentrated sulfuric acid to 125 ml distilled water in an ice bath with constant stirring. This should be done in a dark flask.

   b. In another dark flask, place 125 ml concentrated sulfuric acid and slowly sprinkle 2 grams anthrone into flask at a rate so that clumping does not occur. Stir until dissolved (5-10 minutes).

   c. Slowly add the acid-anthrone solution to the diluted acid solution. Be sure to keep this cold because overheating can destroy the reagent.

   d. Stir until well mixed and store in a dark bottle in refrigerator. Lasts about 2 weeks.

2. Standards.

   1, 2, 3, 4, and 5 mg% inulin, blank is distilled water
   Make fresh weekly.

3. Preparation of Samples.

   a. Sample Collection.

      1. Collect blood into heparinized tube.
         Seal end, spin, and record hematocrit.

      2. Urine is collected into small vial containing distilled water. The amount of water will vary depending upon inulin concentration and urine flow rate.

   b. Deproteinization of Plasma.

      1. Label small cups and add small amount of water and mineral oil.

      2. Add 800 ul of 20% trichloro-acetic acid.

      3. Add 50 ul of plasma.

4. Let stand for 10-15 minutes.

5. Break up precipitated plasma into 20 ul microcap tube and pipette samples into 2 ml of distilled water previously dispensed into plastic tube.

c. Urine.

1. The urine is diluted further by pipetting 2 ul into 4 ml of distilled water.

4. Analysis.

a. Place the 2.5 anthrone reagent in glass test tubes (1 for each sample) and chill in ice water slurry.

b. Add .5 ml distilled urine or diluted deproteinized plasma to each tube, cover, and mix vigorously until dissolved. The stirring should be done after pipetting duplicates. Leave tubes in ice bath until incubation.

c. Incubate sample at 75° C for 5 minutes, return to ice bath for 5 minutes, remove and after samples reach room temperature, read at 620 um. The color is stable for 3 hours.

APPENDIX C

DETERMINATION OF PARA-AMINOHIPPURIC ACID (PAH)

1. Reagents.

   1. 1.2 N HCL: Add 50 ml of 37-38% HCL to 500 ml water.
   2. 100 mg% $NaNO_2$: 100mg/100 ml water; make fresh daily.
   3. Ammonium sulfamate: 500 mg/100 ml water; refrigerate and make fresh every 2 weeks.
   4. 100 mg n-(1-naphthyl) ethylenediamine dihydrochloride / 100 ml water. Refrigerate in dark bottle; lasts 2 to 3 weeks.

2. Standards.

   1. Stock solution: 25 mg PAH/100 ml water.
   2. Working solutions: (1) 1.05% mg% PAH, (2) .10 mg% PAH, (3) .15 mg% PAH and (4) .20 mg% PAH.

3. Preparation of Samples.

   a. Plasma: Use small centrifuge tubes.

      In small centrifuge tube, add 10 ul plasma + 200 ul water.
      Cover. let stand for 10 min. , then centrifuge for 5 min.
      Transfer 175 ul of supernatant into .5 ml storage tube.
      Refrigerate until analysis. Dilution factor is 24.

   b. Urine:

      Add 1 ul urine to 2 ml water in 5 ml test tube. Make duplicate.
      Refrigerate until analysis. Dilution factor is 2001.

4. Analysis.

   Analysis of plasma and urine samples must be run separately using a set of standards with each run.

   a. Plasma: To .5 ml storage tube containing 175 ul of diluted sample (or water blank)

      Add 20 ul 1.2 N HCL.
      Add 10 ul $NaNo_2$ and mix gently.
      Between 3 and 5 min. later, add 10 ul ammonium

sulfamate and mix gently.
Between 3 and 5 min. later, add 10 ul ethylene-diamine dihydrochloride.
Cap and mix vigorously.
Color development is maximal in 5 min. The color is stable for several hours.
Read in micro-cuvettes at 540 nm. Plasma blank (sample taken before PAH infusion started) must be subtracted from plasma readings.

b. Urine: To 5 ml test tube containing 2 ml of diluted sample (or water blank).

Add .5 ml 1.2 N HCL.
Add 200 ul $NaNo_2$ and mix gently.
Between 3 and 5 minutes later, add 200 ul ammonium sulfamate and mix gently.
Between 3 and 5 minutes later, add 200 ul ethylenediamine dihydrochloride.
Cap and mix vigorously.
Color development is maximal at 5 minutes. The color is stable for several hours.
Read in macro-cuvettes at 540 nm.

