The Interactive Effects of Dietary Sodium Chloride and Calcium on the Cardiovascular Responses to Stress

by

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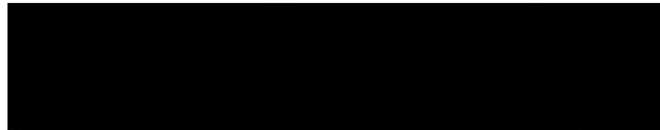

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LIST OF ABBREVIATIONS

General

Analysis of Variance	ANOVA
Anterior Hypothalamus	AH
Central Nervous System	CNS
Cerebral Spinal Fluid	CSF
Counting Period	CP
Epinephrine	EPI
High Pressure Liquid Chromatography	HPLC
Intracerebroventricular	ICV
Norepinephrine	NE
Nucleus Tractus Solitarius	NTS

Conditioning Terms

Conditioned Stimulus	CS
Conditioned Response	CR
Unconditioned Stimulus	US
Unconditioned Response	UR

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ABSTRACT

Dietary sodium chloride and calcium manipulations have been shown to alter blood pressure in human hypertensive subjects. Sodium Chloride exacerbates hypertension while calcium attenuates high blood pressure. Moreover, the ability of either nutrient to alter blood pressure appears to be influenced by the intake of the other. Sodium chloride's effects are most pronounced in hypertensive individuals with lower serum calcium levels and lower calcium intake, while calcium supplementation is more effective in lowering the blood pressure of NaCl-loaded patients. Stress-induced blood pressure responses are also altered with changes in NaCl and calcium intake such that animals fed a high sodium chloride diet or a calcium deficient diet show exaggerated pressor responses. The following study was designed to investigate the possible mechanisms responsible for diet-mediated changes in blood pressure and stress-reactivity.

Spontaneously hypertensive rats (SHR) and their normotensive control strain, the Wistar-Kyoto rat (WKY), were maintained on one of four diets varying in sodium chloride and calcium. The rats were allowed ad lib access to diets containing either normal or high NaCl combined with

either low or high calcium. After 8 weeks of dietary manipulation, blood pressure was measured at rest, directly after handling and during restraint. Blood pressure responses to novel, conditioned and unconditioned stimuli were also assessed. In addition, inhibition of spontaneous locomotor activity upon exposure to a novel environment was used as an indirect measure of stress-reactivity.

In order to investigate the origin of dietary influences on blood pressure and stress-reactivity, both sympathoadrenal and peripheral vascular reactivity were measured. Serum and brain electrolyte levels were determined to provide an index of diet-associated changes in circulatory and central nervous system electrolyte availability.

The results indicated that high NaCl intake was associated with greater blood pressure as well as larger stress responses to novel and conditioned stimuli. In addition, sodium chloride increased the inhibition of locomotor activity upon exposure to a novel environment. Increased calcium intake reduced blood pressure in both strains and attenuated NaCl-induced increases in blood pressure and pressor responses to novel stimuli in the SHR.

Sodium chloride-loaded SHRs showed greater circulating epinephrine levels during rest and restraint, as well as more exaggerated increases in epinephrine release with exposure to stress. Moreover, they showed enhanced blood

pressure responses to exogenous norepinephrine administration. Collectively, these findings indicate that the higher blood pressure and more pronounced stress-responses observed in NaCl-loaded hypertensive animals were likely due to greater tonic adrenal activity combined with exaggerated adrenal and peripheral vascular reactivity to stress.

Though calcium intake did not significantly effect autonomic or vascular reactivity, it did alter serum and brain electrolyte concentrations suggesting that calcium's hypotensive effects may have been mediated by altered electrolyte availability in the blood and within the central nervous system.

Introduction

Overview of Stress and Hypertension

Complications related to hypertension are a leading cause of morbidity and mortality in the US. It is estimated that 50% of the population over the age of 50 is presently afflicted with high blood pressure making it one of the most prevalent diseases in our society (Robins, Cotran, & Kumar, 1984). Unfortunately, the cause of primary or "essential" hypertension is unclear. Consequently, substantial efforts have been made to uncover the origins of the disease.

Accumulating evidence has indicated that stress may make an important contribution to the development of hypertension. However, the link between behavioral stress and high blood pressure is still controversial (Lawler & Baker, 1981). There remains, at present, a lack of direct experimental data supporting a causal relationship between stress and hypertension. Epidemiological studies have provided evidence that an association between stress and high blood pressure does exist.

Reports have shown a lower incidence of hypertension among individuals inhabiting, presumably low stress, non-industrialized cultures (Henry, & Cassel, 1969; Page,

1980). Moreover, long-term studies have demonstrated progressive increases in blood pressure among individuals inhabiting initially low stress, agrarian societies undergoing modernization. (Beaglehole, Salmond, Hooper & Prior, 1973; Henry & Cassel, 1969; Page, Damon & Moellering, 1974). Within cultures, factors such as education level, job pressure and living conditions have been shown to contribute to elevated blood pressure. Moriyana, Krueger & Stamler (1971) noted an association between increased blood pressure and lower educational attainment. Likewise, blood pressure has been found to vary with socioeconomic status, with lower class families tending to have higher blood pressure than more economically stable families (Hypertension Detection, 1977). Additionally, a prospective study of air traffic controllers showed that more frequent hypertensive episodes resulting from job related stressors may have contributed to the increased incidence of hypertension observed among these individuals (Cobbs & Rose, 1973).

Though such reports implicate stress' involvement in hypertension, they have been criticized for their lack of attention to confounding variables such as diet, obesity, smoking, drug and alcohol intake as well as other risk factors (Page, 1983). More tightly controlled animal experiments have yielded little data demonstrating stress-induced hypertension. Baboons exposed to daily

sessions of shock avoidance were shown to have only transient elevations in pressure (Goldstein, Harris & Brady, 1977; Harris, Gillman, Findley & Brady, 1973). Similarly, Murray and Lawler (1978) were unable to change blood pressure in normotensive rats exposed to daily Sidman avoidance schedules for 11 weeks. More recently, Lawler and Cox (1985) failed to induce blood pressure changes in normotensive Wistar-Kyoto rats after 12 weeks of exposure to a conflict-avoidance paradigm. Moreover, daily sessions of hindquarter compression, designed to elicit neurogenic pressor responses, also failed to induce long-term blood pressure changes in dogs (Julius & Brent, 1988).

A few studies have shown marginal success in producing stress-induced hypertension. Mice kept in overcrowded housing conditions have been shown to develop mild hypertension. Removal of the mice from the environment precipitated the reduction of blood pressure back to normal levels. However, chronic hypertension was produced in mice maintained in the stressful environment for more than six months (Henry, Stephens & Santisteban, 1975). Likewise, Long-Evans rats exposed to shock-shock conflict schedules for 11 weeks develop slightly higher blood pressures which fell short of hypertensive levels (Buchholz, Lawler & Baker, 1979). Though these reports demonstrate limited support for the notion of stress-induced hypertension, the majority of the data indicate that stress alone may not be a sufficient

stimulus for hypertension. It is possible that normotensive animals have some protective mechanism that hinders stress from inducing hypertension.

Such a protective mechanism might be compromised in some subjects, leaving them more vulnerable to the effects of stress. For instance, it is conceivable that some inherited genetic defect might reduce the ability of homeostatic mechanisms to regulate blood pressure under conditions of stress (Folkow, 1982). This, in turn, could explain the lack of persuasive epidemiological and experimental data supporting a causal relationship between stress and chronic high blood pressure. Presumably, it would be difficult to demonstrate stress-induced hypertension when only a sub-set of the population has inherited the underlying homeostatic defect. Similarly, normotensive animal models lacking any predisposing regulatory deficiency would not be expected to exhibit hypertension with chronic stress exposure.

Several studies have provided evidence that blood pressure reactivity to stress as well as hypertension are influenced by heredity. Normotensive monozygotic (MZ) twins were shown to have a greater concordance for changes in blood pressure to a cold pressor stress test than less genetically related dizygotic (DZ) twins (McIlhany, Shaffer & Hines, 1975). MZ twins also had a greater concordance for heart rate reactivity to a session of stress provoking video

games. Interestingly, those twins having a parental history of high blood pressure exhibited greater reactivity than non-risk twins (Carroll & Hewitt, 1985). The notion that inherited elevations in reactivity contribute to hypertension was further supported by Falkner, Onesti, and Angelakos (1981) who found that a significantly larger portion of highly reactive subjects with a positive family history went on to develop hypertension compared to low risk, low reactive subjects.

Genetically hypertensive animal strains show a similar hyperresponsiveness to stress. One such experimental model, the spontaneously hypertensive rat (SHR), shows much larger blood pressure and heart rate responses to aversive stimuli than its normotensive control strain, the Wistar-Kyoto rat (WKY) (Chiueh & McCarty, 1981; Hallback & Folkow, 1974; Hatton, Bucholz & Fitzgerald, 1981; Kvetnansky, McCarty, Thoa, Lake & Kopkin, 1979; McCarty & Kopkin, 1978; Yamamoto, Akabane, Yoshimi, Nakai & Ikeda, 1985; Yamamoto, Nakai & Natsume, 1987). Moreover, SHRs show increased blood pressure responses to psychological stress as early as 6 weeks of age, before the onset of hypertension, suggesting that increased reactivity is responsible for initiation of the disease (McCarty & Kopkin, 1978a).

Theories of Stress-Induced Hypertension

Folkow (1978, 1982) has developed a model illustrating

how elevated stress reactivity might precipitate the development of hypertension. He suggested that hyperresponsive individuals have a lower threshold for stress response activation. Consequently, they experience more severe and more frequent activation of cardiovascular stress responses, and thus increased blood pressure, upon exposure to environmental stimuli. Within the vasculature, the resulting elevation in the average pressure load induces structural changes which serve to reduce excessive strain. These changes, though adaptive to the vasculature, narrow the lumen of the vessel causing a sustained increase in pressure by increasing peripheral resistance to flow. Eventually, increased pressure results in end organ damage, primarily in the heart and cerebral vasculature.

An alternative view suggests that hypertension originates from exaggerated renal responses to stress culminating in increased water and sodium retention. In accordance, studies have shown greater stress-induced anti-natriuretic responses among hypertensive rats compared to age-matched, normotensive controls. Such strain differences were abolished with renal denervation or propranolol treatment indicating sympathetic mediation of the anti-natriuretic effect in the hypertensive animal (Koepke & DiBona, 1985; Lawler & Baker, 1981; Lundin & Thoren, 1982). Light, Koepke, Obrist & Willis (1983) have similarly shown a decreased natriuresis among human subjects

at high risk for hypertension. Specifically, individuals with borderline hypertension or a familial history of hypertension who also displayed high heart rate reactivity were shown to have an anti-natriuretic response during exposure to psychological stress. In contrast, low risk, low reactive individuals tended to have increased sodium loss during stress. Furthermore, reduced sodium excretion in the high risk group was strongly correlated with changes in heart rate suggesting that both were mediated by excessive sympathetic reactivity.

Another theory proposes that genetically predisposed individuals exhibiting heightened sympathetic reactivity respond to stress with excessive vascular constriction of renal afferent arterioles. Subsequent reductions in filtration are thought to lead to increased sodium and water retention. The resulting volume mediated increase in cardiac output presumably stimulates autoregulatory mechanisms aimed at reducing excessive perfusion of peripheral tissue (Guyton & Coleman, 1974). In accordance with Folkow's theory (1982), such chronic increases in resistance would stimulate vascular hypertrophy and sustain hypertension.

Sympathetic Outflow vs. Vascular Sensitivity

Though prevailing theories emphasize the importance of cardiovascular reactivity in initiating vascular changes

associated with hypertension (Folkow, 1982; Light & Koepke, 1983), the mechanisms mediating such exaggerated responses are not clear. One view contends that abnormal reactivity is a consequence of excessive activation of sympathetic outflow (Chiueh & McCarty, 1981; Goldstein, 1981; Hallback & Folkow, 1974; Kvetnansky et al., 1979; McCarty & Kopkin, 1978; Sakaguchi, LeDoux & Reis, 1983; Tuck, 1986). It may be that dysfunction of regulatory mechanisms governing autonomic activity allow excessive sympathetic stimulation of the cardiovascular system during stress. Alternatively, elevated reactivity may result from increased vascular responsiveness to stress-related stimuli. In this instance, heightened cardiovascular reactivity would result from excessive vascular constriction, and or abnormally high heart rate in response to normal neural activation.

It should be recognized that altered sympathetic activity and vascular reactivity are not mutually exclusive mechanisms of elevated reactivity. But, it is questioned whether elevated vascular responsiveness significantly contributes to the initiation of hypertension. It has been argued that increased vascular reactivity arises secondarily from blood pressure-induced compensatory structural changes (Folkow, Hallback, Lundgren, Sivertsson & Weis, 1973). More specifically, structural changes causing narrowing of the lumen result in an apparent increase in vascular sensitivity. Because resistance is inversely proportional

to the arteriole radius raised to the 4th power, small decrements in lumen radius result in substantial changes in pressure. Since the hypertrophied lumen is already smaller in comparison to a healthy vessel, blood pressure changes occurring with stress-induced vascular constriction become magnified. As a result, what appears to be a "functional" elevation in vascular sensitivity is, in actuality, a mechanical response to elevated blood pressure (Folkow, 1978).

Alternatively, others believe that a functional vascular hyperreactivity, independent of arteriole structural change, characterizes the pre-hypertensive state (Berecek, Schwertschlag & Gross, 1980; Doyle & Fraser, 1961; Lais & Brody, 1975; Mendlowitz & Naftchi, 1958; Mulvany, Aalkjaer & Christensen, 1980). Such a pre-hypertensive vascular hyperreactivity is difficult to separate from structurally derived changes given that vascular remodeling has already commenced by the time hypertension is recognized. Nevertheless, evidence demonstrating functional differences in vascular reactivity does exist. In 1961, Doyle and Fraser demonstrated a greater vascular reactivity among normotensive children of hypertensive individuals compared to offspring of normotensive subjects. Apparently, some inherited predisposing factor was responsible for increased reactivity since pressure-induced vascular hypertrophy presumably had not yet developed in the

normotensive child. Abnormalities in vascular reactivity have also been found in experimental models of hypertension. The SHR was shown to exhibit an increased vascular reactivity to bolus injections of norepinephrine (NE) at 6, 12 and 24 weeks of age. However, concomitant structural changes were not observed until the 12th week of age indicating a pre-hypertensive vascular hypersensitivity (Mulvany et al., 1980). Similarly, Lais and Brady (1975) demonstrated a three fold increase in NE sensitivity of SHR hindquarter vascular beds compared to WKY controls. The two strains showed no difference in response to other vasoconstrictor agents as would be expected if reactivity differences were due solely to structural hypertrophy.

The majority of evidence suggests that increased stress reactivity is primarily the result of elevated sympathetic outflow. Both essential hypertensive patients and SHRs show elevated catecholamine release upon exposure to mental stress (Chiueh & McCarty, 1981; Goldstein, 1981; Kvetnansky et al., 1979; McCarty & Kopkin, 1978a, 1978b; Tuck, 1986; Yamamoto et al, 1985). In addition, direct recordings of renal efferent nerve activity in the SHR demonstrate exaggerated sympathetic responses during psychological stress (Lundin & Thoren, 1982). The literature indicates that increased neural responsiveness exists prior to the onset of hypertension and likely plays a role in the disease process. McCarty and Kopin (1978a)

found that 6-week-old SHRs had significantly higher catecholamine responses to handling and foot shock than did age matched WKYs. Similarly, enhanced sympathoadrenal reactivity was demonstrated among 8-week-old SHRs exposed to short-term shaker stress (Yamamoto et al, 1987). Sympathetic nerve activity arising from electrical stimulation of the posterior hypothalamic area is also enhanced in young SHRs (Takeda & Bunag, 1978).

More recently, it has been postulated that excessive stress-induced sympathetic outflow leads to vascular structural alterations without chronic elevations in blood pressure. Studies demonstrating the trophic growth factor effect of catecholamines has promoted the notion that sympathoadrenal activity stimulates hypertrophy and hyperplasia of smooth muscle cells lining the vasculature (Beran, 1984; Yamori et al., 1984; Yamori, Mano, Nara & Horie, 1987). Interestingly, in vitro studies have demonstrated that β-adrenergic stimulation of hypertrophy and hyperplasia are increased in SHR smooth vascular cell cultures (Yamori et al., 1984). Moreover, NE induced polyploidy is accelerated in SHR cell cultures suggesting a greater sensitivity to sympathetic growth factors (Yamori et al., 1987). Accordingly, it may be predicted that elevated sympathetic outflow, as well as increased sensitivity to trophic factors combine to promote greater vascular structural changes in the SHR.

Sodium Chloride and Stress Reactivity

Sympathetically mediated vascular changes may be accelerated by dietary modifications. Increased NaCl intake appears to potentiate stress reactivity in a subset of the population. Falkner, Onesti and Angelakos (1981) showed that a high NaCl diet potentiated pressor responses among normotensive subjects with a family history of hypertension. Conversely, sodium chloride restriction among borderline hypertensives reduced blood pressure reactivity to mental stress (Ambrosioni et al., 1982). SHRs also demonstrate enhanced pressor responses to direct sympathetic nerve stimulation when fed a sodium supplemented diet (Gradin, Dahlof & Persson, 1986). Similarly, Koepke and DiBona (1985) found that SHRs given saline to drink showed increased renal nerve activity and consequently more substantial anti-natriuretic responses during stress.

Enhancement of stress reactivity via NaCl loading may result from sodium's effects on cardiovascular regulatory centers in the central nervous system (CNS). Numerous reports describe hypertensive responses with intracerebroventricular (ICV) infusions of sodium (Bunag & Miyajima, 1984; Ikeda, Tobian, Iwai & Goossens, 1978; Kawano & Ferrario, 1984; Miyajima & Bunag, 1984). Acute administration of hypertonic saline as well as chronic infusions of high sodium artificial CSF cause blood pressure

elevations in the rat (Bunag & Miyajima, 1984; Ikeda et al., 1978, Miyajima & Bunag, 1984). Similar infusions produce hypertensive responses in the chloralose anesthetized dog (Kawano & Ferrario, 1984). Accordingly, elevated Na+ cerebral spinal fluid (CSF) levels have been described in NaCl-sensitive essential hypertensive patients. Moreover, a direct correlation has been observed between CSF sodium content and blood pressure (Gotoh, Miyajima, Ohnishi, Fujishima & Kaneko, 1981).

The effects of CNS sodium may originate from altered autonomic integration at higher vasomotor regulatory centers. Localized injections of hypertonic saline directly into the nucleus tractus solitarius (NTS) of the rat cause transient elevations in blood pressure (Gavras, Bain, Bland, Vlahakos & Gavras, 1985). The NTS is responsible for integrating vagal afferent input with other neural activity and relaying the processed information to the hypothalamus (Brody, Barron, Faber, Hartle & Lappe, 1982). Pressor responses arising from hypertonic saline injections in the NTS may result from aberrant integration of various stimuli which, when presented to forebrain regulatory centers, cause changes in sympathetic output.

Sodium chloride induced increases in stress reactivity may result from altered anterior hypothalamic mediation of sympathoinhibitory function. Miyajima and Bunag (1984) found that chronic ICV administration of high sodium CSF

attenuated depressor responses induced with direct electrical stimulation of the anterior hypothalamus. Moreover, electrical stimulation of the ventromedial hypothalamus produced comparable pressor responses in both high and normal sodium fed groups suggesting that sodium dependent elevations in sympathetic tone originated from diminished sympathoinhibitory function rather than direct increases in sympathetic activation.

Wyss, Chen, Jin, Gist and Oparil (1987) have since demonstrated that dietary manipulations of sodium chloride are able to alter neural activity in the anterior hypothalamus (AH). SHRs maintained on a high (8%) sodium chloride diet for two weeks showed significant reductions in both NE and its metabolites within the AH compared to animals on a 1% diet. Such changes were accompanied by a significant increase in blood pressure among the high NaCl animals. These data suggest that sodium chloride reduces tissue stores as well as metabolism rates of neurotransmitters mediating sympathoinhibitory activity. Moreover, an additional 4 weeks of dietary manipulation produced significant elevations in AH NE stores among normotensive animals. Such long term changes were not seen in the SHR suggesting that compensatory mechanisms aimed at increasing sympathoinhibition in the face of high NaCl were impaired in the hypertensive animal.

In vitro studies have provided further evidence that

adrenergic activity is modified by sodium. α_2-adrenergic receptors, which have been linked to sympathoinhibitory depressor activity in brainstem and forebrain vasomotor centers (Young & Kuhar, 1980), show a decreased affinity for agonists when exposed to elevated sodium concentrations (Glossman, Lubbecke, Bellmann, Sattler & Doell, 1982; Greenberg, U'Prichard, Sheehan & Snyder, 1978; Rouot, U'Prichard & Snyder, 1980; Tsai & Lefkowitz, 1978). Theoretically, if increased sodium where able to exert the same effects on CNS adrenergic receptors as demonstrated in vitro, it would be expected that reduced α_2-adrenergic sensitivity, and hence diminished sympatho-inhibition, would result from a high NaCl diet.

Altered CNS catecholamine metabolism, as well as aberrant compensatory responses to sodium chloride load (Wyss et al., 1987), may explain the exaggerated increase in stress reactivity among sodium loaded SHRs (Gradin et al., 1986; Koepke & DiBona, 1985). Since the AH regulates depressor responses involved in blood pressure homeostasis (Brody et al., 1982), it seems likely that NaCl-induced dysfunction of this nucleus would impair reflex compensatory responses to pressor stimuli. Changes in the sensitivity of the baroreceptor reflex arc have been noted in NaCl-loaded hypertensive animals. Miyajima and Bunag (1987) demonstrated diminished reflex bradycardic responses to direct aortic afferent baroreceptor nerve stimulation in

hypertensive, NaCl-sensitive Dahl rats fed a high sodium diet. It is possible that the NaCl-dependent blunting of the baroreceptor reflex diminishes the animal's ability to regulate pressor responses to stress. Sodium acting at the NTS or AH could impair the ability of sympathoinhibitory centers to buffer sympathetic output initiated by stress. If such were the case, long term exposure to high NaCl intake would potentiate the effects of stress, thus providing greater mechanical and or trophic stimulus for vascular hypertrophy and hypertension.

Dietary Calcium and NaCl-Potentiated Reactivity

Increased calcium intake has been shown to reverse some of the neural phenomena found with excess dietary sodium chloride intake. Peuler, Morgan and Mark (1987) have recently demonstrated an amelioration of NaCl induced changes in blood pressure and baroreceptor function in the Dahl NaCl-sensitive rat (DS) using dietary calcium supplementation. They surmised that the calcium-dependent attenuation of hypertension resulted from diminished sympathetic activity since ganglionic blockade abolished blood pressure differences between calcium supplemented and control groups. Interestingly, Peuler et al. noted that reflex baroreceptor inhibition of renal sympathetic nerve activity was augmented in the high calcium group. It was hypothesized that calcium's hypotensive effects were most

likely due to the re-establishment of NaCl-impaired reflex sympathoinhibitory activity.

Further evidence that calcium acts on sympatho-regulatory centers in correcting sodium-induced hypertension was provided by Wyss, Chen, Meng, Jin, Jirakulsomchok and Oparil (1989) who noted that the addition of calcium to a high NaCl diet reversed NaCl-induced reductions in AH NE turnover. Blood pressure and plasma NE were also diminished to levels found in control animals. It may be hypothesized that by re-establishing catecholamine metabolism in sympathoinhibitory centers, calcium is able to restore proper buffering of stress-induced sympathetic activity.

Calcium and Hypertension

Though the molecular mechanisms responsible for calcium's hypotensive effects are not known, some theorists suggest that calcium may alter receptor activity. In vitro studies have demonstrated that divalent cations such as $Ca2+$ and $Mg2+$ are effective in increasing α_2-adrenergic receptor agonist affinity (Tsai & Lefkowitz, 1978). Increased calcium availability afforded through dietary supplementation could potentially counteract sodium-induced reductions in receptor agonist affinity. In so doing, calcium could re-establish adrenergic sympathoinhibitory activity in brainstem and higher forebrain areas.

Alternatively, calcium may compensate for sodium's

hypertensive effects by acting on other regulatory mechanisms. Calcium chloride infused directly into the CNS ventricular system induces a hypotensive response in dogs and rats. However, these responses are unaffected by vagotomy indicating that changes in baroreceptor activity are not responsible (Borowitz, Stebbins & Isom, 1987; Leusen, 1950). Instead, it has been suggested that calcium directly alters sympathetic regulation of vasomotor tone. Early reports showed that ICV calcium infusions in the vagotomized dog attenuated both vasomotor pressor and depressor responses caused by changes in carotid pressure or stimulation of the proximal end of the severed vagal nerve (Leusen, 1950). It may be that increased CNS calcium acts to dampen neural activity regulating cardiovascular reflexes.

These data are consistent with recent theories suggesting that elevated blood pressure reactivity to stress may arise from a primary deficit in calcium availability (McCarron, 1985). Several abnormalities in calcium metabolism have been described among hypertensive patients and animal models including: elevated calciuria (McCarron et al., 1980; McCarron, Yung, Ugoretz & Krutzik, 1981; Strazzullo, Nunziata, Cirillo, Giannattasio & Mancini, 1983), reduced total and serum ionized calcium (McCarron et al., 1981; McCarron, 1982; Wright & Rankin, 1982) as well as diminished bone mineral calcium content (Metz, Karanja &

McCarron, 1985). In addition, cellular alterations involving decreased calcium membrane binding (Orlov & Postnov, 1982, Postnov, Orlov, Sherchenko & Alder, 1977; Postnov, Orlov & Pukudin, 1979), increased ionic flux (Bhalla, Webb, Singh, Ashley & Bhroch, 1978; Noon, Rice & Baldessarini, 1978) as well as elevated intracellular calcium concentrations have been observed in experimental and essential hypertension (Bruschi et al., 1985; Zidek, Vetter, Zunkley & Losse, 1981). Collectively, these observations demonstrate an altered calcium handling, culminating in reduced extracellular calcium availability among hypertensive subjects.

Several studies have indicated that deficient extracellular calcium alters vasoconstrictor activity (Bohr, 1963; Holloway & Bohr, 1973; Webb & Bohr, 1978). A high extracellular calcium concentration has been found to promote binding of calcium ions to the membrane of excitable cells. This, in turn, is postulated to stabilize the membrane, reducing excessive ionic flux across calcium channels during depolarization (Webb & Bohr, 1978). Theoretically, in the calcium deficient state, reduced calcium binding in the sympathetic neural cell would destabilize the cell membrane. Excessive stress-induced depolarization of the destabilized cell would result in elevated calcium influx and exaggerated neurotransmitter release. Consequently, a greater degree of vascular

constriction and thus greater blood pressure reactivity would result.

This hypothesis is consistent with observations of enhanced pressor responses among hypertensive animals maintained on a low calcium diet (Hatton, Huie, Muntzel, Metz & McCarron, 1987; Huie, Hatton & Muntzel, 1987; Hatton, Scrogin, Metz, & McCarron, 1989). Hatton et al. (1989) noted exaggerated responses to handling and restraint stress among SHRs maintained on a calcium deficient diet. Additionally, calcium deprived SHRs have shown significantly greater classically conditioned pressor responses (Hatton et al., 1987) than calcium supplemented animals. A low calcium diet was also found to potentiate the development of hypertension in SHRs exposed to chronic psychosocial stress via overcrowding (Huie et al., 1987). These data support the notion that poor calcium handling combined with a calcium deficient diet facilitate sympathetic pressor responses to stress. Conversely, the same studies demonstrate that increased dietary calcium intake attenuates blood pressure reactivity to stress, possibly by compensating for defective calcium handling (Hatton et al., 1987, 1989; Huie et al., 1987). Dietary calcium dependent changes in stress reactivity may be responsible for reductions in baseline blood pressure seen in clinical and experimental trials with calcium supplementation (Ayachi, 1979; Belizan et al., 1983; McCarron et al., 1981; McCarron

and Morris, 1985; Resnick, Nicholson & Laragh, 1986). It may be hypothesized that decreased stress reactivity afforded by supplemental calcium reduces the stimulus for hypertrophy and hypertension, thereby lowering baseline pressure.

Interactive Effects of NaCl and Calcium

Additional evidence indicates that calcium's ability to lower baseline blood pressure may also depend upon sodium intake. In separate studies, Metz, Karanja and Morris (1986) as well as McCarron, Lucas, Schneidman, LaCour and Drueke (1985), have shown that increased dietary sodium is able to potentiate calcium's hypotensive effects in the SHR. Moreover, animals maintained on both high and low calcium diets demonstrate decreased pressure with increased NaCl intake. These data, coupled with experimental and clinical reports demonstrating calcium-dependent mediation of sodium-induced hypertension, suggest the existence of an important interaction between these nutrients (Peuler et al., 1987; Resnick et al., 1986; Wyss et al., 1989). Evidently, NaCl's hypertensive effects are most prominent when calcium intake is low (Resnick et al, 1986). In contrast, a higher sodium intake may enhance calcium's hypotensive effects (Metz et al., 1986).

If exaggerated stress reactivity potentiates the hypertensive process then changes in stress reactivity induced by calcium and NaCl should be accompanied by

parallel dietary influences on baseline blood pressure. It may be hypothesized than that supplemental dietary NaCl's ability to magnify calcium's hypotensive effects are mediated initially by a change in stress reactivity. Conversely, the exaggerated hypertensive response to increased NaCl intake among calcium deficient animals would presumably be reflected in an initial elevation in blood pressure reactivity. As yet, there are no published reports which have examined the effects of concurrent manipulations in NaCl and calcium on cardiovascular stress reactivity.

In summary, it has been suggested that the hypertensive process is accelerated by excessive pressor responses to stress. Elevations in dietary NaCl intake also facilitate the development of high blood pressure (Singh, Singh & Cameron, 1987). It is possible that this occurs through a NaCl dependent enhancement of stress-induced pressor responses (Ambrosioni et al., 1982; Falkner et al., 1981; Gradin et al., 1986; Koepke & DiBona, 1985). Specifically, elevated NaCl may hamper CNS sympathoinhibitory mechanisms presumed to regulate stress- induced sympathetic outflow to the cardiovascular system (Wyss et al., 1987). Sodium's CNS effects depend upon the state of calcium homeostasis (Peuler et al., 1987; Resnick et al., 1986; Wyss et al., 1989). Increased CNS calcium availability has an important effect on vasomotor activity which may counteract the CNS pressor effects of sodium (Leusen, 1950). When calcium levels are

elevated, sodium is less effective as a pressor agent and may even facilitate calcium's CNS hypotensive action (Metz et al., 1986). Whether the interaction of these nutrients is able to facilitate changes in stress reactivity is unknown. If stress reactivity does indeed influence the progression of hypertension, it would be expected that changes in blood pressure afforded by dietary manipulation would be accompanied by parallel changes in blood pressure eactivity.

Rationale

Both dietary NaCl and calcium manipulations alter cardiovascular responses to stress in hypertensive animals. Increased NaCl intake has been demonstrated to enhance blood pressure responses to stress (Ambrosioni et al., 1982; Falkner et al., 1981; Gradin et al., 1986; Koepke & DiBona, 1985), while calcium supplementation reduces cardiovascular stress responses (Hatton et al., 1987, 1989; Huie et al., 1987). Dietary manipulations of NaCl and calcium alter baseline blood pressure in a manner parallel to the dietary effects observed on stress reactivity (Singh et al., 1984). In addition, there appears to be an interactive effect of these nutrients such that the blood pressure effect of one is dependent on the intake level of the other. Specifically, calcium's hypotensive effects are exaggerated under conditions of NaCl load. Conversely, NaCl's ability to increase blood pressure is influenced by calcium intake, i.e., increased dietary calcium attenuates NaCl's hypertensive effects (McCarron et al., 1985). The interactive effect of these nutrients on stress reactivity is presently unknown.

Recent studies on the NaCl-sensitive SHR demonstrate that increased NaCl intake affects the neural activity of hypothalamic areas involved in cardiovascular depressor responses. Decreased NE stores and turnover rates induced

by the high NaCl diet were associated with increased plasma NE and blood pressure. It was hypothesized that the sympathoinhibitory abilities of the hypothalamus were impaired by excessive NaCl intake culminating in elevated blood pressure. Further, it was found that supplementing the high NaCl diet with calcium reversed NaCl's effects.

These reports suggest that the interactive effects of NaCl and calcium on blood pressure would be accompanied by parallel changes in cardiovascular reactivity. Given the possibility that NaCl interferes with depressor activity aimed at regulating blood pressure, it seems that pressor responses to stress would be enhanced due to impaired homeostatic responses. Alternatively, normalization of sympathoinhibitory activity with supplemental calcium would presumably correct aberrant pressor responses.

The current study was designed to assess the hypothesis that the hypertensive effects of NaCl and the hypotensive effects of calcium as well as interactive effects of these nutrients would be accompanied by parallel changes in cardiovascular reactivity. In order to assess the effects of NaCl and calcium on stress reactivity and blood pressure, both NaCl-sensitive SHRs and their normotensive control strain, the WKY, were maintained on one of four diets including two high NaCl diets combined with either high or low calcium as well as two low NaCl diets combine with the same high or low calcium content.

The content of sodium chloride in the "high" NaCl diet was chosen based on reports by Wyss et al. (1989) which demonstrated significant changes in sympathetic activity accompanied by blood pressure changes with an 8% NaCl diet. The level of NaCl chosen for the "low" sodium chloride diet actually contains a relatively normal amount of NaCl. It will herein be referred to as "low" simply as a contrast to the "high" NaCl diet, but should not be interpreted as a NaCl deficient diet. The content of calcium used in the "low" calcium diets was chosen based on consultation with a nutritionist who suggested that a 0.2% calcium diet would be deficient in calcium while providing enough of the nutrient to maintain relative health in the animals (Karanja, personal communication). The content of calcium in the high calcium diets was determined based on reports by Wyss et al. (1989) which demonstrated that a 2.0% calcium diet was sufficient to reverse elevations in blood pressure associated with NaCl loading.

These dietary variations were chosen to: 1. replicate the blood pressure and CNS effects observed with the high NaCl diets used by Wyss et al. (1989), 2. replicate the hypotensive effect of supplemental calcium on NaCl-loaded animals as described by Wyss et a. and, 3. investigate the effects of a calcium deficient diet under conditions of normal and high NaCl intake. A control diet using normal levels of both NaCl and calcium was not employed in this

study because repeated studies have failed to show a consistent blood pressure difference between normal and calcium supplemented hypertensive rats (Hatton et al., 1987, 1989).

Because high NaCl intake was found to increase blood pressure when calcium intake was not supplemented (Wyss et al., 1989), it was hypothesized that animals maintained on a high NaCl/low calcium diet would show a more exaggerated blood pressure response to stressful stimuli than animals maintained on a normal NaCl/low calcium diet. Furthermore, supplementation with calcium was hypothesized to attenuate blood pressure as well as stress responses among NaCl-loaded animals. Since a calcium deficient diet has been shown to increase blood pressure in the absence of elevated NaCl (Hatton et al., 1987, 1989), it was hypothesized that a normal NaCl/low calcium diet would elevate blood pressure and induce greater pressor responses than a normal NaCl/high calcium diet.

In order to assess the effects of diet on stress reactivity, blood pressure was measured directly after handling, during unrestrained rest as well as during restraint stress. A comparison of blood pressure over the different stress measures provided an indication of stress reactivity. Responses to stress were also assessed by observing blood pressure changes with exposure to novel, conditioned as well as unconditioned stimuli. A third study

indirectly assessed stress reactivity by examining behavioral inhibition in a novel open field.

In order to determine whether dietary influences on blood pressure and stress responses were mediated by changes in autonomic nervous system activity, repeated blood samples were taken for plasma catecholamine determination from animals during rest and restraint. Plasma norepinephrine was thought to be an appropriate measure of sympathetic activity given that it comes principally from neurotransmitter released from sympathetic postganglionic nerve endings, and its concentration correlates well with other indices of sympathetic function (Katholi, Winternitz & Oparil, 1980). Plasma epinephrine was also measured because its release from the adrenal medulla and its plasma concentrations have been found to vary directly with sympathetic activation (McCarty & Kopin, 1978b).

To determine whether diet-induced changes in blood pressure reactivity were the result of peripheral vascular alterations, blood pressure responses to bolus i.v. injections of NE were assessed. Dietary differences in vascular reactivity were presumed to indicate altered peripheral control of blood pressure such as changes in vasoconstrictor receptor sensitivity, vascular structure or alterations in hormonal balance that might influence vascular contractility.

Serum and brain electrolyte determinations were made in

order to assess the effects of diet on electrolyte availability to peripheral vascular tissue as well as to CNS cardiovascular regulatory areas. Altered serum electrolyte availability has been associated with changes in calcium binding thought to regulate vascular smooth muscle function (Webb & Bohr, 1978). In addition, infusions of electrolytes into the CNS are associated with alterations in cardiovascular regulation. Findings of altered serum or CNS electrolyte concentrations were expected to provide supporting evidence that electrolyte availability might have a significant impact on cardiovascular functioning.

Methods

The protocol is depicted in Figures 1 and 2.

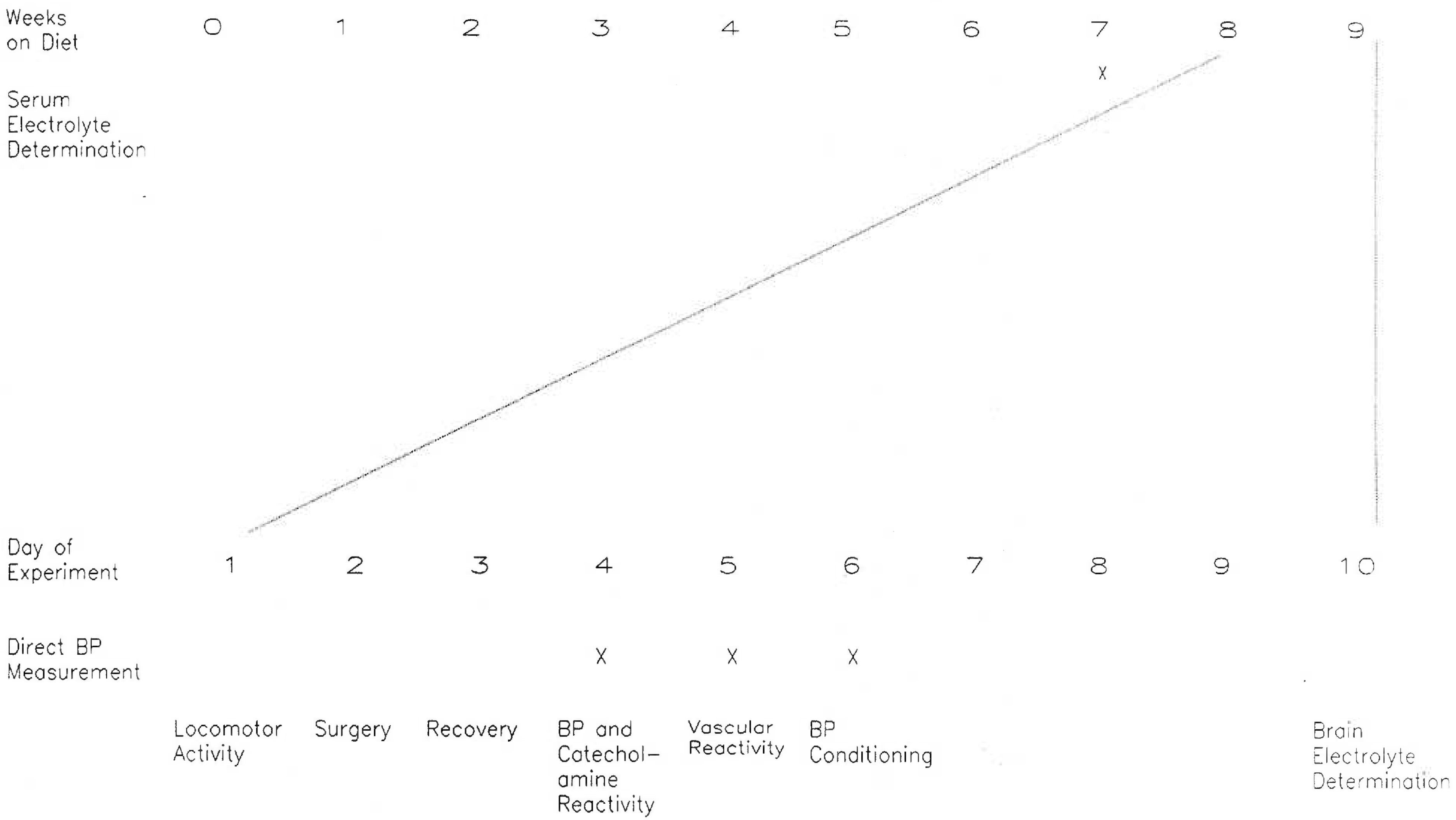
Subjects

Forty male NaCl-sensitive (IBU3 colony, Taconic Farms, Germantown, New York) spontaneously hypertensive rats (SHR) and equal numbers of their normotensive control strain, the Wistar-Kyoto rat (WKY), were obtained at 4 weeks of age. Upon arrival the animals were randomly assigned to one of four diets (Teklad, Madison, WI) varying in calcium and sodium chloride content as follows: high NaCl, low calcium (8.0 NaCl/0.2 Ca++ percent dry weight); normal NaCl, high calcium (0.73% NaCl/2.0% Ca++); high NaCl, high calcium (8.0% NaCl/2.0% Ca++); and normal NaCl, low calcium (0.73% NaCl/0.2% Ca++). Aside from differences in sodium chloride and calcium, the diets were equivalent in all other respects: phosphorus, 0.43%; potassium, 0.39%; magnesium, 0.05%; zinc, 0.0033%; vitamin D, 20,000 U/Kg. Animals were maintained on the diets for 8 weeks while housed in the animal care facility on a light/dark cycle of 12:12 hours. The facility was kept at 23 C. Food and water were

Figure 1. Experimental design and cell sizes.

NaCl \ Calcium	0.2%	2.0%
0.73%	SHR n=10 WKY n=10 Rats were fed diet from 4 to 12 weeks of age.	
8.0%		

Figure 2. Procedure for all subjects. Weeks on diet are displayed at top of figure. Weeks 8 and 9 are expanded to show procedures performed on given day of experiment.



Experimental Design
Weeks on Diet
0
1
2
3
4
5
6
7
8
9
Serum Electrolyte Determination
X
Day of Experiment
1
2
3
4
5
6
7
8
9
10
Direct BP Measurement
X
X
X
Locomotor Activity
Surgery
Recovery
BP and Catechol-amine Reactivity
Vascular Reactivity
BP Conditioning
Brain Electrolyte Determination

provided ad libitum. The facility was AAALAC approved and all animals were housed under guidelines specified in the PHS Guide for the Care and Use of Laboratory Animals (1985).

Determination of Serum Electrolytes

After seven weeks of exposure to the dietary condition, blood samples were taken to determine serum electrolyte concentrations. Each subject was lightly anesthetized with ether before withdrawing 3.0 mls of blood via subclavian venepuncture. After phlebotomy, a 3.0 ml bolus of saline was injected subcutaneously in the abdominal area for volume replacement. The Blood samples were allowed to coagulate in glass test tubes for 40 min. The tubes were then spun in a centrifuge (Philips-Drucker, Astoria, Oregon) at 20-25 thousand RPM for 15 min. The serum was removed with a glass pipette and placed in a second test tube and centrifuged again for 15 min. After the second spin, the supernatant was removed and placed in labeled plastic test tubes for storage in a -20°C freezer until electrolyte assays were performed. Serum was assayed for ionized Ca++ using a calcium specific electrode (Radiometer America, Westlake, OH). Total calcium, magnesium and phosphorus were determined by spectrophotometry (COBAS-BIO centrifugal analyzer, Roche Analytical Inst., Nutley, NJ). Sodium and potassium were determined by flame photometry (Instrumentation Laboratories, Tustin, CA). All serum

electrolyte assays were performed by the core laboratory technical support group in the Division of Nephrology of the Oregon Health Sciences University.

Activity Measurement

Apparatus

After 8 weeks of exposure to the diets, spontaneous locomotor activity was tested using a Digiscan Animal Activity Monitor (Model No. RXYZCM(8), Omnitech electronics, Inc. Columbus, OH). The apparatus consisted of a 42.0 x 42.0 x 30.5 cm plexiglass enclosure placed in a dark, sound attenuation chamber. Three sets of eight photoelectric beams positioned in both horizontal dimensions and the vertical plane detected the animal's movement. The activity monitor automatically scored and reported activity after each trial.

Procedure

Activity was assessed during three successive 5 min trials. During the trials, total distance, vertical activity and center time were measured. The order in which animals were tested was randomized across diets in order to control for the effects of circadian rhythm. Activity measures were determined between 11:00 a.m. and 2:00 p.m.

Surgery

After 8 weeks of dietary exposure each rat underwent surgery for placement of femoral artery and jugular vein catheters as well as shock electrodes.

Catheter construction

The femoral catheter consisted of a single 25-cm length of polyethylene (PE 50) tubing (0.58 mm I.D.; 0.96 mm O.D). The tubing was dipped in boiling water and stretched. The stretched portion was cut with a scalpel to provide a tapered end having a diameter approximately half that of the unstretched segment. The tapered portion was approximately 4.0 cm in length. A 1-cc saline-filled syringe fitted with a 23-gauge needle was inserted into the untapered end allowing for catheter flushing.

The jugular vein catheter was constructed using a hot air stream to first flare the end of 160-mm piece of PE-50 tubing. A 5-cm piece of stainless steel wire extending from a 23-ga blunt tipped needle was inserted into the flared end of the PE 50. The wire was inserted through the PE 50 until the flared end met the needle hub. A piece of heat shrink tubing, 17 mm (3/64 in. diameter) long was placed over the flared end of the PE 50 up to, but not over, the hub of the adjacent needle. A second, 10-mm (1/16 in. diameter) length of heat shrink tubing was placed over the first. The heat shrink tubing was then shrunk around the tubing and needle using a hot air stream. When the heat shrink tubing had

cooled the needle tip and wire were removed. The heat shrink end of the cannula constituted the infusion portion of the catheter. Three knobs were then formed 25, 35 and 45 mm from the infusion end of the catheter. Two more knobs were formed, one at the center of the PE 50 and one knob 10 mm from the other end. When these knobs were cool, the wire was removed and the PE 50 set aside.

The intravascular portion of the catheter was made of a 37-mm piece of Dow Corning Medical Grade silastic tubing (0.51 mm i.d. x .94 mm o.d.). The end to be inserted in the vein was cut at a 45-degree bevel. A piece of 9-ga wire was then inserted through the lumen of the silastic tubing. The silastic tubing was inserted over 5 mm of a 6.5-cm piece of PE 10, (0.28 mm i.d.; 0.61 o.d.). The PE 10 was then quickly melted to the silastic using the hot air stream. A 5-mm piece of heat shrink tubing (3/64-in. diameter) was centered over the end of the silastic and shrunk tightly over the silastic-PE 10 junction. When this junction had cooled, the 9-ga wire was inserted into the unflared end of the PE 50 until it abutted the PE 10. The connection was then melted forming a knob.

The catheter was checked for leaks by infusing air through it while submerged under water. When it had been established that there were no leaks, the PE-10 portion of the catheter was wrapped 1 and 1/2 times around a glass rod such that the free end of the silastic paralleled the PE 50.

The glass rod was then dipped in boiling water. Upon cooling the rod was removed. This formed a loop in the PE 10.

The PE-50 portion was bent in a U-shape and dipped into hot water. The portion of the catheter containing the three knobs was bent in a U-shape in the opposite direction and dipped in boiling water. The loop and bends in the PE tubing reduced clotting, increased flexibility and facilitated surgical insertion of the catheter.

Anesthesia

All surgeries were performed on subjects anesthetized with halothane gas in oxygen. Oxygen flow was maintained at a constant rate of 0.5 L/min using an Airco oxygen flowmeter. Halothane delivery was controlled using a calibrated vaporizer (Ohio Medical Products). The subjects were anesthetized initially in a Nalgene bottle with a loading dose of 5% halothane in oxygen. A maintenance dose of approximately 1.5-2.5% halothane was employed through the remainder of the surgery.

Catheter Placement

For insertion of the femoral catheter, a 4-cm square area on the ventral hind limb surface was shaved and washed with an antiseptic solution (Betadine). A small 2-cm incision was made superficial and parallel to the femoral artery. The artery was isolated from the femoral vein and nerve using blunt tipped forceps, with care not to make

contact with the sciatic nerve. Two 4-cm lengths of 000 silk were placed under the artery isolating it from adjacent vascular and nervous tissue. The distal ligature was tied off. The proximal ligature was loosely tied near the medial end of the incision exposing 1.0-1.5 cm of the vessel. Both ligatures were held taut using straight tipped hemostats in order to occlude blood flow.

A 20-ga hypodermic needle bent at a 90-degree angle 2 mm from the tip was inserted at the distal end of the exposed vessel. The needle was inserted medially into the artery with the beveled end of the needle facing the artery lumen. The femoral catheter was placed into the arterial incision beneath the needle and advanced until it met the proximal ligature. The needle was removed and the proximal ligature loosened to allow catheter advancement into the abdominal aorta, approximately 4 cm from the insertion site.

Both proximal and distal ligatures were tied securely around the catheter and trimmed. The catheter was flushed with a 1:1 solution of saline and heparin (100 units/ml) after which the needle and syringe were replaced by a 2-cm length of #10 stainless steel piano wire in order to occlude flow. Five mgs of antibacterial agent (Nitrofurazone) were applied topically to the wound.

For insertion of the jugular catheter, the animal was shaved in a 2- x 2-cm area just above the right clavicle. Betadine antiseptic solution was used to cleanse the shaved

area. A 1-cm incision parallel to the midline over the right clavicle was made. Using two fine-tipped curved tissue forceps, the fascia was pulled away to expose 5-7 mm of the right jugular vein. The tissue surrounding the vein was removed. One pair of forceps was inserted underneath the vein, separating it from surrounding connective tissue. Two 5-cm strands of 000 silk suture were pulled underneath the vein using the forceps. The ligatures were situated at both the distal and proximal ends of the exposed portion of the vein, and tied loosely around the vein. A pair of straight hemostatic forceps were attached to both ligatures in order to keep the vein taut and to occlude blood flow.