APPENDIX D

PROCEDURE FOR CATECHOLAMINE ASSAY

1. Reagents

   1. THAM--Fisher T395
   2. EGTA Na_2--Sigma E3251
   3. $MgCl_2$ $6H_2O$--Fisher M33
   4. Glutathione--Sigma G4251
   5. Boric Acid--Matheson Coleman & Bell (MCB) BX865
   6. EDTA Na_2--Mallinckrodt 4931
   7. Metanephrine HCl--Sigma M8625
   8. Normetanephrine HCl--Sigma 7127
   9. Toulene--Fisher T-324
   10. Isoamyl Alcohol--Fisher A-393
   11. Glaceol Acetic Acid--Fisher A-38
   12. Sodium Periodate--Fisher S-398
   13. Glycerin--Fisher G33
   14. Ammonium Hydroxide--Fisher A669
   15. HCl--Fisher A144
   16. Epinephrine--Fisher SO-F-148
   17. Norepinephrine (100 ug/ml) BIORAD
   18. ^3H SAM--New England Nuclear (5-15 uCi)
   19. Ethanol--Chem Stores
   20. Methanol--Chema Stores
   21. Silica Gel Plates (10 x 20 cm 25 u) ANALTECH
   22. Benzene--Fisher B-242
   23. Tert Amyl Alcohol--Fisher A-730
   24. Methyl Amine (40%)--Fisher M223
   25. Liquifluor--New England Nuclear NEF903
   26. H_2SO_4 (pH 2):ethanol (9:1)

2. Daily Working Solutions

   a. Assay Buffer: Tris
      Add 6.05 gm of THAM, 1.9 gm EGTA-Na_2, and 3.05 gm $MgCl$-L_2-$6H_2O$ to approximately 20 ml distilled water. Adjust pH to 8.3 with concentrated HCl or NaOH. Bring volume to 25 ml with water.

   b. Glutathione Solution: .1 Molar
      Add 30.7 gm of reduced glutathione to a small test tube and add 1 ml of water. Vortex lightly and keep on ice.

c. Stopping Solution: 1.0 m Boric acid + .2 m EDTA-Na_2
1. Add: 1.55 gm boric acid + .93 gm EDTA-$2H_2O$-Na to

d. Metanephrine & Normetanephrine: .2 umoles/50 ul of borate buffer, which is .934 mg/ml of Met and .88 mg/ml of NorMet. For every 5 ml of the stopping solution add 4.67 mg of metanephrine and 4.4 mg of normetanephrine. Prepare prior to to use.

e. Toluene-isoamyl alcohol (3:2)
Combine 300 ml of toulene and 200 ml of isoamly alcohol.

f. Acetic Acid: .1 N
Bring 2.95 ml of glacial acetic acid up to 500 ml with water.

g. Sodium Periodate Solution: 4% w/v $NaIO_4$
1 gm of $NaIO_4$ up to 25 ml with water. Prepare fresh.

h. Ammonium Hydroxide Solution: .05 M NH_4OH
1 ml of NH_4OH up to 300 ml with water.

i. HCl: 1.0 N
1. 8.3 ml of concentrated HCl up to 100 ml.
2. .01 HCl: 1.0 ml of 1.0 N HCl to 100 ml.
3. .001 HCl: 1.0 ml of 1.0 N HCl to 1000 ml.

j. Epinephrine and Norepinephrine Standards: 100 pg/10 ul
1. 200 ul of Epinephrine (50 ug/ml) and 100 ul of norepinephrine (100 ug/ml) are brought to 50 ml with .01 N HCl in a volumetric.
2. Bring 5 ml of this solution to 100 ml with .001 N HCl in a volumetric.
3. 10 ul of (2) will give 100 pg of E and NE.

k. Enzyme: COMT
Prepared according to the procedure of Axelrod and Tomchick (1958).

l. H_2SO_4-Ethanol Solution: (9:1)
Prepare a H_2SO_4 solution of pH=2. Add concentrated H_2SO_4 to water (250 ml) until pH=2. Use 9 ml of this for every 1 ml of Ethanol (95%).

m. Toulene--Liquifluor (1000:50 v/v)
Add 50 ml of Liquifluor to 1000 ml of toulene.

3. Reaction

a. Turn on water bath and set at 37 degrees C.

b. Prepare two sets of test tubes (13 x 100 cm). Each sample should be done in duplicate. Label tubes prior to this part of the procedure.

c. Remove enzyme, samples, buffer, and standard plasma from freezer. Thaw and keep on ice. Thaw $_3$H SAM only before use.

d. Prepare the enzyme cocktail in a round bottom 13 x 100 mm tube. Add in the following order in amounts sufficient for the assay.