A 20-ga hypodermic needle tip was used to make an incision in the vein between the two ligatures. The needle was inserted such that the tip projected toward the heart. The silastic end of a saline-filled jugular catheter was inserted into the vein under the needle tip. The needle tip was then removed and pressure released from the proximally placed ligature such that the silastic portion of the catheter could be advanced into the vein. Using curve tipped forceps the catheter was advanced approximately 5.0 cm. The caudal ligature was tied just medial to the incision, around the vein and silastic portion of the catheter, being careful not to occlude flow. To prevent slippage, the loose ends of the proximal ligature were tied tightly around the heat shrink tubing which covers the

silastic-PE-10 junction.

The rostral ligature was tied once tightly around the vein to occlude blood flow and again around the PE-10 portion of the catheter just distal to the silastic, PE-10 junction. Patency of the catheter was checked to ensure that the ligatures had not been tied too tightly.

The fascia covering the midline muscle was then removed and a pocket formed just rostral to the midline muscles. A 5-cm piece of suture was tied around the catheter between the two knobs closest to the PE-10 portion of the cannula. The end of this suture was loosely tied around a small portion of midline muscle. A final check of catheter patency was then made prior to closure of the wound.

The animal was turned over and a 15-cm piece of 13-ga stainless steel hypodermic tubing was used to tunnel subcutaneously from the dorsal neck incision underneath the right foreleg to the ventral midline incision. The infusion end of the jugular catheter was pulled through the hypodermic tubing and exteriorized dorsally at the nape of the neck. At this time, the femoral catheter was exteriorized through a subcutaneous tunnel made from the same dorsal incision, along the left side of the animal, curving ventrally at the abdominal region and ending at the ventral incision. The catheters were anchored to the subcutaneous tissue on the back of the neck using a 5-cm strand of suture tied around the knobs at the infusion end

of the catheter. Both catheters were occluded with a 2-cm piece of #10 music wire. The incisions were then filled with anti-bacterial agent and sutured with 000 silk.

Placement of shock electrodes

The shock electrodes were made of 34-ga stainless-steel wire and butt connectors. The electrodes were placed on either side of the animal's thoracic region. The wires were threaded through the butt connectors and subcutaneously through the skin. The wires were looped three times and crimped with the butt connector against the skin.

After the procedure the anesthesia was discontinued and the animal was allowed to recover in a plastic shoe box cage lined with bedding.

Blood Pressure and Plasma Catecholamine Assessment

Two days following surgery, blood pressure was assessed during rest as well as during handling and restraint stress. Blood samples for plasma catecholamine assay were drawn following the baseline and restraint stress blood pressure measurements.

Apparatus

The animal was placed in a clear plexiglas cylinder (26-cm diameter, 31-cm tall) lined with corn cobb bedding. The animal's femoral catheter was connected to a saline filled extension tubing made up of a 45-cm length of PE 50. The extension tubing was connected to the catheter with a 2-

cm piece of 23-ga stainless steel hypodermic tubing while the other end was extended out of the sound proof box. The extension tubing was used to record blood pressure during the habituation period and for the collection of blood.

During blood pressure recording, the extension tubing was connected in line with a Statham p23ID pressure transducer attached to a Grass model 7D polygraph. The analog signal was displayed on a Grass polygraph chart recorder while simultaneously being fed to a Keithley 570 data acquisition unit which converted the analog signal to digital data. The digitized signal was sent to an IBM personal computer. Custom software was used to convert the digital input into mm Hg. The program sampled digital data 128 times per sec and stored 2 sec interval averages of systolic and diastolic blood pressure.

Analysis of plasma catecholamines was performed by high performance liquid chromatography using electrochemical detection (Bio-Analytical Systems, Model BAS 400). All catecholamine assays were performed by the laboratory of Dr. Hatton in the Department of Medical Psychology at the Oregon Health Sciences University.

Procedure

The animal was allowed to habituate for 30 min while blood pressure was recorded. The first 15 min recording period was collapsed to obtain a measure of handling stress blood pressure. An average of the following 15 min

recording period provided a measure of unrestrained resting baseline blood pressure. After habituation, the extension tubing was disconnected from the recording apparatus and the dead space was bled from the free end. A 1-cc syringe fitted with a 23-ga needle was attached to the extension tubing in order to draw an additional 0.2 cc of blood. This blood was set aside. A second syringe was used to draw an additional 0.5 ml of blood which was immediately put on ice. At this point the original 0.2 ml of blood was replaced and additional saline was infused to replace both the dead space and the 0.5-ml blood sample. The iced blood sample was spun in a refrigerated centrifuge for 10 min. The plasma was stored at -80 C, until a catecholamine assay was performed.

Following the unrestrained recording period, blood pressure was recorded while the animal was tightly restrained. The rat was put in an inverted U-shaped plexiglass restrainer (19 x 6 cm). Blood pressure was recorded for 15 min, after which time a second blood sample was drawn in the same manner as described for the non-stressed blood sample. Following the second blood draw, the animal was returned to the home cage. The blood pressure measure taken during 15-min restraint period was averaged to provide a single restraint stress blood pressure value.

For analysis of plasma NE, 100 μl of freshly thawed

plasma along with 800 µl of distilled, deionized water was added to 15-mg acid washed alumina and 50 µl of 5-mM sodium metabisulfite in a 1.5-cc plastic conical bottom tube. To this was added 50 µl of 100 mg/ml dihydroxybenzylamine (DHBA) and 200 µl of 3M Tris 4 gm%-disodium ethylenediamine tetraacetate (EDTA) which was adjusted with hydrochloride to a pH of 8.6. The metabisulfite was added to minimize oxidation of the catecholamines during sample preparation. The alumina acted to selectively separate the catecholamines from other constituents in the plasma by exclusively binding DHBA, NE and epinephrine (EPI) at a neutral pH which was provided by the addition of Tris. DHBA acted as an internal standard from which catecholamine concentrations were determined. The sample was shaken for 15 min and spun in a micro-centrifuge to settle the alumina. The supernatant was discarded and the alumina washed three times with distilled, deionized water. After the third washing, 100 µl of 0.1 N perchloric acid (PCA) was added. The change in pH allowed desorption of the catecholamines into the purified supernatant. Finally, 50 µl of the supernatant was injected onto the HPLC column for assay.

Vascular Reactivity Assessment

Three days following surgery, the animals were assessed for vascular responsiveness to bolus i.v. injections of NE.

Apparatus

The animal were restrained in an inverted U-shaped plexiglas restrainer. The femoral catheter was connected to the Grass polygraph as previously described. A 2-cm piece of hollow 23-ga hypodermic tubing connected to 3-cm piece of saline-filled PE 50 was attached to the infusion end of the jugular catheter.

Procedure

The animal was habituated to the apparatus for 30 min. After habituation, blood pressure acquisition commenced and continued through the duration of the experiment. After 10 mins of baseline recording, cardiovascular responses to i.v. bolus NE injections were assessed. One five-hundredth, 0.1, 0.3 and 3.0 μg/kg doses were made up in separate 0.1-ml volumes of saline. For each injection, a 1-cc syringe fitted with a 23-ga needle was used to draw up the 0.1-ml sample. The needle was fitted into the free end of the PE 50 previously attached to the jugular catheter of the animal. The sample was injected and followed by a 0.1-ml saline flush to ensure administration of the full dose. Blood pressure was allowed to return to baseline before each successive injection. The order of dose administered was randomized in order to control for volume changes as well as learned responses to the injection procedure. Responses were determined as the difference between baseline and the peak response to catecholamine injection.

Conditioned Blood Pressure Responses

One day following assessment of vascular reactivity, blood pressure responses were measured during classical conditioning. Measurements of blood pressure during the conditioning procedure provided analysis of cardiovascular reactivity to the conditioned and unconditioned stimuli.

Apparatus

During conditioning, the rat was restrained in an inverted U-shaped plexiglas restrainer. In order to prevent spurious sounds from reaching the rats, the holder was placed in a sound-attenuating chamber (Industrial Acoustics). Ventilation was provided by a 7.5-cm fan which also aided in masking extraneous noise. The rat was placed approximately 10 cm away from two 8-cm speakers used to present the conditioning stimuli.

One conditioned stimulus (CS) was a continuous 6.5-sec, 5-kHz, 85-dB tone while the other consisted of a continuous 6.5-sec, 1-kHz, 85 dB tone. The unconditioned stimulus (US) was a continuous 0.5-sec, 0.8-mA shock pulse produced by a Grason Stadler shock generator (W. Concord, Mass.) delivered through the shock electrodes during the last 0.5 sec of the reinforced CS.

Automated acquisition of blood pressure data was controlled by an Apple II Plus microcomputer. Blood pressure was detected by a Statham p23ID pressure transducer (Gould) which converted changes in blood pressure to an

electrical signal. The electrical signal was fed to a Grass low level D.C. preamplifier. An analog-to-digital device (Interactive structures, Inc., model AI13) connected in line with the Grass model 7 D polygraph converted electrical analog blood pressure data to digital output. An assembly language program (Cunningham, 1982) sampled the digital output every 10 msec and averaged the values over selected intervals. The averaged digital value was then converted to blood pressure and stored to disk.

Procedure

Two rats, maintained on different diets, were classically conditioned at the same time in separate sound-attenuating chambers. Stimulus events and recording procedures were programmed to alternate between the two subjects such that one conditioning trial was administered to one subject followed immediately by an identical trial administered to the second subject.

Fifty-six conditioning trials were given to each animal with an average intertrial interval of 180 sec. The first eight trials were CS-alone trials in which the two tones were given by themselves 4 times each in order to habituate orienting responses to the tones. Each animal then received 48 trials of discrimination training consisting of 24 trials in which the CS+ was paired with the US, and 24 trials in which the CS- was presented alone. The order of CS+ and CS- trial presentation was quasi-random such that no more than

three trials of a given type occurred consecutively. The use of either tone as a CS+ or CS- was counterbalanced over diet groups as was the use of a particular conditioning chamber.

Seven measures of blood pressure were obtained on each trial. The first two measures were taken from two 5-sec intervals recorded immediately prior to onset of the CS. The two values were averaged to provide a single pre-CS baseline of BP. The next three measures came from successive 2-sec intervals taken during the first 6 secs of the 6.5-sec CS. The last two measures consisted of consecutive 2-sec intervals recorded immediately following termination of the US. Difference scores were computed off line by subtracting the pre-CS value from each 2-sec measure, thus providing data on novel conditioned and unconditioned blood pressure responses. Following the conditioning procedure, the animals were returned to the animal care facility and provided with food and water *ad libitum*.

Measurement of Brain Electrolytes

Four days following conditioning, the rats were sacrificed and the brains quickly removed and placed on a cooled metal block and dissected into three parts including: cerebral cortex, brainstem and cerebellum. The tissue was quickly frozen in liquid nitrogen and stored at -80°C until

electrolyte assays could be performed. The cerebral cortex, brainstem and cerebellum portions were weighed after drying in a low heat oven for 24 hours. The tissue was then ashed at 600°C for 12 hours. Assays for calcium content were performed by atomic absorption at the Chemical Agriculture Laboratory, Oregon State University. Magnesium and phosphorus content were determined by spectrophotometry (COBAS-BIO centrifugal analyzer. Roche Analytical Inst., Nutley NJ). Sodium and potassium were assayed by flame photometry (Instrumentation Laboratories, Tustin, Ca.). All brain electrolyte assays, with the exception of calcium, were performed by the core technical support laboratory in the Division of Nephrology at the Oregon Health Sciences University.

Data Analysis

All parameters including serum electrolyte concentrations, weight, cardiovascular responses to novel conditioned and unconditioned stimuli, vascular reactivity, sympathetic activity, as well as brain electrolytes and plasma catecholamines were evaluated for dietary and strain differences using an SPSS/PC data analysis software package (SPSS Inc., Chicago, IL).

Cardiovascular responses to the CS and US stimuli were measured as the change in blood pressure between the pre-stimulus baseline and the stimulus response. The

difference scores were analyzed using a Strain x NaCl x Calcium x Trial Blocks x Counting Periods (CP) design with Trial Blocks and CPs assessed as repeated measures. Vascular reactivity was measured as the difference between baseline and the peak response to drug. The difference scores were analyzed using a Strain x NaCl x Calcium x Dose design with Dose assessed as a repeated measure. Analysis of blood pressure and plasma catecholamines during baseline and restraint consisted of a Strain x NaCl x Calcium x Stress Condition with a repeated measures design for the Stress Condition. Activity was analyzed with a Strain x NaCl x Calcium x CP design with CP used as a repeated measure. Brain and serum electrolytes as well as weight were all assessed using a Strain x NaCl x Calcium analysis of variance (ANOVA).

In order to investigate the origin of particular interactions and effects, data were often averaged across levels of a given factor. This is referred to throughout the text as "collapsed" and may be considered interchangeable with "averaged". Follow-up ANOVAs were performed on all significant interactions. Further post-hoc modified Tukey's tests were performed on main effects in order to determine the origin of group differences.

Results

Body Weight

Body weights determined at 13 weeks of age are presented for all the groups in Figure 3. A three-way analysis of variance ANOVA with Strain (2), NaCl (2) and Calcium (2) levels as between group factors was used to determine group differences. Main effects were revealed for both strain ($F\{1,47\}=199.68$, $p<0.001$) and NaCl ($F\{1,47\}=25.29$, $p<0.001$). The strain effect was due to an overall greater body weight in the normotensive strain. As shown by Figure 3, elevated NaCl in the diet significantly attenuated growth when assessed across strains.

Serum Electrolytes

Serum electrolyte data are illustrated in Figure 4. Group differences in ionized calcium, total calcium, magnesium, sodium, phosphorus and potassium were assessed using three-way ANOVAs with Strain, NaCl and Calcium as between group factors. Though there were no significant interactions between factors, electrolyte levels were influenced by strain, NaCl and calcium independently.

Figure 3. Weight after 8 weeks of dietary exposure. Values are expressed as group means + SEM. Subject n for each group was 6, 6, 7, 7 for SHRs on low NaCl/low calcium (Diet 1), low NaCl/high calcium (Diet 2), high NaCl/low calcium (Diet 3) and high NaCl/high calcium (Diet 4) respectively. Subject n's for the WKY strain was 7, 7, 7, 6 for Diets 1, 2, 3 and 4 respectively.

Weight After Eight Weeks of Dietary Exposure

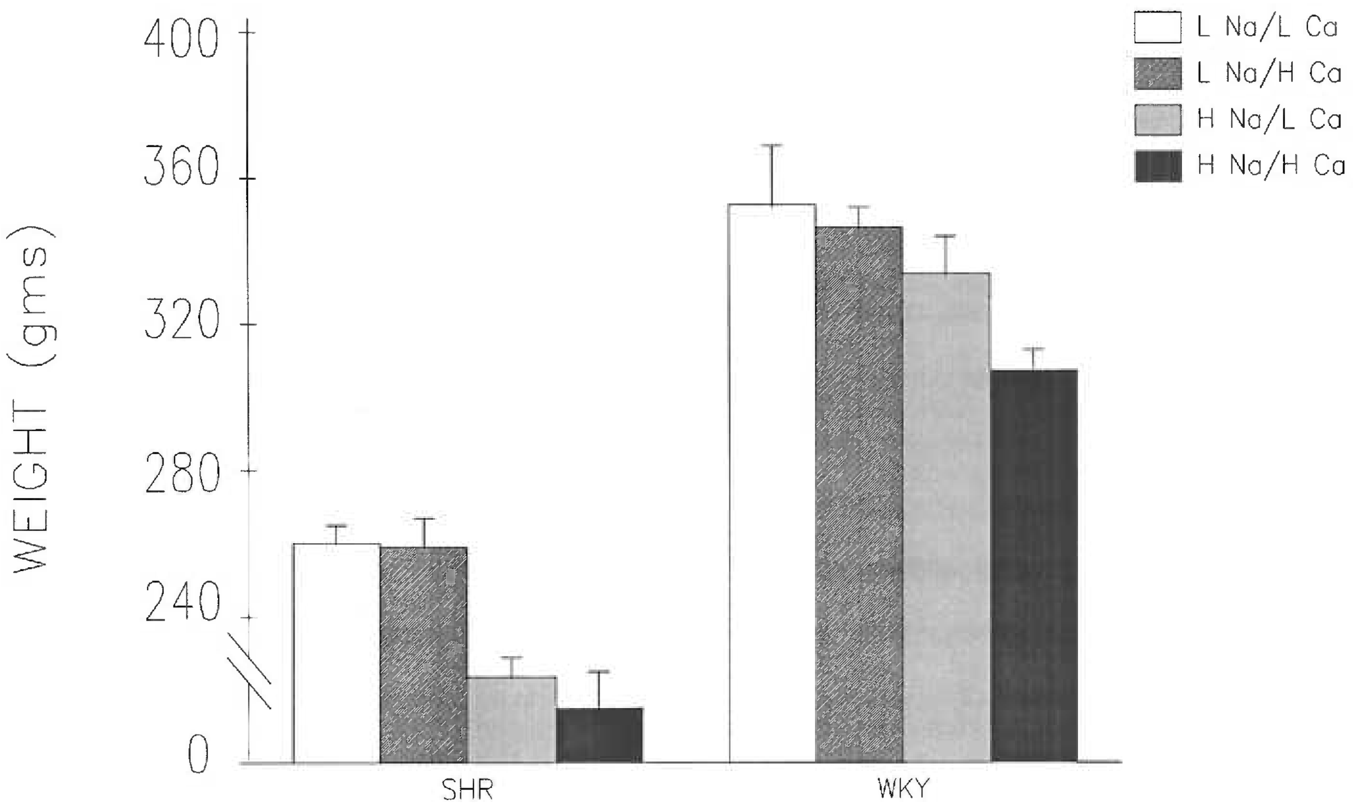
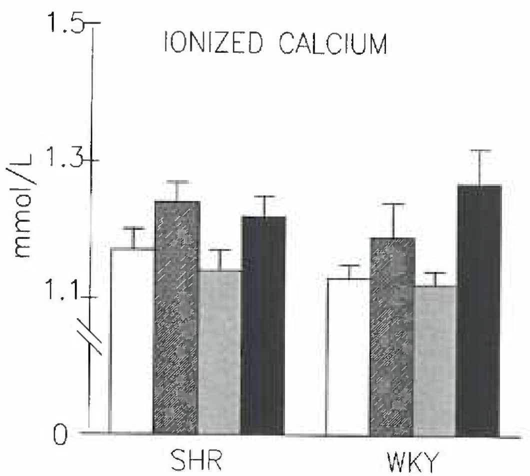
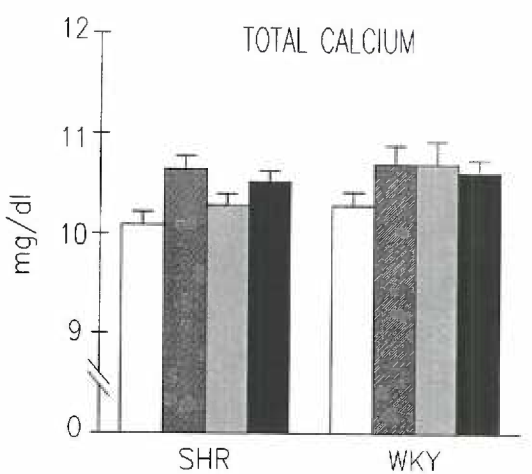


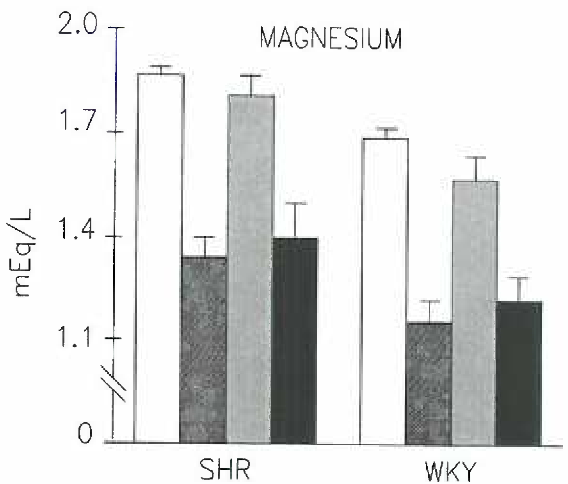
Figure 4. Serum ionized calcium, total calcium, magnesium, phosphorus, sodium and potassium for each diet group in each strain. Values expressed as group means + SEM. Subject n's for rats on Diets 1, 2, 3 and 4 were 9, 9, 10 and 10 for the SHR strain and 6, 6, 6 and 5 for WKY strain respectively.

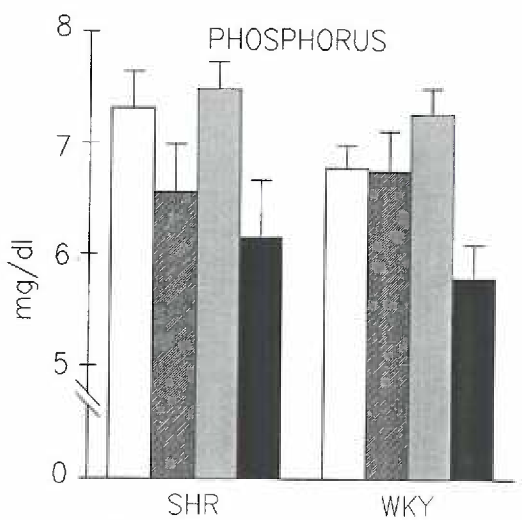
SERUM ELECTROLYTES

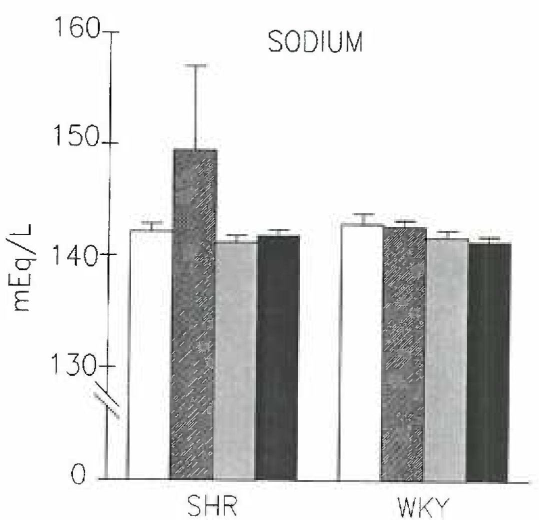
L NA/L CA
L NA/H CA
H NA/L CA
H NA/H CA

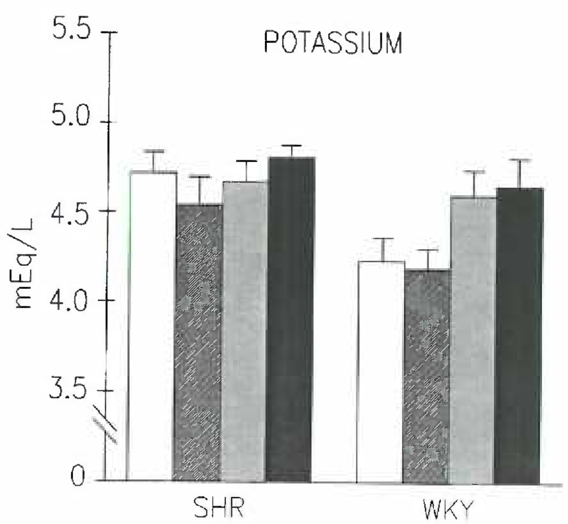












Both ionized and total calcium differed with calcium intake. As would be predicted, animals fed the high calcium diets showed greater amounts of both ionized and total calcium ($F\{1,50\}=11.76$, $p=0.001$; $F\{1,50\}=10.71$, $p=0.002$). Furthermore, total calcium was also greater among WKYs as indicated by a significant main effect for strain ($F\{1,50\}=5.05$, $p=0.029$).

Serum magnesium levels were likewise influenced by both calcium intake and strain type. In contrast to total calcium, magnesium was significantly lower in the WKY strain ($F\{1,50\}=14.5$, $p<0.001$), as well as among subjects fed the high calcium diets ($F\{1,50\}=81.99$, $p<0.001$).

A significant main effect of calcium intake was also found in serum phosphate levels. Like magnesium, higher calcium intake was associated with reduced levels of phosphate ($F\{1,53\}=12.26$, $p=0.001$).

There were no significant effects of strain, NaCl or calcium on serum sodium levels. In contrast, potassium was found to differ with strain as well as with NaCl intake. SHR subjects showed significantly greater serum potassium than did normotensive animals ($F\{1,53\}=7.83$, $p<0.007$). Potassium was also found to be elevated among animals fed the high NaCl diets ($F\{1,53\}=5.55$, $p=0.022$).

In summary, all serum electrolytes with the exception of sodium differed with strain, NaCl or calcium. Ionized and total calcium as well as magnesium and phosphorus were

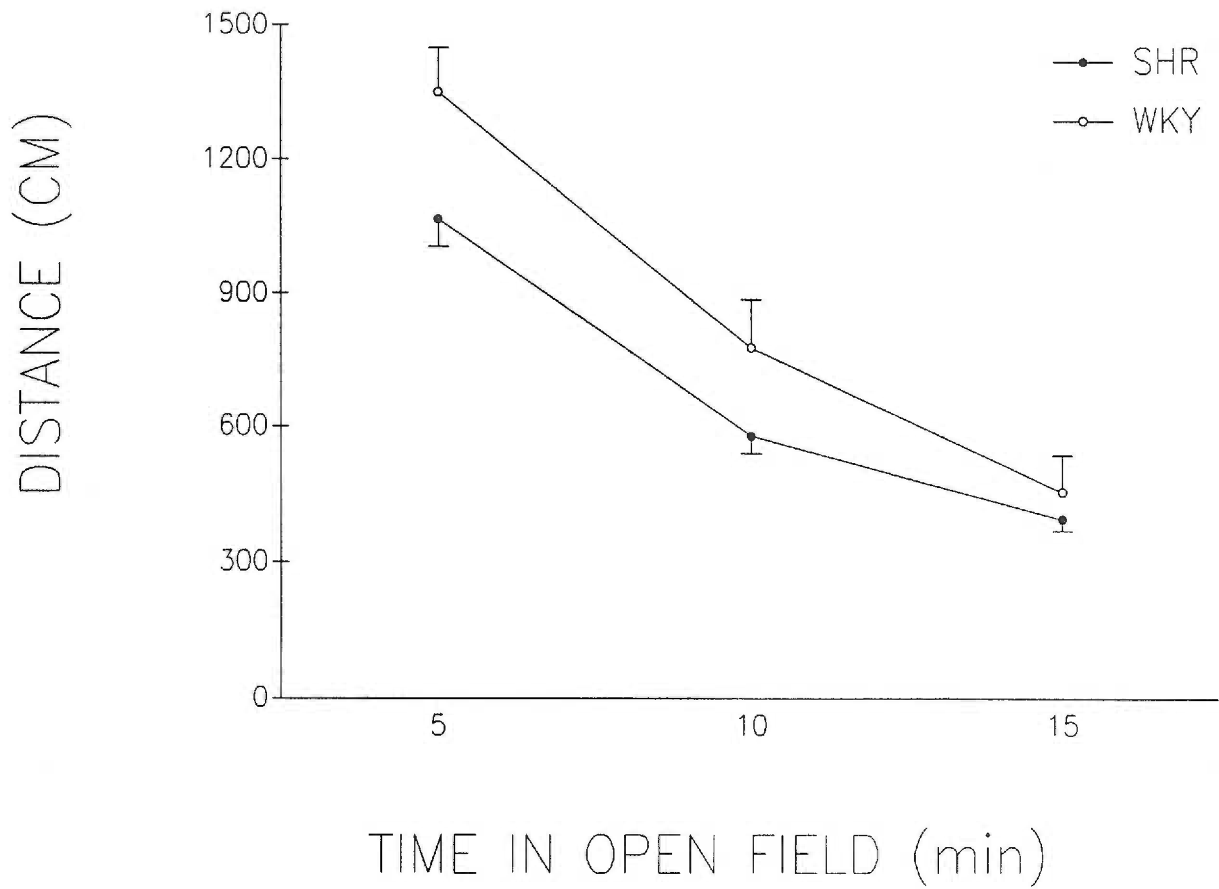
influenced by calcium intake. In addition, total calcium, magnesium, phosphorus and potassium all differed with strain. Potassium was unique in that it was the only electrolyte influenced by NaCl intake. As indicated by the data, serum electrolytes in all subjects were influenced by diet. But, the lack of Diet by Strain interaction indicated that the dietary manipulations had similar effects on both strains.

Locomotor Activity

Three measures were used to determine activity: including vertical activity, center time as well as total distance. Four way ANOVAs using Strain, NaCl and Calcium as between groups factors and Time as a within group factor were performed on data obtained from each of the three activity measures.

Total distance of both strains collapsed across diet groups are shown in Figure 5. A significant Strain by Time interaction was revealed ($F\{2,134\}=5.20$, $p<0.007$) in the four-way analysis of total distance. As can be seen in the figure, WKY animals covered more distance than did the hypertensive strain. Follow-up analyses of individual CP showed significant strain differences during the first ($F\{1,75\}=10.26$, $p=0.002$) and second ($F\{1,74\}=9.77$, $p=0.003$), but not the third CP indicating a diminished strain difference with habituation. Also found in the four-way

Figure 5. Total distance covered in the open field. Data are expressed as strain means collapsed across diet for each measurement interval + SEM. Subject n's for all activity measures for rats on Diets 1, 2, 3, and 4 were 10, 10, 8 and 10 for the SHR strain and 7, 7, 8 and 9 for the WKY strain respectively.


TOTAL DISTANCE
DISTANCE (CM)
1500
1200
900
600
300
0
5
10
15
TIME IN OPEN FIELD (min)
SHR
WKY

analysis were main effects for strain ($\underline{F}\{1,67\}=11.25$, $\underline{p}=0.001$), NaCl ($\underline{F}\{1,67\}=6.13$, $\underline{p}=0.016$) and time ($\underline{F}\{2,134\}=238.99$, $\underline{p}<0.001$). The main effect for strain was due to an overall greater total distance covered by WKYs. In addition, higher NaCl intake caused a decrease in total distance across all the groups (data not shown). The main effect for time resulted from a progressive decrease in total distance with each successive CP, as indicated in Figure 5.

Data for the results of the center time analysis are illustrated in Figures 6-8. A four-way analysis revealed a significant three-way interaction between Strain, NaCl and Time ($\underline{F}\{2,132\}=3.45, \underline{p}=0.035$). Analyses of the individual strains demonstrated a significant NaCl by Time effect only in the hypertensive strain ($\underline{F}\{2,72\}=3.33$, $\underline{p}=0.041$). Follow-up analyses of data from SHRs maintained on the high and low NaCl diets indicated a significant main effect for time among hypertensive subjects fed the low NaCl diets ($\underline{F}\{2,36\}=19.73$, $\underline{p}<0.001$). The source of the Strain by NaCl by Time interaction is shown in Figure 7. As can be seen, SHRs maintained on the high NaCl diet showed a constant level of center time, while center time declined in successive CPs among animals fed the low NaCl diet. In addition to the three-way interaction found in the four-way center time analysis, there was also a strain by time effect

Figure 6. Time spent in center of open field. Data are expressed as strain means collapsed across diet groups for each measurement period + SEM.

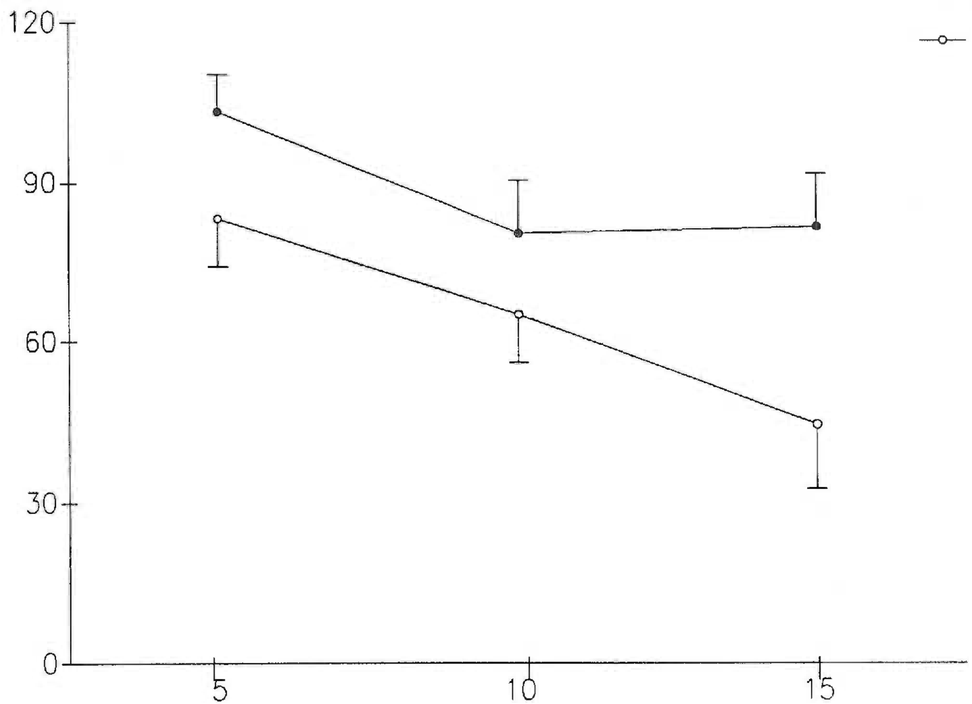

CENTER TIME
SHR
WKY
120
90
60
30
0
CENTER TIME (SEC)
5
10
15
TIME IN OPEN FIELD (MIN)

Figure 7. Time spent in center of open field for the high and low NaCl groups of the SHR strain. Activity for the SHR strain collapsed into high and low NaCl diet groups are shown. Data are expressed as group means + SEM during each measurement period.

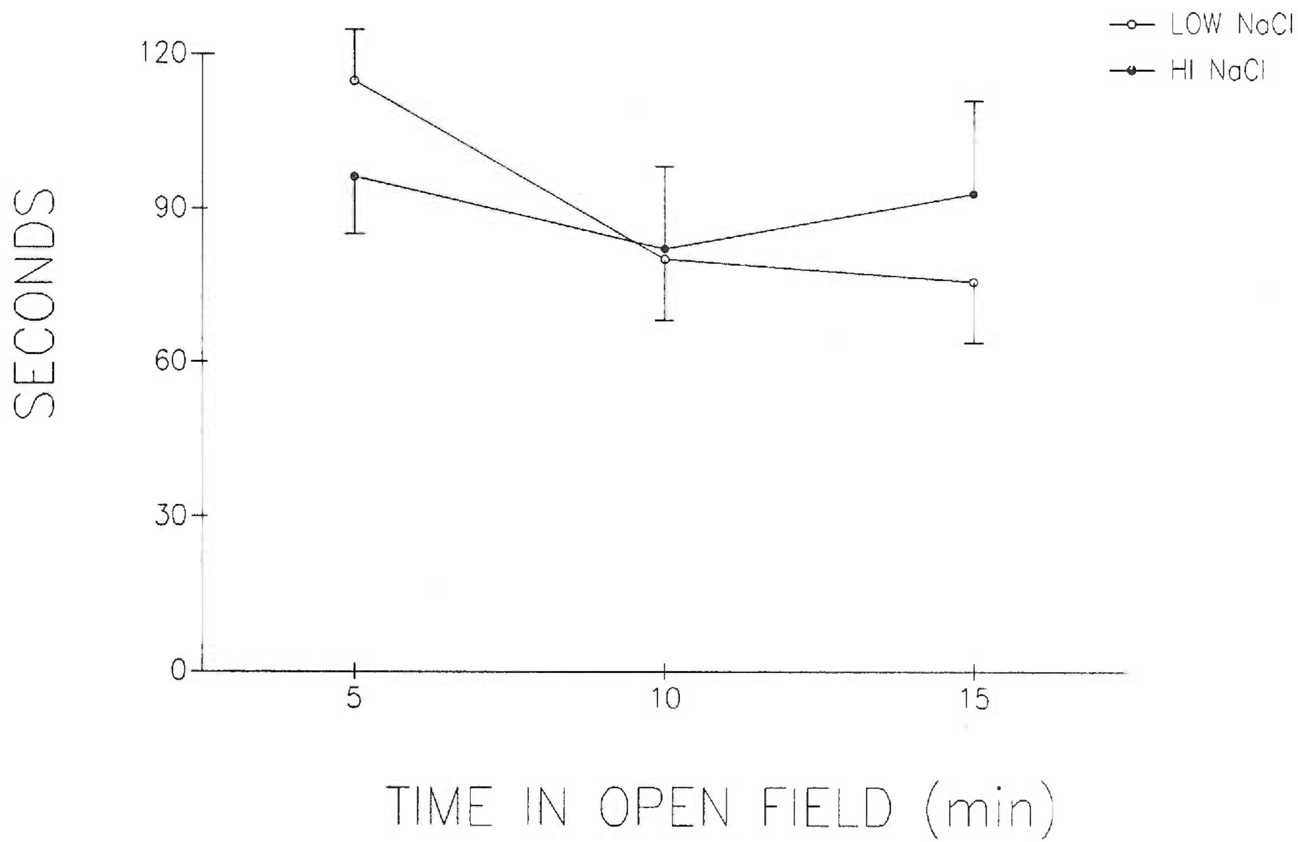
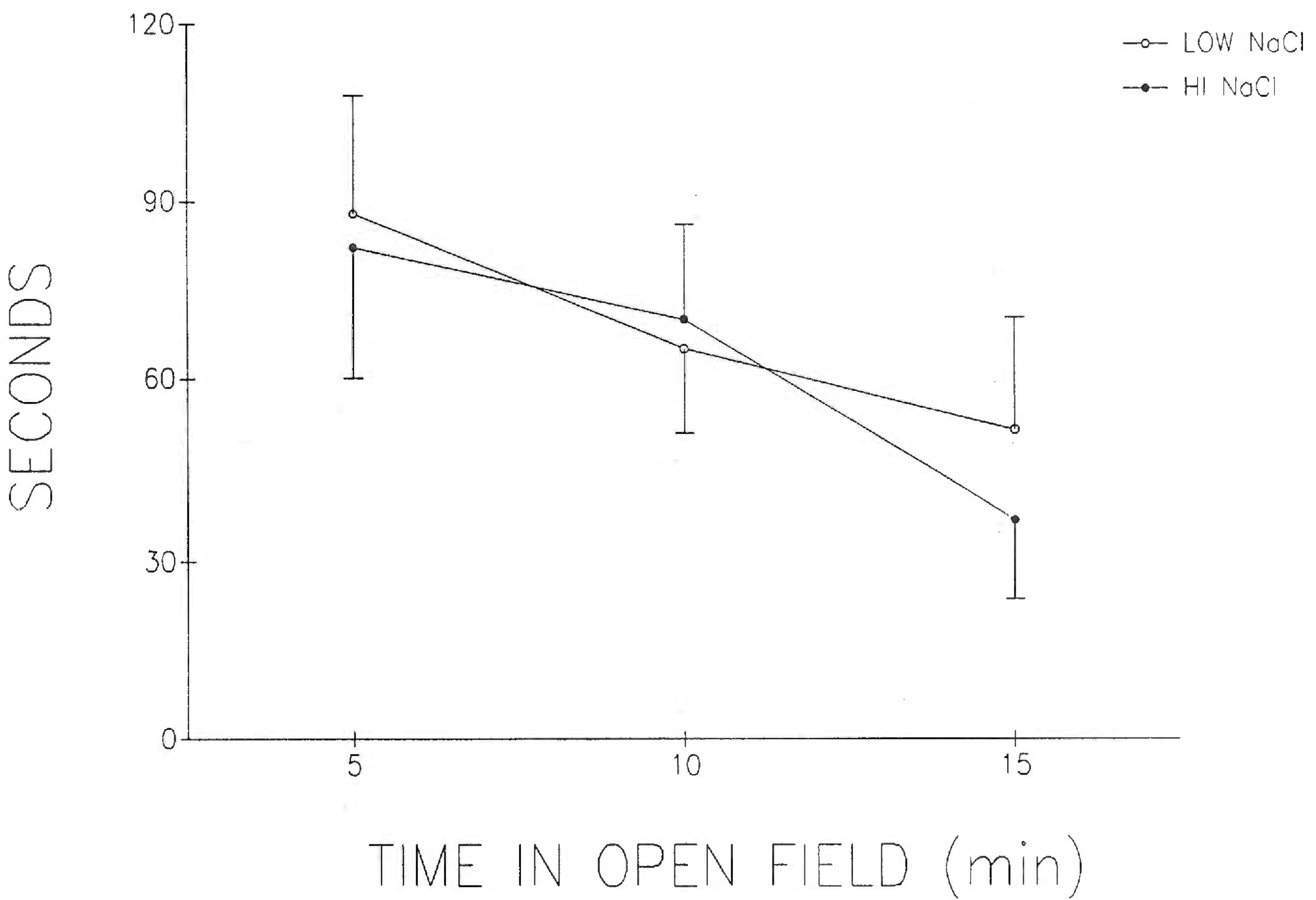

CENTER TIME IN THE SHR
LOW NaCl
HI NaCl
SECONDS
120
90
60
30
0
5
10
15
TIME IN OPEN FIELD (min)

Figure 8. Time spent in center of open field for the high and low NaCl groups of the WKY strain. Activity for the WKY strain collapsed into high and low NaCl diet groups are shown. Data are expressed as group means + SEM during each measurement period.


CENTER TIME IN WKY
SECONDS
120
90
60
30
0
5
10
15
TIME IN OPEN FIELD (min)
LOW NaCl
HI NaCl

($F\{2,132\}=3.91$, $p=0.022$) as illustrated in Figure 6. While center time for both strains diminished slightly with time, the strain difference, characterized by a greater center time among hypertensive animals, did not reach significance until the third CP ($F\{1,72\}=6.95$, $p=0.01$). As expected, there was a significant main effect for time ($F\{2,132\}=22.82$, $p<0.001$) due to a progressive decrease in center time with increased exposure to the apparatus.

Rearing behavior was assessed by examining vertical activity (data not shown). A four-way analysis showed a significant interaction between Strain, NaCl, Calcium and Time ($F\{2,134\}=3.14$, $p=0.047$). Follow-up analyses demonstrated a Strain by Calcium by Time interaction ($F\{2,66\}=5.42$, $p=0.007$) among animals fed the low NaCl diets. Analyses of the low NaCl data further partitioned by calcium diet indicated a significant interaction of Strain and Time in animals fed the low NaCl/high calcium diet ($F\{2,32\}=7.43$, $p=0.002$). Analyses performed at each time point showed a main effect for strain only during the first CP ($F\{1,17\}=9.73$, $p=0.006$). The Strain by NaCl by Calcium by Time interaction resulted from a significantly lower degree of vertical activity among WKYs fed the low NaCl/high calcium diet. This difference disappeared as levels of vertical activity diminished with time. Furthermore, the decline of vertical activity over time was consistent across all groups as indicated by a main effect for time

($F(2,134)=162.49$, $p<0.001$) revealed in the initial four-way analysis.

The three measures of activity: total distance, center time and vertical activity, differed with strain, NaCl and calcium intake. All measures of activity declined with time as would be expected with habituation. The effects of strain varied across the different activity measures with the normotensive strain covering more total distance and the SHR strain spending more time in the center of the apparatus. Vertical time did not differ with respect to strain with the exception of the low NaCl/high calcium WKY animals which showed diminished activity initially that subsequently diminished with habituation.

Stress-Induced Blood Pressure Responses

Mean arterial blood pressure recorded during handling, baseline and restraint conditions are presented for both SHRs and WKYs in Figures 9 and 10 respectively. As can be seen, SHRs had higher blood pressure than normotensive animals during each condition. SHRs also showed a more pronounced effect of the dietary manipulation. Specifically, SHRs fed the high NaCl diets had higher blood pressure than the low NaCl hypertensive groups during all stress conditions. Normotensive animals, in contrast, showed only small elevations in blood pressure with increased NaCl intake. A four-way ANOVA using Strain, NaCl

Figure 9. Blood pressure response of the SHR strain during handling, rest and restraint stress. Group means for each diet in each strain are shown + SEM. Subject n's for SHRs on Diets 1, 2, 3 and 4 were 9, 9, 8 and 9 respectively.

BLOOD PRESSURE RESPONSE IN THE SHR

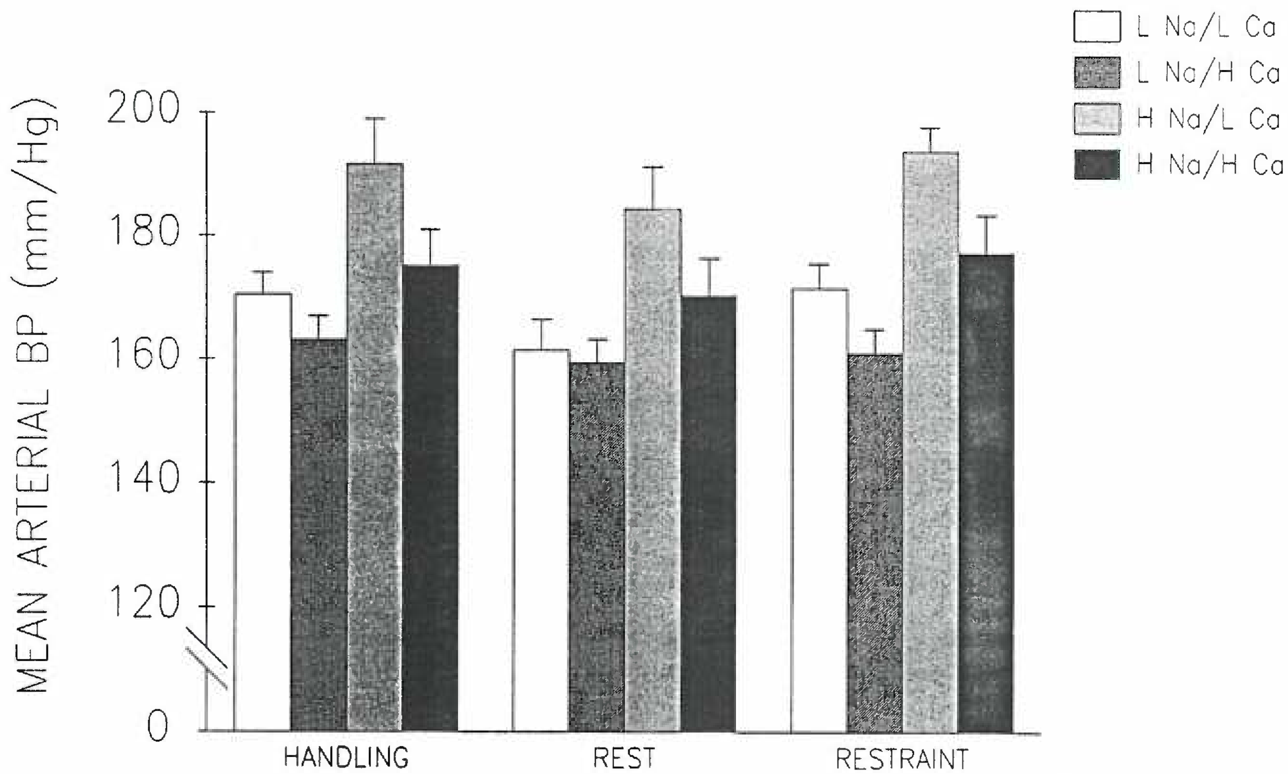
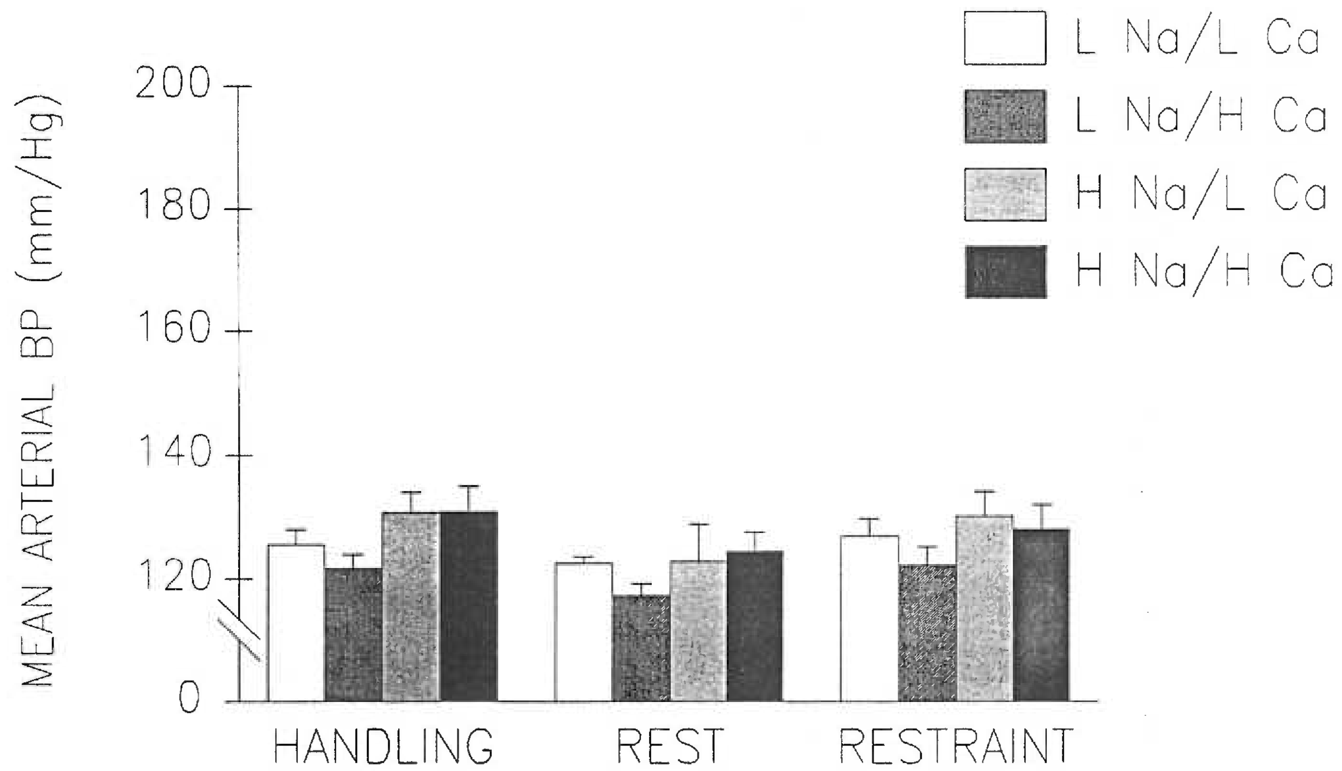


Figure 10. Blood pressure response of the WKY strain during handling, rest and restraint stress. Group means for each diet in each strain are shown + SEM. Subject n's for WKYs on Diets 1, 2, 3 and 4 were 9, 8, 7 and 6 respectively.