Assay	x1	x20	x50	x100
Buffer	5 ul	100 ul	250 ul	500 ul
Glutathione	1 ul	20 ul	50 ul	100 ul
^{3}H SAM	5 ul	100 ul	25 ul	500 ul
H_2SO_4-Ethanol	5 ul	100 ul	250 ul	500 ul
Enzyme	24 ul	480 ul	1.2 ul	2.4 ul

e. Incubation mixture
The first set of tubes will receive the following:
1. Blanks
   a. 50 ul of sample vehicle (i.e., water that is mixed with EGTA-Glutathione in the same concentration as that of the blood sample).
   b. 40 ul of enzyme cocktail.
   c. 10 ul of .001 N HCl.
2. Standards (internal)
   a. 50 ul of plasma.
   b. 40 ul of enzyme cocktail.
   c. 10 ul of 100 pg standard.
3. Plasma samples
   a. 50 ul of sample plasma.
   b. 40 ul of enzyme cocktail.
   c. 10 ul of .001 N HCl.

f. Incubation
75 minutes at 37 degrees C in oscillating water bath.

g. Stopping the Reaction
Place reaction tubes in ice bath and add 50 ul of the Borate stopping solution that contains metanephrine and normetanephrine carriers.
Gently shake the tube rack.

4. Extraction

The second set of tubes will be used in this step. They will each receive 100 ul of .1 N acetic acid.

a. Add 2ml of toulene-isoamyl alcohol (3:2) to incubation tubes. Vortex well and centrifuge.

b. Quick freeze:
Prepare an ice bath of methanol and dry ice. Methanol level should only be deep enough to cover the bottom of test tubes (.5-1 inch).
   1. Submerge tube to level of aqueous phase.
   2. Blot outside of tube. Decant the organic phase into its corresponding tube containing the .1 N acetic acid.
   3. Decant one tube while next tube is in ice bath.

c. Vortex and centrifuge the acetic acid, organic solution.

d. Quick freeze and aspirate off arganic phase without touching suction pipette to pellet.

e. Add 1 ml of toulene-isoamyl alcohol to thawed aqueous phase.

f. Vortex and centrifuge.

G. Quick freeze, aspiration, and discard organic phase as in D above. The assay may be stopped at this point. Cap tubes and keep at -20 degrees C.

5. Thin Layer Chromatography

a. Add 100-150 ul of ethanol to the thawed acetic acid from section IV-G above.

b. Slowly pull acetic acid-ethanol solution into syringe. Do not pull excess air into syringe. Should have small bubble on end of plunger.

c. Turn on dryer and spotter.

d. Place dryer approximately 3 cm from plates and set temperature control between 200-300.

e. Position syringes so that tips touch TLC plates. Set plunger speed to slowest speed.

f. Prepare 44 ml of developing solution (6:2:3) in a 50 ml graduate.
   1. 24 ml of tertiary amyl alcohol.
   2. 8 ml benzene.
   3. 12 ml methylamine.
   4. Mix gently.

g. Put developing solution in developing tank immediately before use. Carefully place plates in tank. Develop until solvent runs the entire plate (40-50 minutes).

h. Dry plates and visualize spots under UV. Utilizing a scapel, carefully outline the spots.

i. Scrape spots into acid washed scintillation vials. The metanephrines are the top zone and normetanephrines are the bottom zone.

6. Oxidation

a. Add 1 ml of .05 M NH_4OH to vials. Vortex vigorously.

b. Line up vials and add 50 ul of 4% periodate solution at time intervals. Vortex vigorously.

c. Stop reaction by adding 50 ul of 10% glycerol solution, five minutes after step B.

d. Add 1 ml of .1 M acetic acid to each vial and vortex.

e. Add 10 ml of toulene-liquifluous (1000:50 v/v) to each scintillation vial . Shake by hand for about 10 seconds.

f. Count each vial for 2-10 minutes.

VITA

Brian John Sanders was born in Peoria, IL on August 19, 1958. He was educated in the Carthage, Illinois school system and was graduated from Carthage High School in 1976. After attending Western Illinois University in Macomb, Illinois for one year, he transferred to Cornell College where he received a Bachelor of Arts degree in 1980. The author entered graduate school at the University of Tennessee in the fall of 1980 and received a Doctor of Philosophy degree with a major in psychology in June of 1986. Following his graduate training, the author was awarded a training fellowship from the Iowa Cardiovascular Center at the University of Iowa in Iowa City, Iowa.