BLOOD PRESSURE RESPONSE IN THE WKY



and Calcium as between groups factors and Stress Condition as the within groups factor showed a Strain by NaCl interaction ($F\{1,57\}=4.29$, $p=0.02$) as well as main effects for strain ($F\{1,57\}=255.3$, $p<0.0001$), NaCl ($F\{1,57\}=14.44$, $p<0.0001$), calcium ($F\{1,57\}=5.10$, $p<0.025$) and stress ($F\{2,114\}=25.95$, $p<0.0001$).

As demonstrated in the figures, high dietary NaCl appeared to increase blood pressure in both strains. However, NaCl had a more pronounced hypertensive effect on SHRs. This was substantiated by separate one-way ANOVAs performed on each strain comparing blood pressure at the two NaCl levels. SHRs showed a significant main effect for NaCl ($F\{1,33\}=11.50$, $p<0.002$), while NaCl did not influence blood pressure in WKYs. The main calcium effect revealed in the original analysis was due to a lower blood pressure among rats fed the high calcium diets.

As can be seen, particularly in Figure 9, the main effect for stress condition resulted from consistent blood pressure elevations during handling and restraint stress. A Tukey's test comparing blood pressure means of the three stress conditions collapsed across diet and strain indicated significant differences between resting and handling blood pressure ($p<0.05$) as well as between resting and restraint blood pressure ($p<0.05$).

There was little dietary influence on stress-induced blood pressure reactivity as indicated by the lack of

interaction between either NaCl or Calcium diet factor and Stress Condition. However, since it was initially planned to assess the dietary effects during handling, rest and restraint separately, post-hoc Tukey's tests comparing group were performed at each stress condition. Despite a clear trend for higher blood pressure among SHRs maintained on the high NaCl/low calcium diet, there was no difference between the two high NaCl groups during either handling or baseline conditions. However, during restraint stress, the high NaCl/low calcium group was found to have higher blood pressure than the high NaCl group supplemented with calcium ($p<0.05$).

Plasma Catecholamine Response

Plasma catecholamine levels assessed immediately following baseline and restraint stress periods are presented for each strain in Figures 11 and 12. Strain differences in plasma NE were apparent during both baseline and stress as exemplified by the larger NE levels found among the hypertensive animals. As expected, there were substantial increases in plasma NE after exposure to restraint stress across all the dietary groups. However, diet did not influence baseline or stress-induced release of NE in either strain. A four-way ANOVA using Strain, NaCl and Calcium level as between groups factors and Stress Condition as the within groups factor revealed a main effect

Figure 11. Plasma catecholamine values determined during rest and restraint stress in the SHR strain. Mean levels of NE and EPI are shown for each diet group + SEM. Subject n's for SHRs maintained on Diets 1, 2, 3 and 4 were 9, 6, 7 and 7 for baseline measures and 9, 6, 6 and 6 for restraint measures respectively.

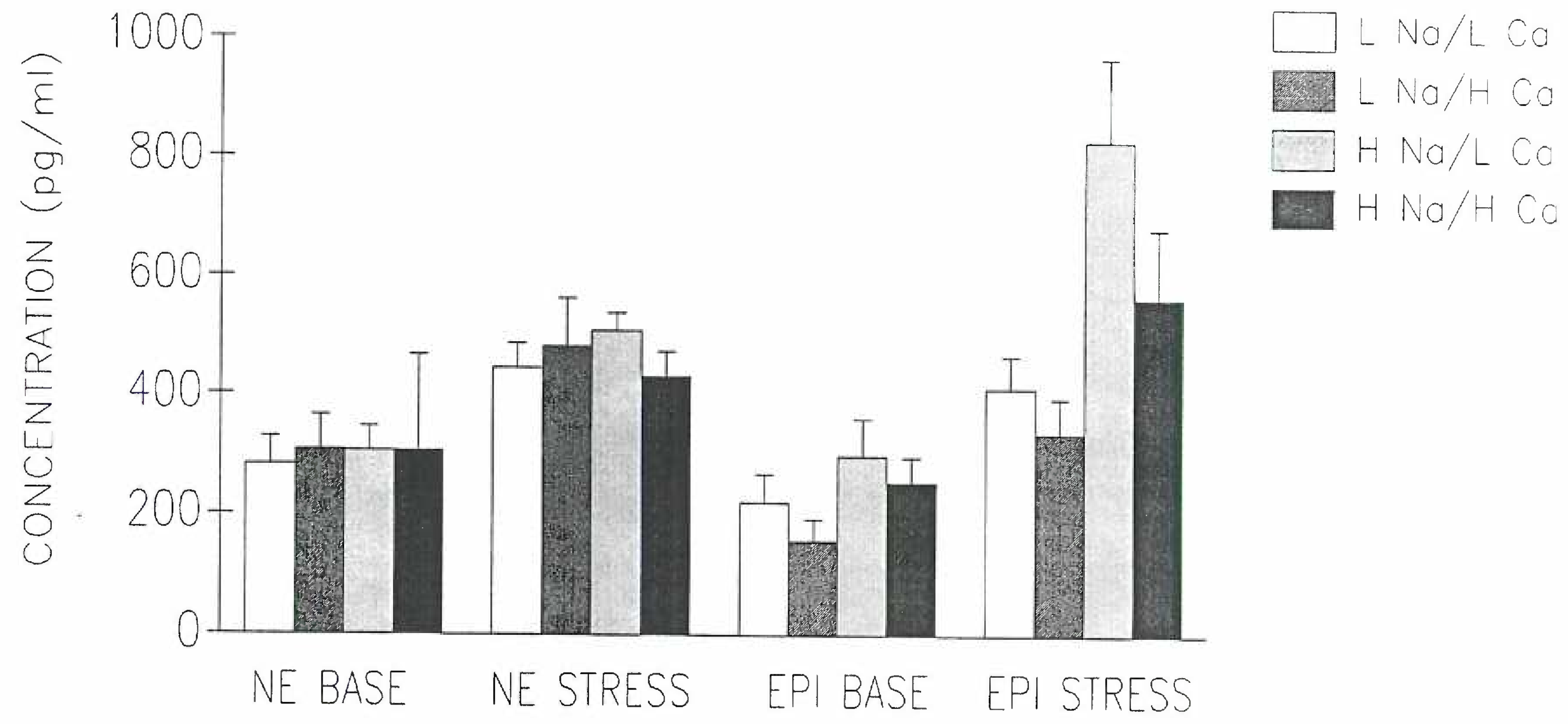
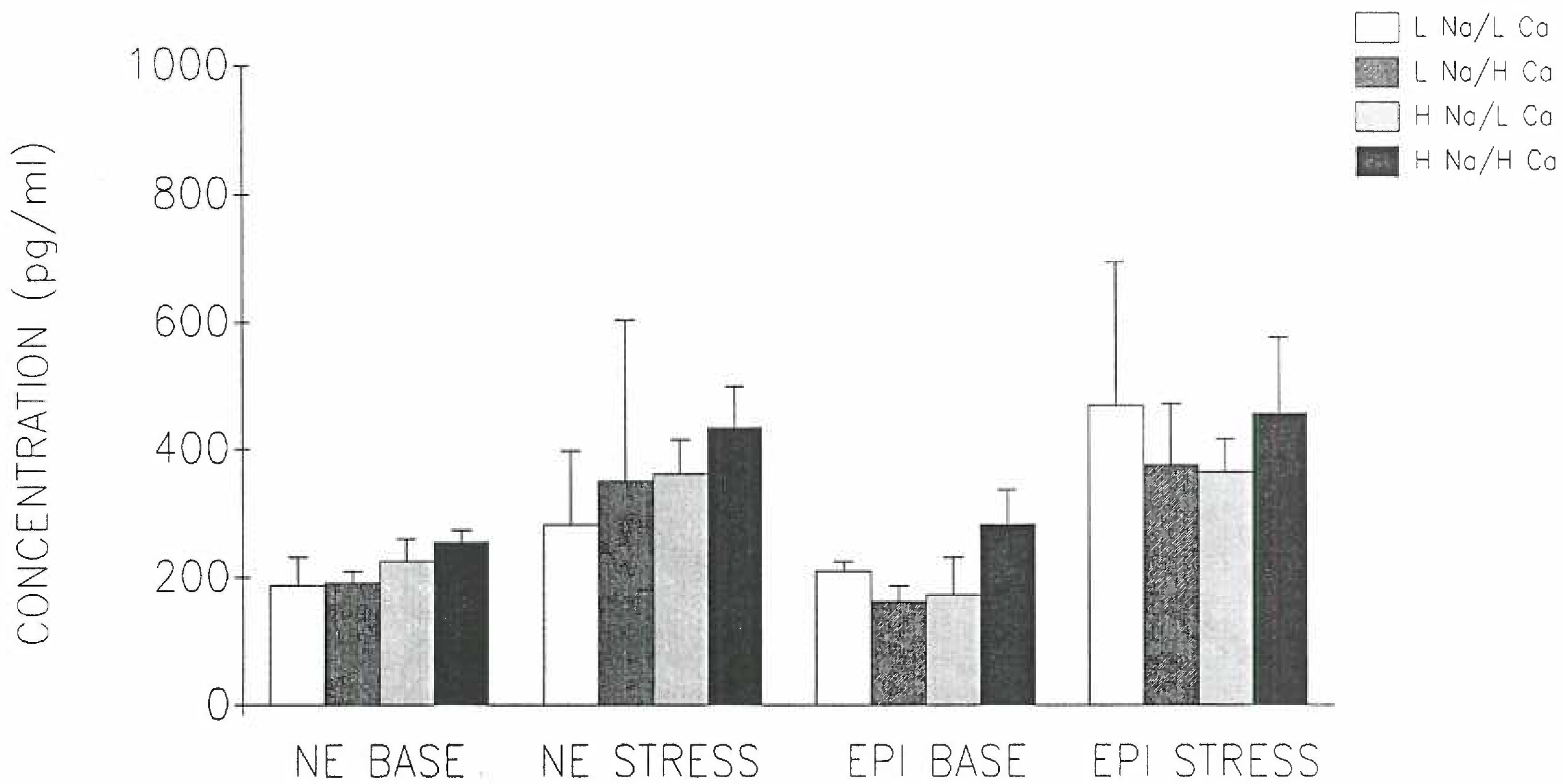

PLASMA CATECHOLAMINE RESPONSE IN THE SHR
CONCENTRATION (pg/ml)
1000
800
600
400
200
0
NE BASE
NE STRESS
EPI BASE
EPI STRESS
L Na/L Ca
L Na/H Ca
H Na/L Ca
H Na/H Ca

Figure 12. Plasma catecholamine values determined during rest and restraint stress in the WKY strain. Mean levels of NE and EPI are shown for each diet group + SEM. Subject n's for WKYs maintained on Diets 1, 2, 3 and 4 were 5, 8, 5 and 5 for baseline measures and 4, 4, 6 and 4 for restraint measures respectively.


PLASMA CATECHOLAMINE RESPONSE IN THE WKY
L Na/L Ca
L Na/H Ca
H Na/L Ca
H Na/H Ca
CONCENTRATION (pg/ml)
1000
800
600
400
200
0
NE BASE
NE STRESS
EPI BASE
EPI STRESS

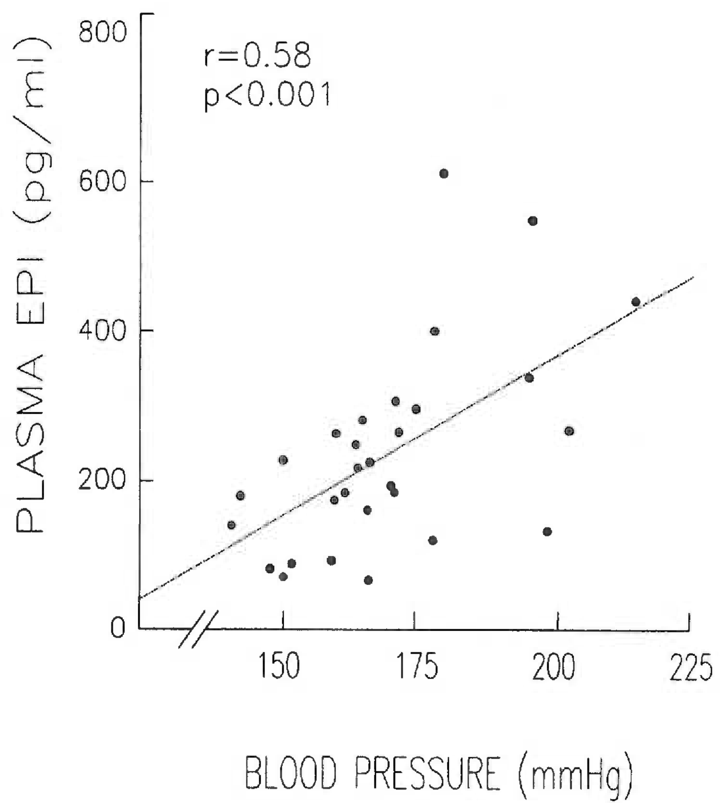
for strain ($\underline{F}\{1,36\}=7.89$, $\underline{p}=0.008$) as well as for stress condition ($\underline{F}\{1,36\}=66.87$, $\underline{p}<0.001$), while no significant effects of diet were found.

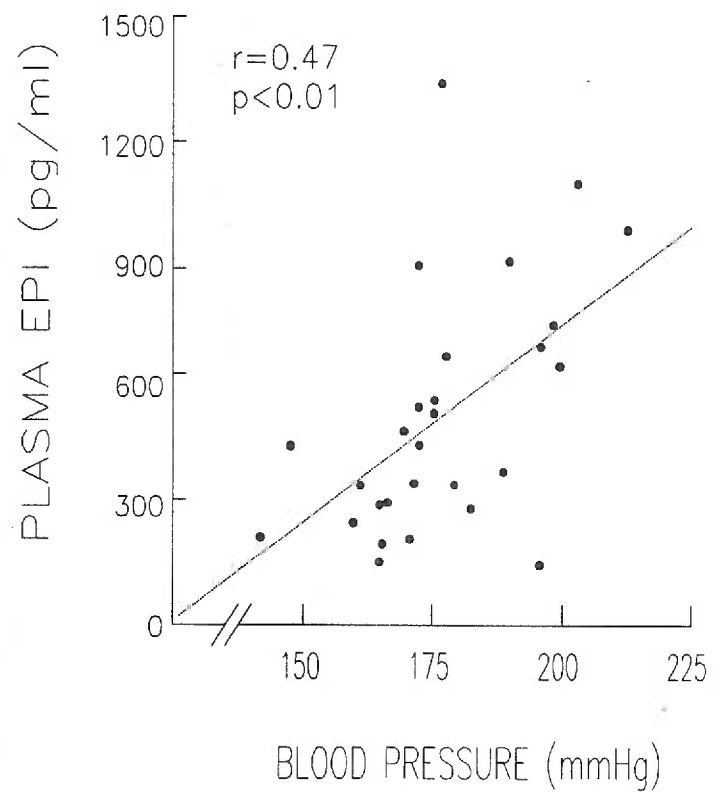
Though both strains showed significant increases in plasma EPI levels after exposure to stress, there were no strain differences in EPI levels during either baseline or stress. Hypertensive animals were influenced by the dietary manipulation as indicated by a greater stress-induced release of EPI among SHRs fed the high NaCl diets. WKYs showed little dietary effect in plasma EPI levels. A four-way ANOVA revealed a significant main effect for stress ($\underline{F}\{1,36\}=65.26$, $\underline{p}<0.001$) as well as a three-way interaction between Strain, NaCl and Stress ($\underline{F}\{1,36\}=5.01$, $\underline{p}=0.032$). Follow-up analyses in each strain demonstrated a significant NaCl by Stress interaction ($\underline{F}\{1,23\}=13.41$, $\underline{p}=0.001$) among the hypertensive animals while WKYs showed only a main effect for stress ($\underline{F}\{1,13\}=11.53$, $\underline{p}=0.005$). Within the SHR strain, only the high NaCl diet groups demonstrated a main effect for stress ($\underline{F}\{1,8\}=10.63$, $\underline{p}=0.012$).

Because the diets had parallel effects on both blood pressure and circulating EPI in the SHR, correlative analyses between the two variables were run for values obtained from both the resting and restraint measures. Figure 13 demonstrates the correlations between blood pressure and EPI both during rest ($\underline{r}=0.58$, $\underline{p}<0.001$) and

Figure 13. Correlation of blood pressure and circulating EPI concentrations during rest and restraint in the SHR.

CORRELATION OF MEAN BLOOD PRESSURE AND CIRCULATING EPINEPHRINE





restraint (r=0.47, $p<0.01$).

Blood Pressure Reactivity to Exogenous Norepinephrine

Dose responses to bolus injections of NE are shown in Figures 14 and 15. Figure 14 demonstrates an exaggerated reactivity to the highest dose of NE among SHRs fed the high sodium diets. In contrast, the normotensive strain showed no dietary differences in response to the catecholamine.

A four-way ANOVA, assessing pressor responses to NE injections, using Strain, NaCl and Calcium as between groups factors and Dose as a within group measure revealed a Strain by NaCl by Dose interaction ($F\{3,87\}=7.06$, $p<0.001$). Follow-up analyses within each strain showed a significant NaCl by dose interaction in the hypertensive strain ($F\{1,51\}=9.71$, $p<0.001$), while WKYs showed no effect of diet. Hypertensive rats maintained on the high NaCl diets showed greater pressor responses to the 3.0 μg/Kg dose than did SHRs fed the low NaCl diets as indicated by a main effect for NaCl found only at the 3 μg/Kg dose ($F\{1,35\}=6.96$, $p=0.012$).

Conditioned Blood Pressure Responses

Habituation:

Figures 16 and 17 show the blood pressure responses recorded during the first four CS alone trials for each CS type collapsed into two trial blocks. Figure 16 illustrates

Figure 14. Blood pressure response to exogenous, i.v. injection of NE at four doses in the SHR strain. Values are expressed as maximum change in mean blood pressure after injection for each diet group + SEM. Subject n's for SHRs maintained on Diets 1, 2, 3 and 4 were 5, 5, 5 and 4 respectively.

BLOOD PRESSURE RESPONSE TO NE IN THE SHR

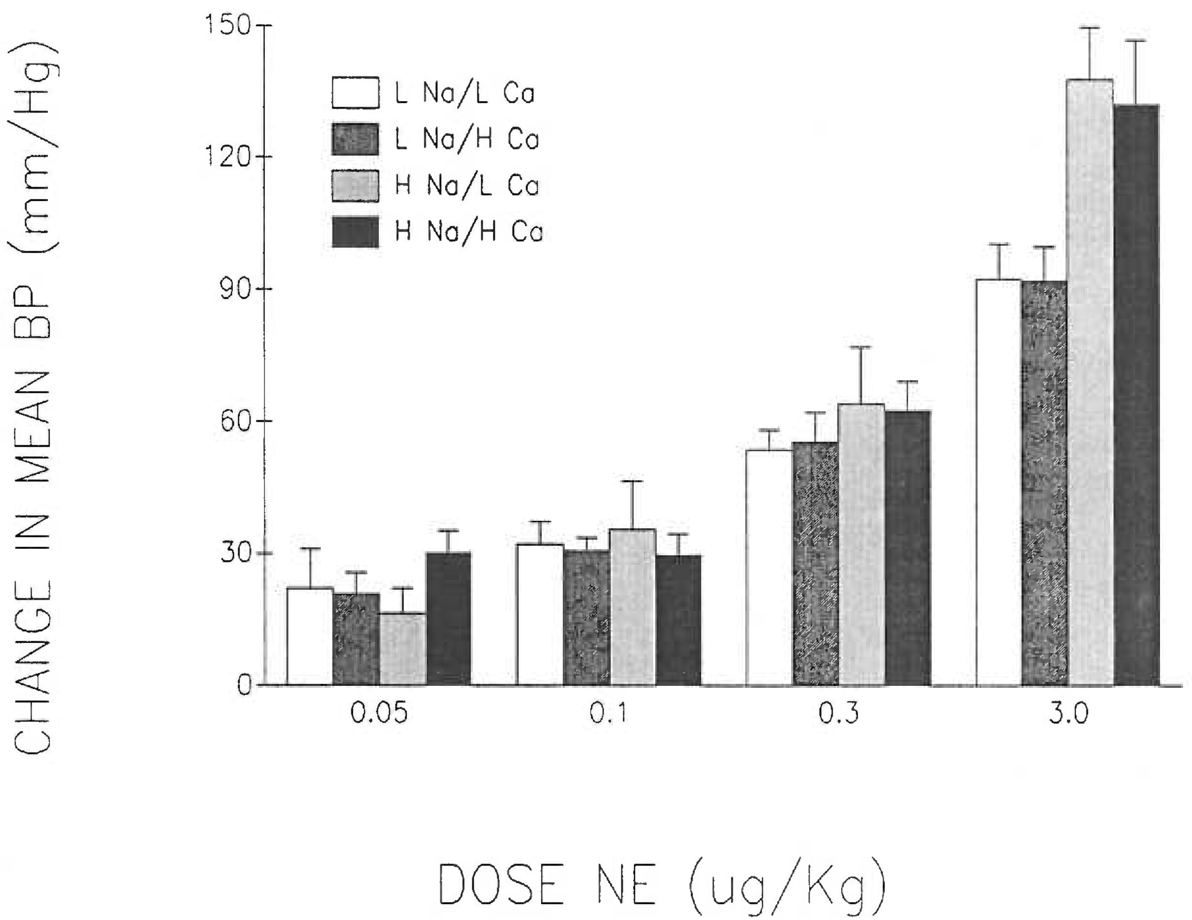


Figure 15. Blood pressure response to exogenous, i.v. injections of NE at four doses in the WKY strain. Values are expressed as maximum change in mean blood pressure after injection for each diet group + SEM. Subject n's for WKYs maintained on Diets 1, 2, 3 and 4 were 6, 4, 5 and 3.

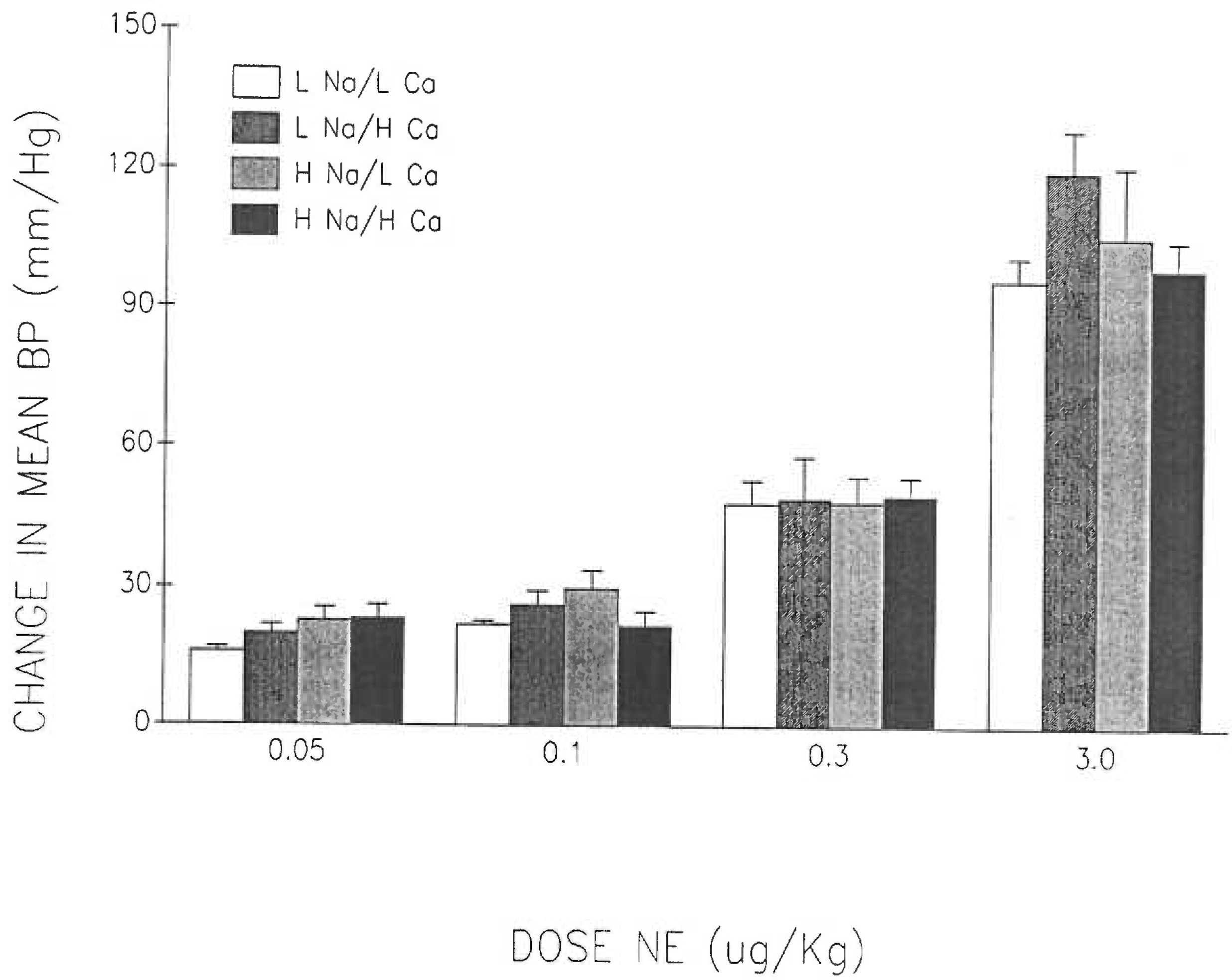

BLOOD PRESSURE RESPONSE TO NE IN THE WKY
CHANGE IN MEAN BP (mm/Hg)
150
120
90
60
30
0
L Na/L Ca
L Na/H Ca
H Na/L Ca
H Na/H Ca
0.05
0.1
0.3
3.0
DOSE NE (ug/Kg)

Figure 16. Orienting responses of the SHR strain to conditioned stimuli presented without shock. Values are expressed as change in mean blood pressure during each CP averaged across two trials into two trial blocks. Responses of each diet group are shown separately. Subject n's for the SHR diet groups 1, 2, 3 and 4 were 5, 4, 3 and 3 respectively for all conditioning data.

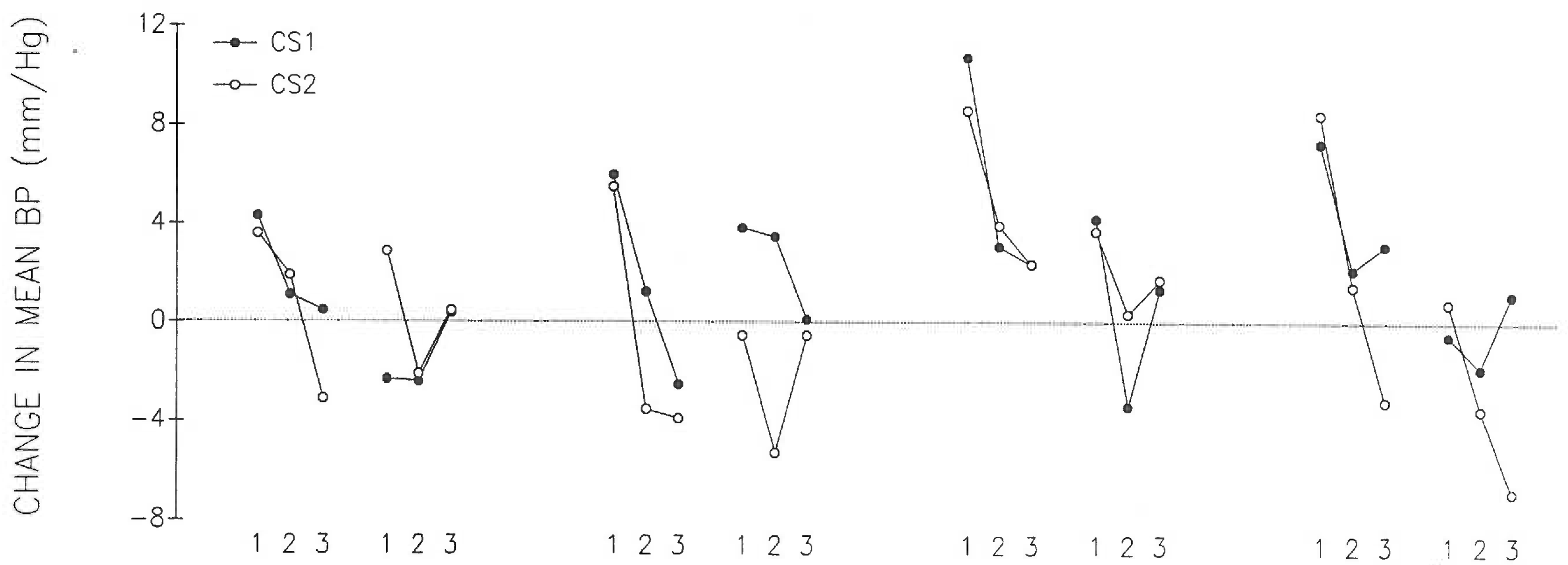
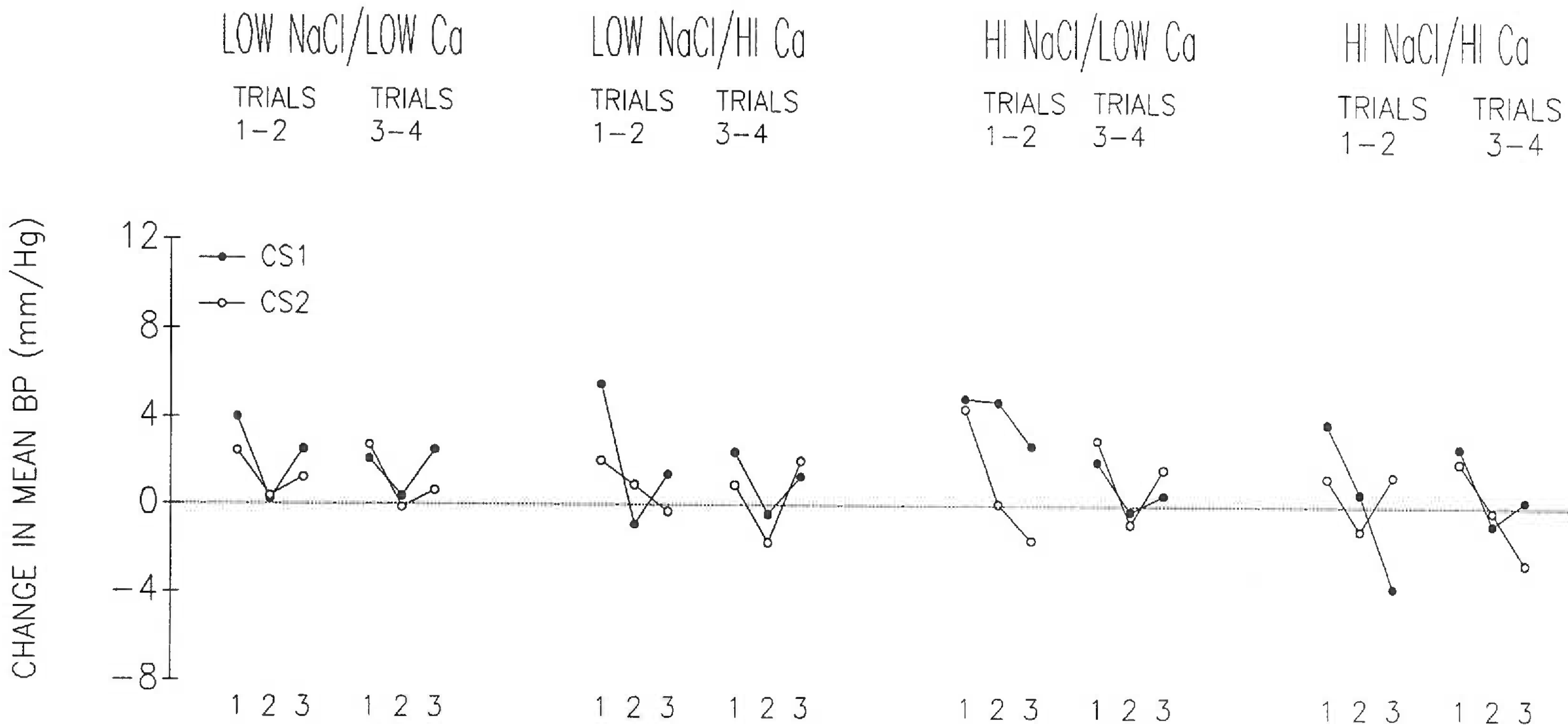

SHR BP RESPONSE TO CS ALONE TRIALS
LOW NaCL/LOW CA
LOW NaCL/HI CA
HI NaCl/LOW CA
HI NaCl/HI CA
TRIALS 1-2
TRIALS 3-4
CS1
CS2
CHANGE IN MEAN BP (mm/Hg)
12
8
4
0
-4
-8
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

Figure 17. Orienting responses of the WKY strain to conditioned stimuli presented without shock. Values are expressed as change in mean blood pressure during each CP averaged across two trials into two trial blocks. Responses of each diet group are shown separately. Subject n's for the WKY diet groups 1, 2, 3 and 4 were 7, 5, 6 and 5 respectively for all conditioning data.



WKY BP RESPONSE TO CS ALONE TRIALS
LOW NaCl/LOW Ca
LOW NaCl/HI Ca
HI NaCl/LOW Ca
HI NaCl/HI Ca
TRIALS 1-2
TRIALS 3-4
CS1
CS2
CHANGE IN MEAN BP (mm/Hg)
12
8
4
0
-4
-8
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

the exaggerated reactivity of the SHR strain as indicated by their more pronounced pressor and depressor responses. Examination of the figure suggests that blood pressure reactivity among the SHR subjects was affected by both NaCl and calcium. In contrast, as shown in Figure 17, the normotensive strain showed little evidence of any dietary influence on blood pressure reactivity.

Results from a six-way ANOVA using Strain, NaCl and Calcium levels as between groups factors and CS type, Trial blocks and CPs as within groups factors showed a significant five-way interaction between Strain, NaCl, Calcium, CS type and CPs ($\underline{F}(2,62)=4.27$, $\underline{p}=0.018$). Analyses performed on the individual strains revealed a significant NaCl by Calcium by CS type by CP interaction ($\underline{F}(2,24)=4.54$, $\underline{p}=0.021$) only in the SHRs.

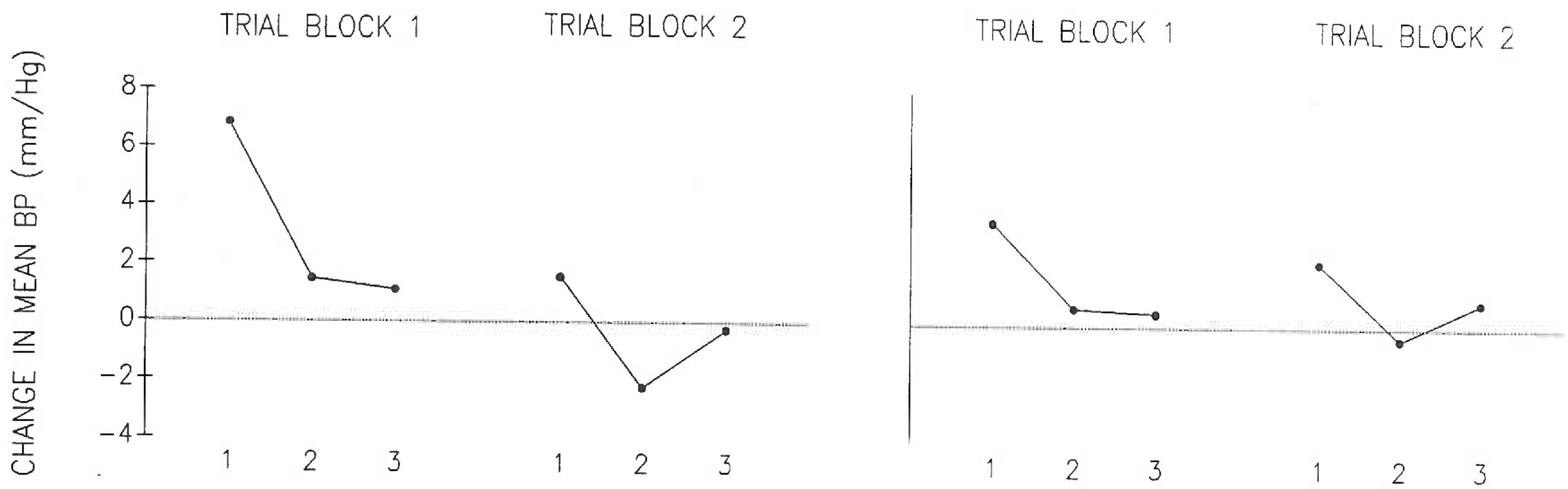
Separate three-way analyses assessing calcium, CS type and CP were performed on the high and low NaCl diet groups. Only the high NaCl groups showed a significant three-way interaction ($\underline{F}(2,10)=4.65$, $\underline{p}=0.037$) for Calcium, CS type and CPs. Using data from the two high NaCl groups, each CP was examined independently for any Calcium by CS type interactions. A two-way interaction which approached significance ($\underline{F}(1,5)=5.42$, $\underline{p}=0.067$) was found only in the third CP. As can be seen in Figure 16, the two high NaCl groups fed differing amounts of calcium showed divergent

responses during the third CP. Specifically, the high calcium group showed a more pronounced blood pressure deceleration in response to CS 2 which was not evident in the high NaCl/low calcium group.

Similar analyses of the SHR data examining NaCl by CS type by CP interactions in the individual calcium diet groups demonstrated a NaCl by CS by CP effect only in the high calcium group ($F\{2,10\}=5.33$; $p=0.027$). Such findings would be predicted given the Calcium by CS interaction found in the high NaCl diet group. Interestingly, within the low calcium group, a main effect for NaCl was found ($F\{1,7\}=7.95$; $p=0.026$). As indicated by Figure 15, pressor responses in animals fed a low calcium diet were potentiated by the high NaCl diet. Responses within the high calcium diet did not differ with respect to NaCl, indicating that supplemental calcium was able to attenuate the NaCl-dependent exaggeration in pressor responses.

Aside from the dietary effects found in the initial six-way ANOVA there was also a Strain by Trial Blocks by CP interaction ($F\{2,62\}=3.90$, $p=0.025$). Figure 18 illustrates the blood pressure responses during the first two trial blocks for each strain collapsed across diets and CS type. The SHR strain showed substantial pressor activity during the first trial block while WKYs showed only mild pressor responses. However, during the second trial block, SHR pressor responses diminished to levels

Figure 18 Orienting responses of both the SHR and the WKY strain collapsed across CS type. Data are expressed as change in mean blood pressure during each CP averaged across two trials into two trial blocks.


BP RESPONSE TO CS ALONE AVERAGED OVER CS TYPE AND DIET
SHR
WKY
TRIAL BLOCK 1
TRIAL BLOCK 2
TRIAL BLOCK 1
TRIAL BLOCK 2
CHANGE IN MEAN BP (mm/Hg)
8
6
4
2
0
−2
−4
1 2 3
1 2 3
1 2 3
1 2 3
TWO SECOND COUNTING PERIODS PER TRIAL BLOCK

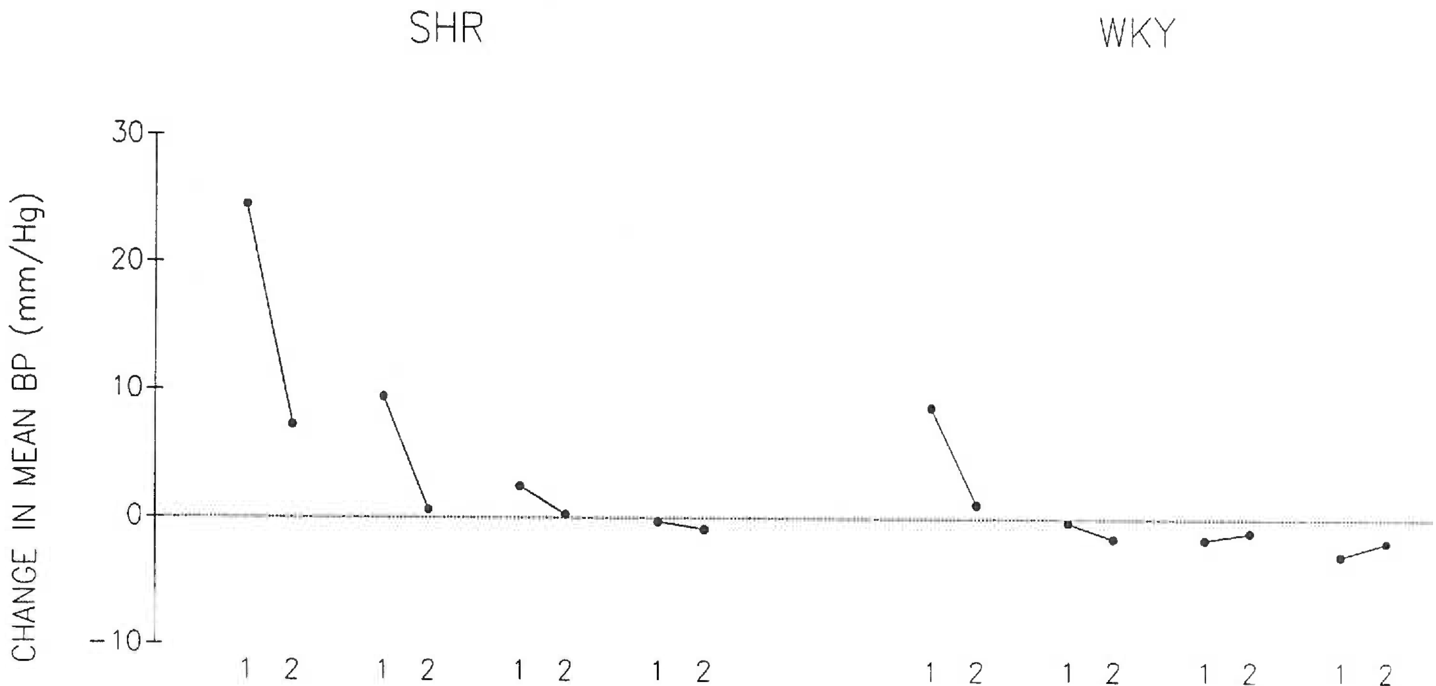
comparable to those of the WKY. Strain by CP analyses run on the individual trial blocks demonstrated a two-way interaction during the first trial block ($\underline{F}\{2,72\}=6.58$, $\underline{p}=0.02$). A one-way ANOVA comparing strains at each CP of the first trial block showed a strain difference only during the first CP ($\underline{F}\{1,37\}=5.87$, $\underline{p}=0.02$). As evidenced by follow-up analyses, the original Strain by Trial Block by CP interaction resulted from a greater reactivity to the stimulus onset in the hypertensive strain. Furthermore, SHR pressor responses were habituated with repeated exposure to the stimuli, while the normotensive strain showed little change across trial blocks.

In summary, SHRs showed orienting responses which varied with NaCl and calcium intake. Increased dietary NaCl intake was found to elevate pressure responses in the low calcium SHR diet groups. In addition, the hypertensive strain showed exaggerated pressor responses initially which were effectively habituated by the second trial block. WKY animals showed very limited responses to the stimuli initially and thus showed little habituation.

Unconditioned Blood Pressure Responses:

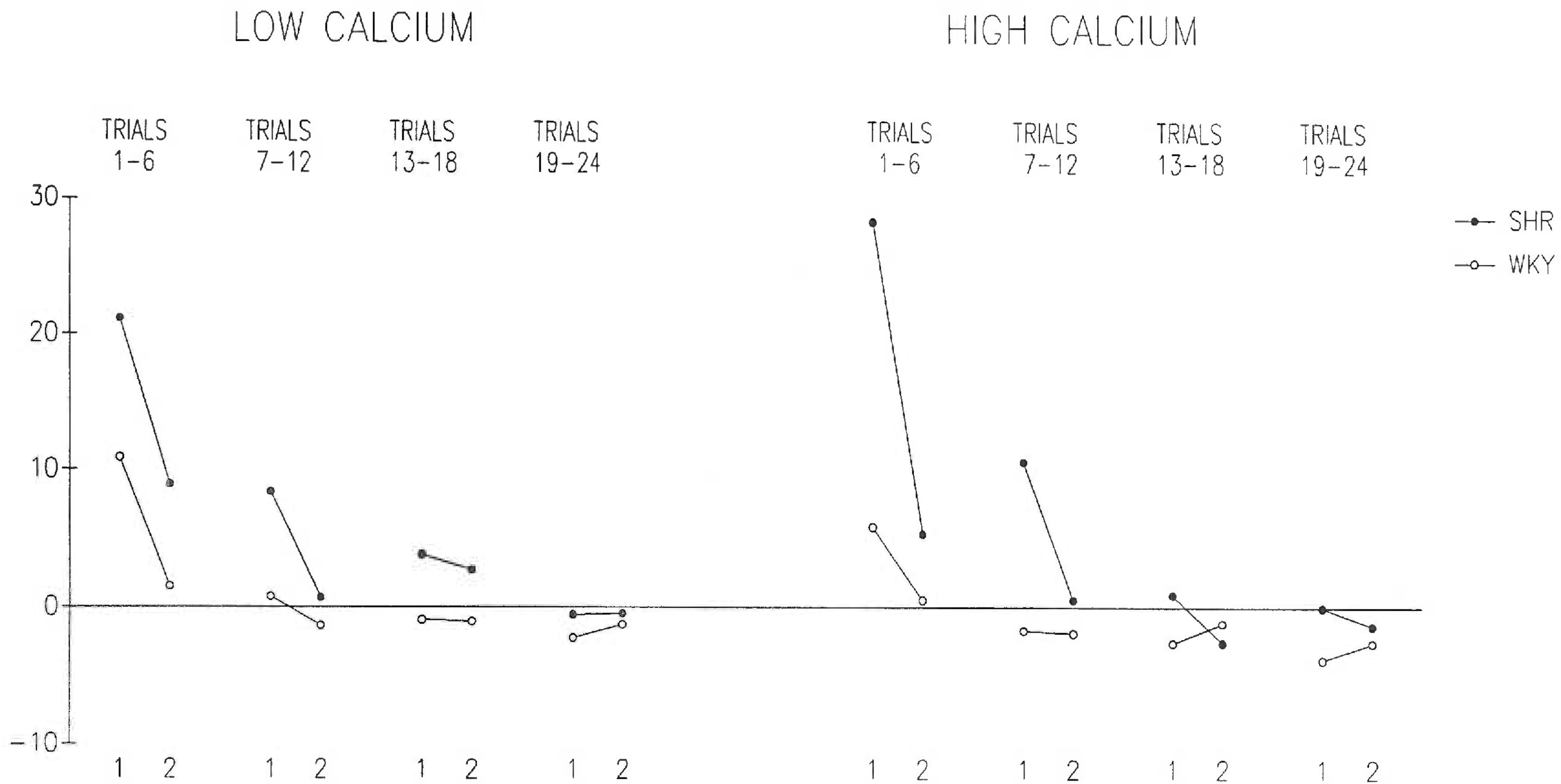
Unconditioned blood pressure responses (UR) of each strain collapsed into four 6-trial blocks are shown in Figure 19. As can be seen in the early trials, SHRs showed dramatic pressor responses directly after shock followed by

Figure 19. Blood pressure response to shock for both the SHR and WKY strain. Values are expressed as change in mean blood pressure for each CP averaged across six trials into four trial blocks. Data were further collapsed across diet group to give an overall strain mean.


BP RESPONSE TO SHOCK COLLAPSED ACROSS DIET
SHR
WKY
CHANGE IN MEAN BP (mm/Hg)
30
20
10
0
-10
1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

a precipitous fall in blood pressure during the second CP. The WKY strain demonstrated a rapid habituation to the US as evidenced by the small pressor responses elicited during the second, third and fourth trial blocks. In contrast, the hypertensive animals showed pressor responses through the first three trial blocks, which were almost fully habituated by the fourth trial block. A five-way ANOVA using Strain, NaCl and Calcium as between groups factors with Trial Blocks and CPs as the within groups factors revealed a significant Strain by Calcium by Trial Blocks by CP interaction ($F\{3,90\}=4.18$, $p=0.008$). Unconditioned responses of each strain averaged into high and low calcium diets are presented in Figure 20 . Follow-up analyses assessing the high and low calcium groups independently demonstrated a significant Strain by Trial Blocks by CP interaction ($F\{3,45\}=10.58$, $p<0.001$) among animals fed the high calcium diet. Each trial block from the high calcium animals was then assessed separately to determine the origin of the strain effect. Both the first and second trial blocks demonstrated significant strain by CP interactions ($F\{1,15\}=80.86$, $p<0.001$; $F\{1,15\}=12.72$, $p=0.003$). One-way ANOVAs comparing strain responses during the first and second CPs separately showed a significant strain effect only during the initial CP for both the first and second trial blocks ($F\{1,15\}=47.30$, $p<0.001$;$F\{1,15\}=25.60$, $p<0.001$). Thus, the original Strain by Calcium by Trial

Figure 20. Blood pressure response to shock for each strain collapsed into high and low calcium diet groups. Data were averaged across six trials into four trial blocks and expressed as change in mean blood pressure.


BP RESPONSE TO SHOCK IN EACH STRAIN AVERAGED INTO CALCIUM DIET GROUPS
LOW CALCIUM
HIGH CALCIUM
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
SHR
WKY
30
20
10
0
-10
1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2
TWO SECOND COUNTING PERIODS FOR EACH TRIAL BLOCK

Blocks by CP interaction was due to larger pressor responses directly following shock presentation in the first two trial blocks among hypertensive animals fed the high calcium diet. In contrast, when fed the low calcium diet, the two strains did not show a significant difference in reactivity.

Additionally, as can be seen in Figure 19, there was a significant Strain by Trial Blocks by CP interaction independent of any dietary influence ($F\{3,90\}=8.38$, $p<0.001$). Follow-up analyses performed on the individual trial blocks showed significant Strain by CP interactions during the first ($F\{1,36\}=17.55$, $p<0.001$) and second trial blocks ($F\{1,36\}=14.14; p=0.001$). In turn, ANOVAs run on data from the individual CPs for each of the significant trial blocks demonstrated main effects for strain during both the first ($F\{1,36\}=20.38$, $p<0.001$) and second CPs ($F\{1,36\}=6.49$, $p=0.015$) of the first trial block and the first CP of the second trial block ($F\{1,36\}=26.56$, $p<0.001$). These results demonstrate a greater reactivity coupled with a slower habituation of response in the hypertensive strain. In addition, strain differences in response to the shock were potentiated with the high calcium diet though neither strain showed a significant main effect for calcium.

Conditioned Blood Pressure Responses:

Conditioned blood pressure responses (CR) recorded from SHR and WKY animals maintained on the four different diets

are represented in Figures 21 and 22. The data were collapsed into four 6-trial blocks for each the four diet groups. As can be seen, the hypertensive strain showed more pronounced conditioned responses than did the WKY strain. In general, responses to the CS+ in both strains were characterized by a large blood pressure rise during the stimulus onset followed by a rapid decline and rebound in blood pressure during the second and third CPs respectively. In both strains, CS- responses were similar in topography to the reinforced stimulus response. However, both strains showed attenuated responses to the neutral stimulus in comparison to their CS+ response.

A six-way analysis using Strain, NaCl and Calcium as between groups factors and CS type, Trial Blocks and CPs as within groups factors demonstrated significant main effects for strain ($F\{1,30\}=18.76$, $p<0.001$) and CS type ($F\{1,30\}=42.99$, $p<0.001$) as well as a Strain by CS type by Trial Block interaction ($F\{3,90\}=3.07$, $p=0.032$). Figure 23 demonstrates CS+ and CS- responses of the two strains collapsed across diet and CPs. As indicated by the figure, SHRs had a greater discriminative response to the two stimuli than did normotensive animals. This was confirmed by follow-up analyses of the individual strains which revealed a CS type by Trial Blocks interaction only among SHRs ($F\{3,33\}=4.46$, $p=0.01$).

The CS type by Trial Blocks interaction seen in the

Figure 21. Conditioned blood pressure responses to both reinforced and neutral conditioning stimuli of each diet group in the SHR strain. Data for CP period are presented as mean scores averaged across six trials. Four trial blocks are presented for each diet group.

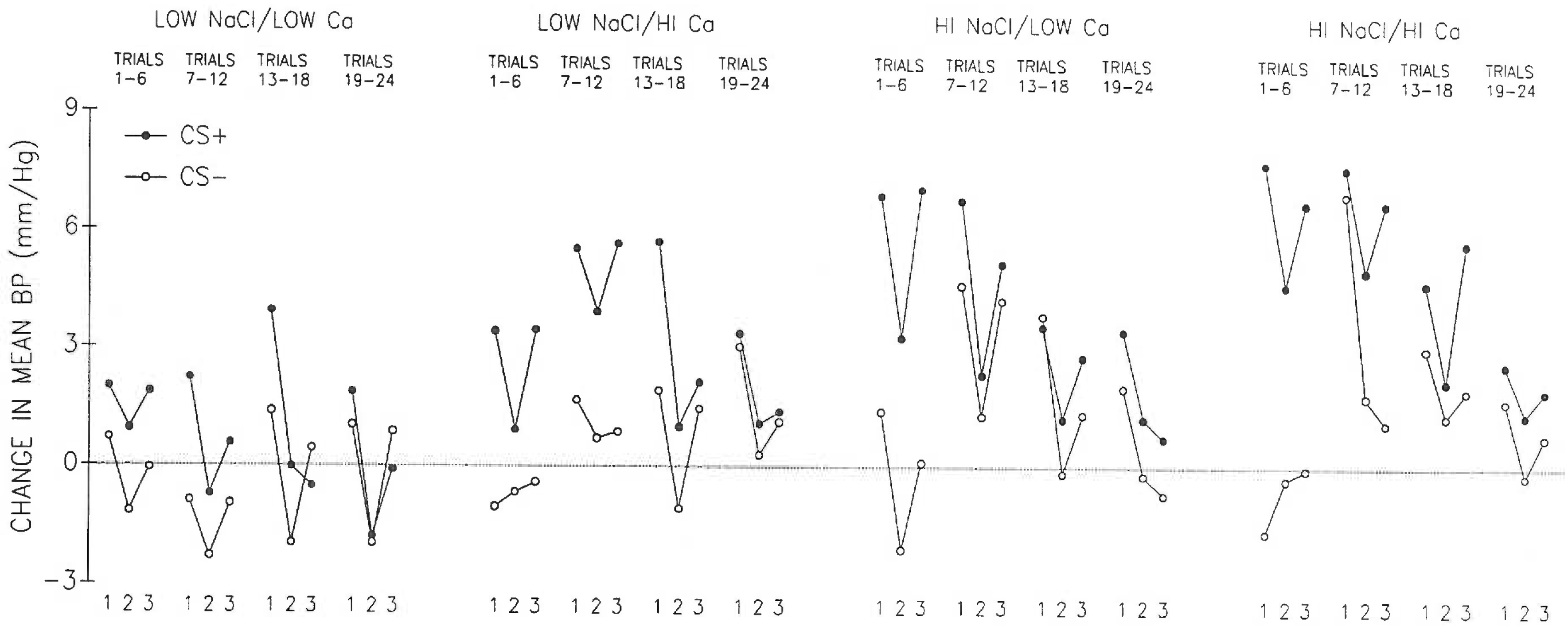
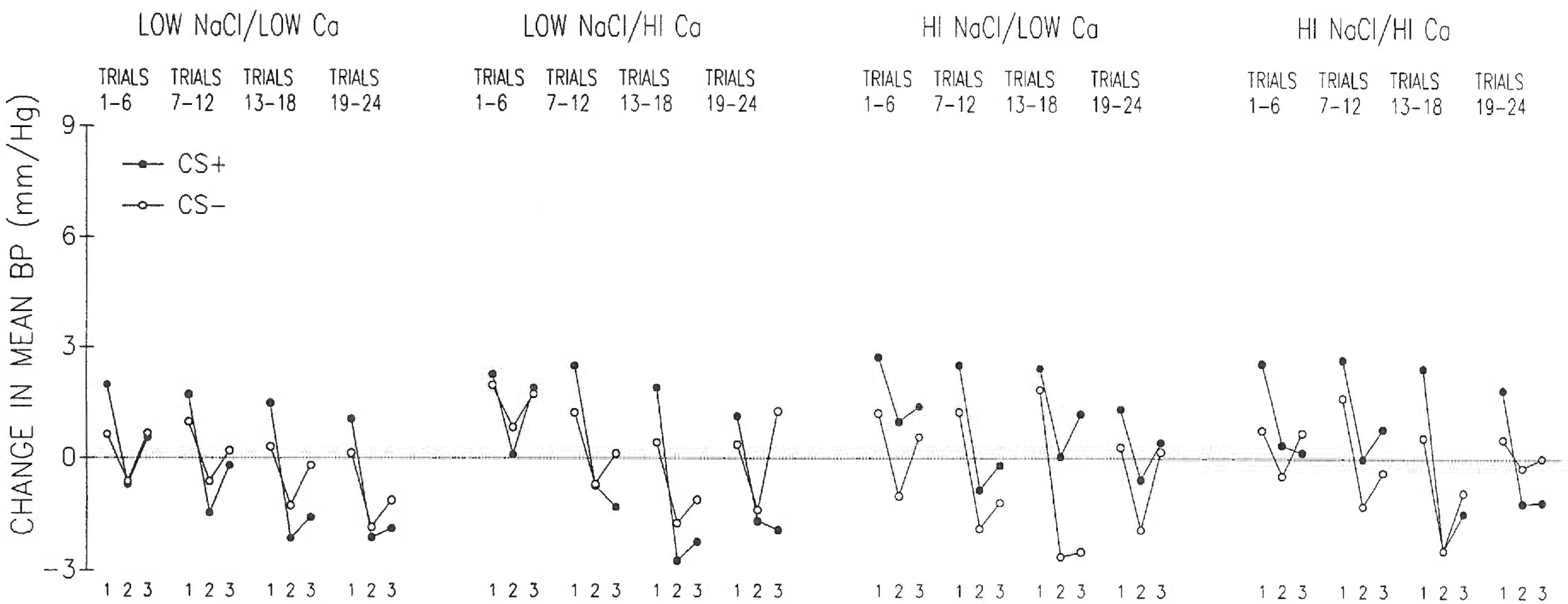

CONDITIONED BP RESPONSE IN THE SHR
LOW NaCl/LOW Ca
LOW NaCl/HI Ca
HI NaCl/LOW Ca
HI NaCl/HI Ca
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
CS+
CS-
CHANGE IN MEAN BP (mm/Hg)
9
6
3
0
-3
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

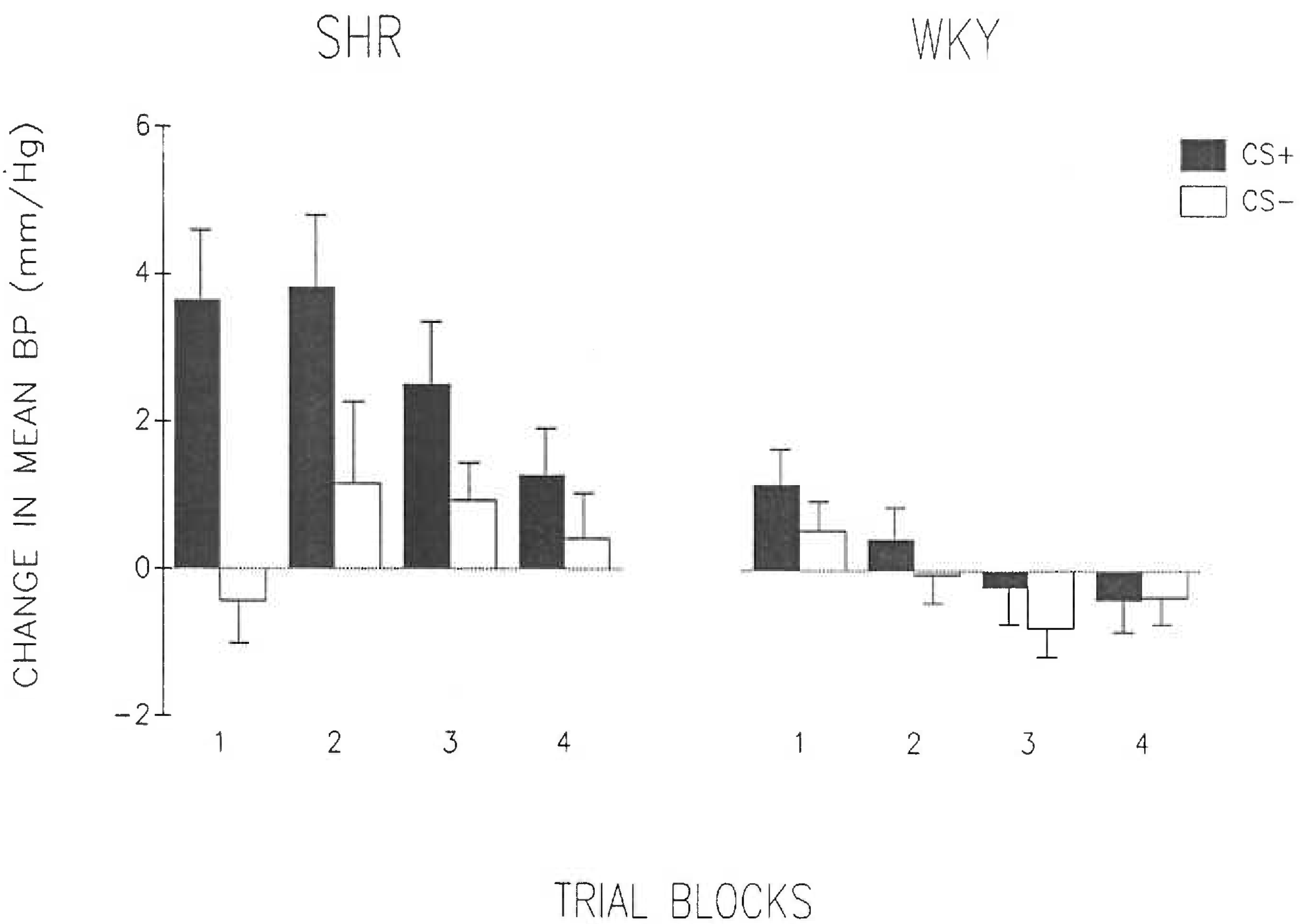
Figure 22. Conditioned blood pressure responses to both reinforced and neutral conditioning stimuli of each diet group in the WKY strain. Data for each CP are presented as mean scores averaged across six trials. Four trial blocks are presented for each diet group.



CONDITIONED BP RESPONSE IN THE WKY
LOW NaCl/LOW Ca
LOW NaCl/HI Ca
HI NaCl/LOW Ca
HI NaCl/HI Ca
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
CS+
CS-
CHANGE IN MEAN BP (mm/Hg)
9
6
3
0
-3
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

Figure 23. Conditioned blood pressure responses to the reinforced and neutral conditioning stimuli of both strains collapsed across diet groups. Data were averaged across CPs for six trials into four trial blocks. Values are expressed as group means + SEM.

CONDITIONED BP RESPONSE COLLAPSED ACROSS DIET AND COUNTING PERIODS

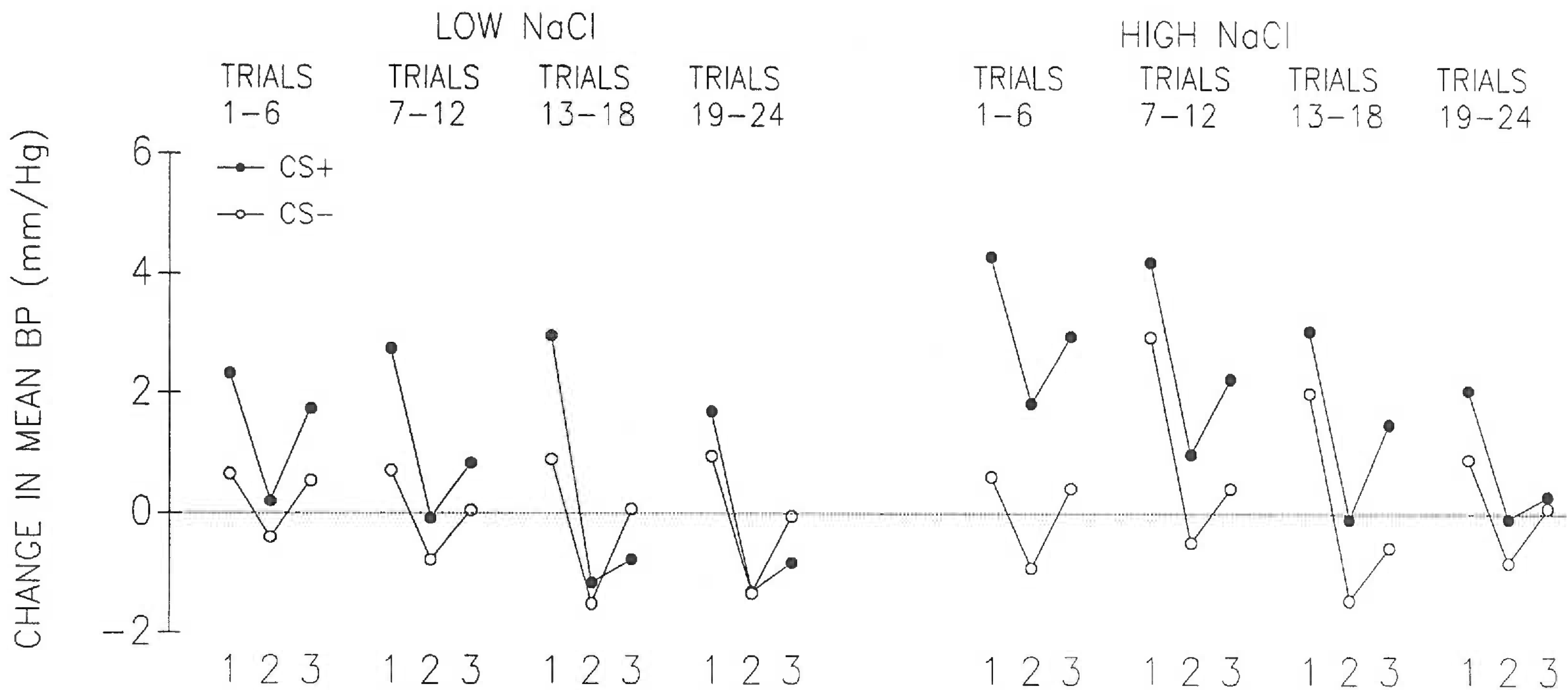


hypertensive strain is suggestive of discriminative learning. However, examination of Figure 22 indicates that the CS by Trial Block interaction was most likely due to a decrease in discriminative responding with repeated exposure to the two stimuli. Follow-up analyses showed significant CS type response differences at each trial block in the hypertensive strain (Fs{1,14}=24.37, p<0.001; 8.2, p=0.013; 5.94, p=0.029; 5.13, p=0.05 respectively). The diminished degree of differential responding to the two CS types was reflected statistically in the decreased reliability of the CS type differences in successive trial blocks. No such CS type differences were observed during the habituation phase, indicating that learned responses among the hypertensive strain were most likely acquired during the first trial block.

Conditioned responses were also found to differ with NaCl intake. The high NaCl diet potentiated blood pressure responses to stimuli over the entire conditioning session. This was validated statistically by the presence of a main effect for NaCl (F{1,30}=5.64, p=0.024) in the overall analysis.

The original overall analysis also showed a significant NaCl by CS type by Trial Block by CP interaction (F{6,180}=2.46,p=0.026). This interaction is illustrated in Figure 24 which demonstrates CRs of the high and low NaCl diet groups collapsed over strains. Separate CS type by

Figure 24. Conditioned blood pressure responses to reinforced and neutral stimuli collapsed across strain into low and high NaCl diet groups. Data are shown for each CP averaged across six trials into four trial blocks and expressed as change in mean blood pressure.



CONDITION RESPONSE AVERAGED ACROSS STRAINS INTO NaCl GROUPS
LOW NaCl
HIGH NaCl
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
TRIALS 1-6
TRIALS 7-12
TRIALS 13-18
TRIALS 19-24
CS+
CS-
CHANGE IN MEAN BP (mm/Hg)
6
4
2
0
-2
1 2 3
TWO SECOND COUNTING PERIODS PER TRIAL BLOCK

Trial Blocks by CP analyses run on the high and low NaCl groups revealed a significant three-way interaction within the low NaCl group ($\underline{F}\{6,102\}=2.19$, $\underline{p}=0.05$). Analyses of CRs from each trial block of the low NaCl group showed a significant CS type by CP interaction ($\underline{F}\{2,40\}=15.65$, $\underline{p}<0.001$) during the third trial block. During the first two trial blocks the CS+ response was characterized by an initial rise in blood pressure followed by a steep decrease during the second CP which then rebounded above baseline. However, during the third trial block, blood pressure responses of low NaCl subjects to the CS+ did not rebound back to baseline within the third CP and, in fact, were exceeded by responses to the unreinforced tone. Conditioned Stimulus Type by Trial Block analyses run at each CP demonstrated a significant interaction only during the third CP ($\underline{F}\{3,60\}=3.76$, $\underline{p}=0.015$) confirming the notion that the change in CS response during the final CP was likely responsible for the CS type by Trial Block by CP interaction in the low NaCl group.

Further inspection of Figure 24 indicated that animals maintained on the high NaCl diets exhibited greater discriminative responses to the two stimulus types than did the low NaCl animals. In fact, only the high NaCl group showed a significant main effect for CS type ($\underline{F}\{1,13\}=36.90$, $\underline{p}<0.001$). Such discriminative responses were likely acquired within the first trial block given the lack of CS

type difference during habituation coupled with the diminishing difference in CS type response over the duration of the conditioning session.

In order to determine whether response differences developed during the first trial block, data from the first six trials were collapsed into three, 2-trial blocks for analysis. Figures 25 and 26 show the CRs of each strain on each of the four diets during the three early trial blocks. Within the first six trials, SHRs (Figure 25) showed evidence of discriminative learning as indicated by the progressively greater pressor responses elicited by the CS+ stimulus coupled with the relatively constant response to the neutral tone. In contrast, normotensive animals showed nearly the same responses to both stimuli throughout the three trial blocks (Figure 26). Dietary influences were also evident within the early trial blocks. As seen in Figure 27, hypertensive animals fed the two high NaCl diets showed progressively more pronounced pressor responses to the CS+ than did SHRs fed the low NaCl diets. In the WKY strain, such dietary effects were less apparent.

An analysis of variance using Strain, NaCl and Calcium as between groups factors with CS type, the three early Trial blocks and CPs as within groups factors revealed a significant interaction between NaCl, Strain, CS type and Trial blocks ($F(2,60)=3.41$, $p=0.04$). Follow-up analyses of the early trials within the individual strains revealed a

Figure 25. Conditioned blood pressure responses during the early conditioning trials for each diet group in the SHR strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values are expressed as change in mean blood pressure.

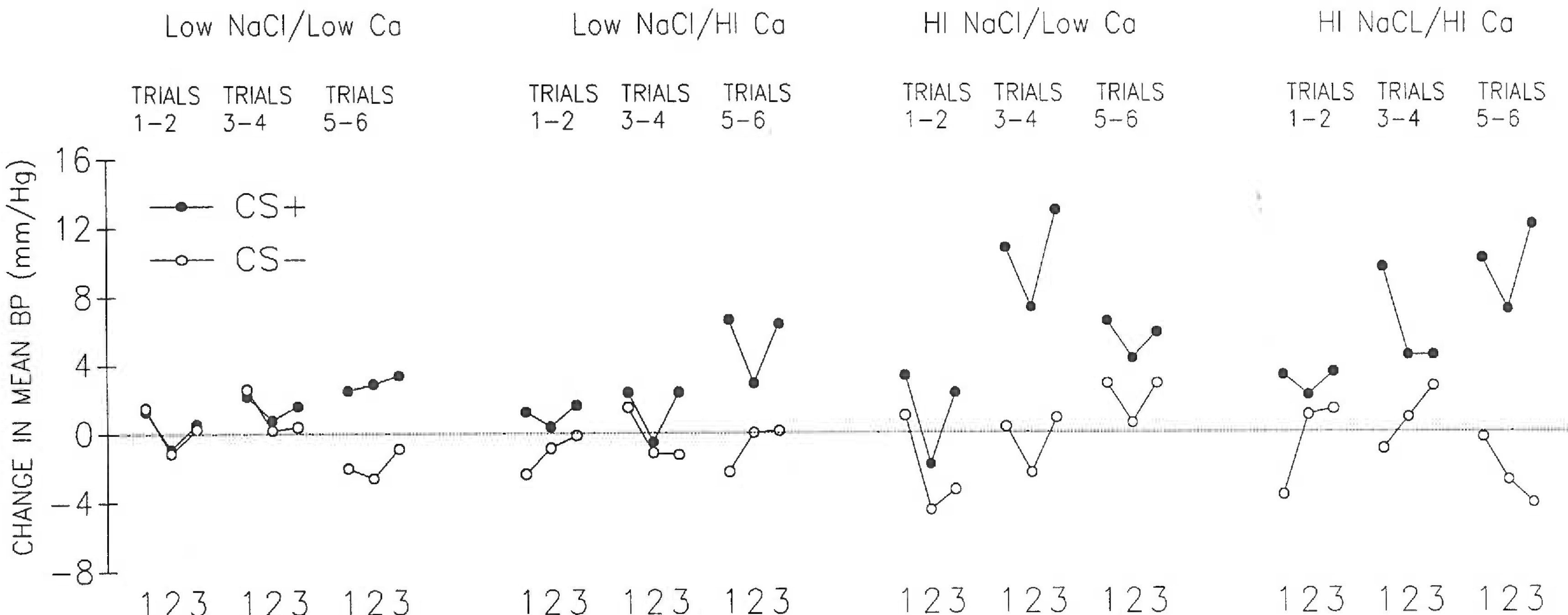

SHR BP CR DURING THE FIRST SIX TRIALS
Low NaCl/Low Ca
Low NaCl/HI Ca
HI NaCl/Low Ca
HI NaCL/HI Ca
TRIALS 1–2
TRIALS 3–4
TRIALS 5–6
CS+
CS–
CHANGE IN MEAN BP (mm/Hg)
16
12
8
4
0
–4
–8
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

Figure 26. Conditioned blood pressure responses during the early conditioning trials for each diet group in the WKY strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values are expressed as change in mean blood pressure.

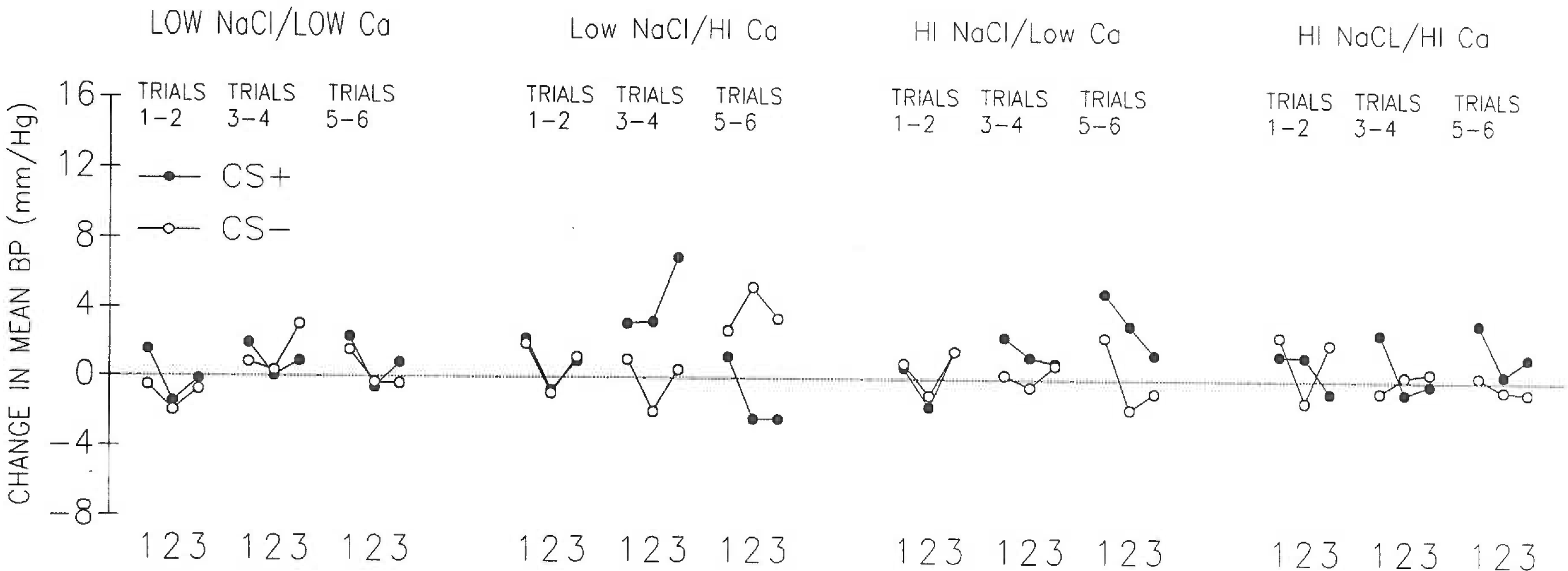
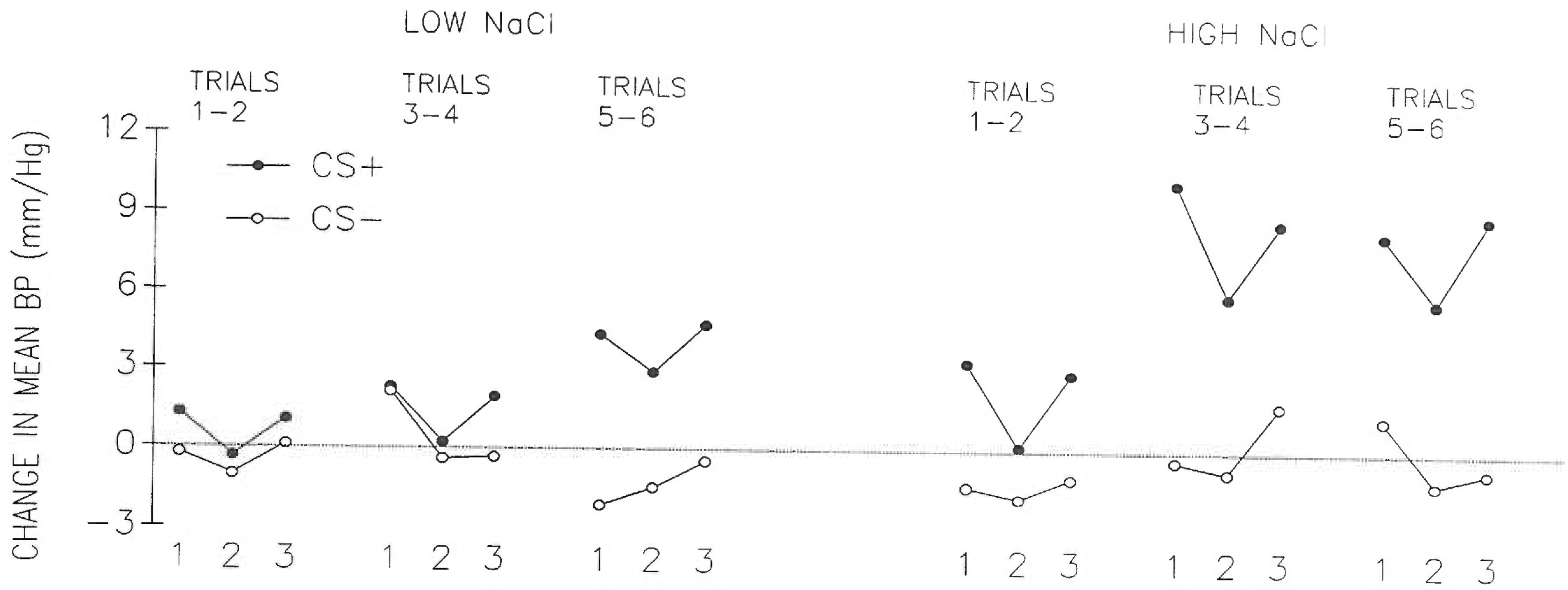

WKY BP CR DURING FIRST SIX TRIALS
LOW NaCl/LOW Ca
Low NaCl/HI Ca
HI NaCl/Low Ca
HI NaCL/HI Ca
TRIALS 1-2
TRIALS 3-4
TRIALS 5-6
CS+
CS-
CHANGE IN MEAN BP (mm/Hg)
16
12
8
4
0
-4
-8
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

Figure 27. Conditioned blood pressure responses during the early trials collapsed into high and low NaCl diet groups for the SHR strain. Data for each CP are shown averaged across 2 trials into three-trial blocks. Values are expressed as change in mean blood pressure.

SHR BP CR DURING FIRST SIX TRIALS AVERAGED INTO NaCl DIET GROUPS



ST SIX TRIALS COLLAPSED ACROSS SALT DIETS IN THE SHR

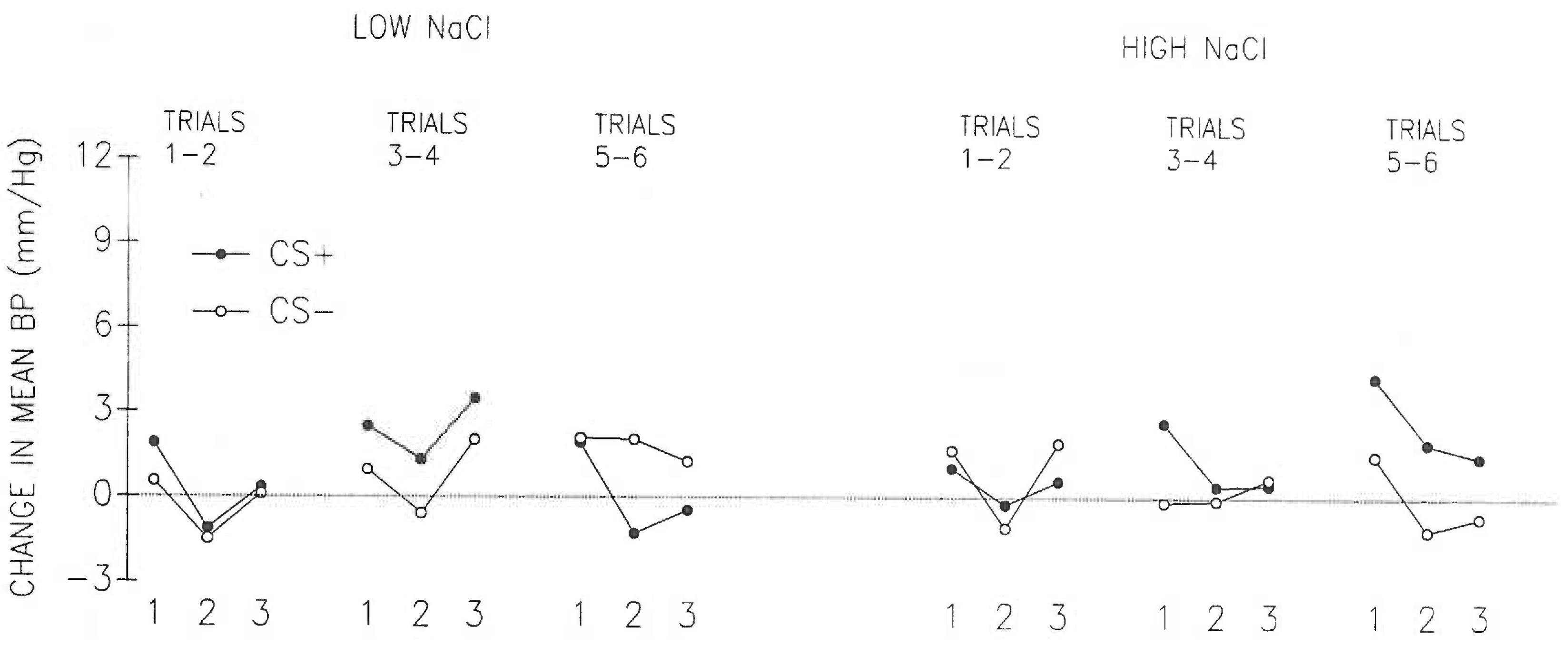
significant interaction for NaCl and CS type ($\underline{F}\{1,11\}=7.96$, $\underline{p}=0.017$) as well as for CS type and Trial Blocks ($\underline{F}\{2,22\}=4.14$, $\underline{p}=0.03$) in the hypertensive strain. Examination of Figure 27 suggests that the NaCl by CS type interaction resulted from greater reactivity to the CS+ among the high NaCl SHRs. This was confirmed by a one-way ANOVA comparing CS+ responses in the high and low NaCl groups which revealed a significant effect for NaCl ($\underline{F}\{1,13\}=8.567$, $\underline{p}<0.05$). A similar analysis showed the unreinforced stimulus response to be unaffected by NaCl intake.

The origin of the CS type by Trial Block interaction found among SHRs in the early trials can be seen in Figure 25. Responses to the reinforced tone increased in magnitude with repeated exposures while responses to the CS- did not differ across trials. This was substantiated by an analysis examining SHR CS+ responses collapsed over CPs which demonstrated a significant effect for trial blocks ($\underline{F}\{2,28\}=4.51$, $\underline{p}=0.02$). No significant trial blocks effect was observed in an analysis of unreinforced stimulus responses.

A follow-up analysis of NaCl, CS type and Trial Blocks for the early trials was also performed on the normotensive strain. WKYs demonstrated a significant interaction between all three variables ($\underline{F}\{2,38\}=3.54$, $\underline{p}=0.039$). Figure 28 demonstrates that WKY animals fed the high NaCl diets had

Figure 28. Conditioned blood pressure responses during the early trials collapsed into high and low NaCl diet groups for the WKY strain. Data for each CP are shown averaged across 2 trials into three-trial blocks. Values are expressed as change in mean blood pressure.

WKY BP CR DURING FIRST SIX TRIALS AVERAGED INTO NaCl DIET GROUPS

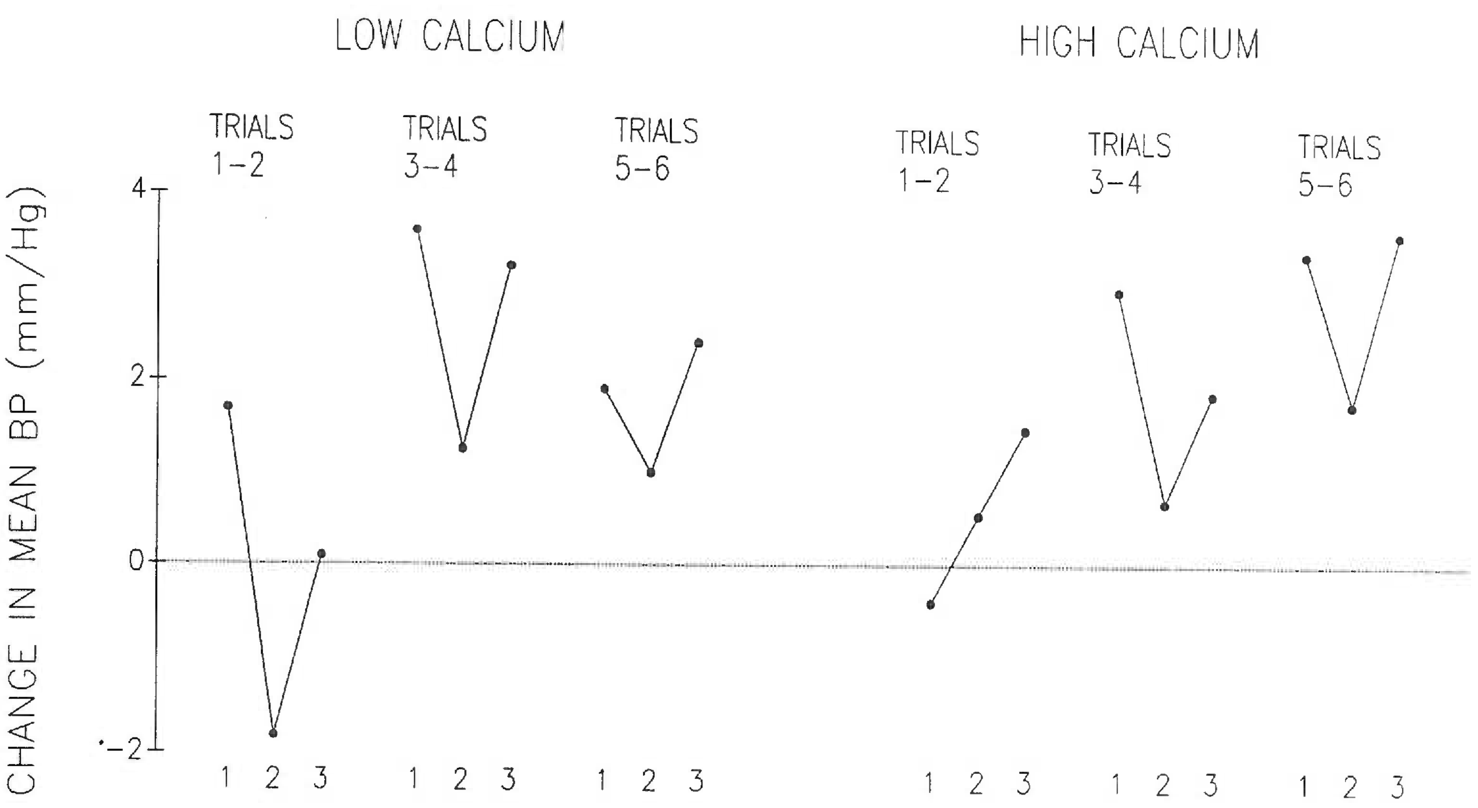


differential responses to the two CS types after repeated exposure to the stimuli. In contrast, WKYs fed the low NaCl diets did not show consistent evidence of discriminative responding. This was confirmed by analyses of the low and high NaCl groups which showed a significant CS by Trial Blocks effect for WKYs fed the two high NaCl diets ($F\{2,20\}=4.23$, $p=0.029$). Analyses of CRs from the normotensive animals fed the low NaCl diet showed no CS type by Trials interaction. Analysis of separate trial blocks revealed a main effect for CS type during the third trial block in the normotensive high NaCl group ($F\{1,10\}=8.86$, $p=0.014$) indicating that the discriminative response was acquired over trials. These data coupled with results from the SHR strain suggest that the CS type differences observed in the overall analysis of the entire 28-trial conditioning session were most likely due to discriminative learning which developed during the first six trials in both strains.

A Strain by Calcium by Trial Blocks by CP interaction ($F\{4,120\}=3.63$, $p=0.008$) was also found within the overall six-way analysis of the early trials. Follow-up analyses looking at Calcium, Trial Blocks and CPs in the individual strains revealed a significant three-way interaction among SHRs ($F\{4,44\}=3.49$, $p=0.015$) while the normotensive strain showed no significant differences. Figure 29 shows SHR blood pressure responses collapsed across CS types for each

Figure 29. Conditioned blood pressure responses of the early trials collapsed into low and high calcium diet groups for the SHR strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values were further collapsed across CS type and expressed as change in mean blood pressure.

SHR BP RESPONSE AVERAGED ACROSS CS TYPE INTO CALCIUM DIET GROUPS



trial block and CP in the high and low calcium diet groups. Separate analyses run on the high and low calcium SHR subjects demonstrated a significant Trial Blocks by CP interaction among the high calcium animals ($F\{4,24\}=3.47$, $p=0.023$). One-way ANOVAs performed on data from each CP within the high calcium group revealed a significant trial blocks effect during the first CP ($F\{2,12\}=5.65$, $p=0.019$). Thus, the Calcium by Trial Block by CP interaction was due to a progressive increase in responsiveness only during the stimulus onset among the high calcium animals. The low calcium hypertensive animals, in contrast, did not show a consistent change in responsiveness over trials.

The original six-way analysis on the early trial blocks also demonstrated a significant Strain by Calcium by CS type by Trial Block interaction ($F\{2,60\}=5.01$, $p=0.013$). Figures 30 and 31 show the CRs of SHRs and WKYs collapsed into high and low calcium groups. Consistent with the previous findings, SHRs showed a greater pressor response to the reinforced CS than did the normotensive strain. Interestingly, during the third trial block WKY animals maintained on the high calcium diet had a paradoxical pressor response to the unreinforced stimulus which exceeded the responses to the CS+. A significant Strain by Calcium by CS type interaction was found only during the third trial block ($F\{1,34\}=5.84$, $p=0.021$) indicating that the reversal in magnitude of the two CRs in the high calcium normotensive

Figure 30. Conditioned blood pressure responses to reinforced and neutral conditioning stimuli during the early trials collapsed into high and low calcium diet groups for the SHR strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values are expressed as change in mean blood pressure.

SHR BP CR DURING THE FIRST SIX TRIALS AVERAGED INTO CALCIUM DIET GROUPS

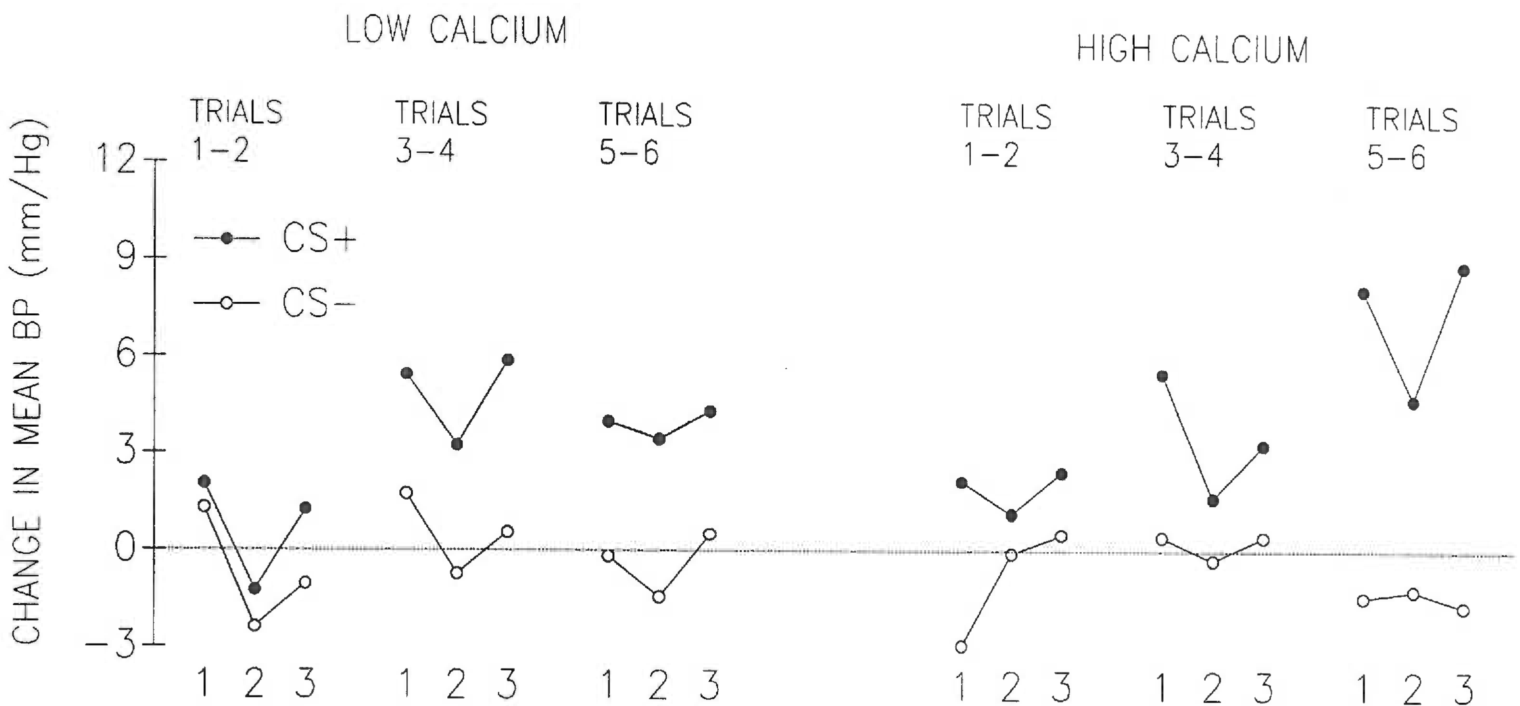
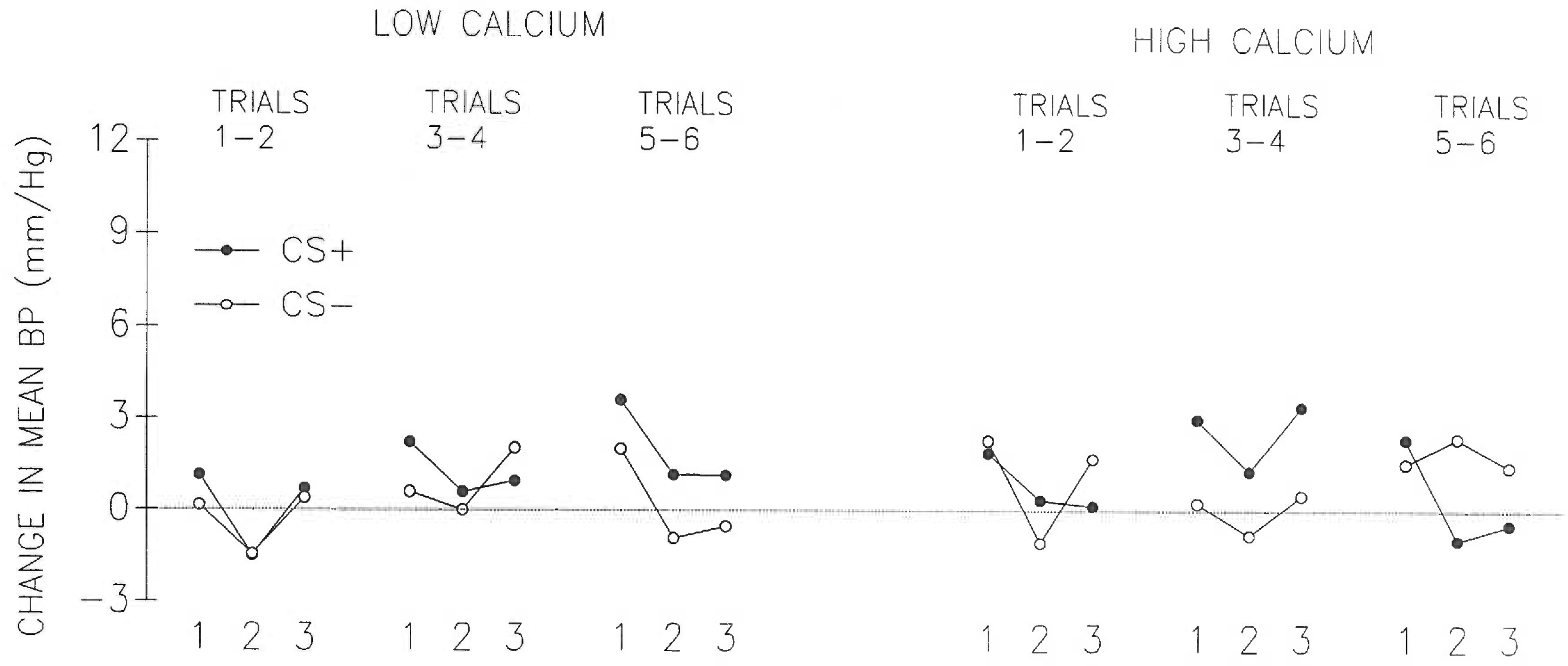


Figure 31. Conditioned blood pressure responses to reinforced and neutral conditioning stimuli during the early trials collapsed into high and low calcium diet groups for the WKY strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values are expressed as change in mean blood pressure.

WKY BP CR DURING FIRST SIX TRIALS AVERAGED INTO CALCIUM DIET GROUPS

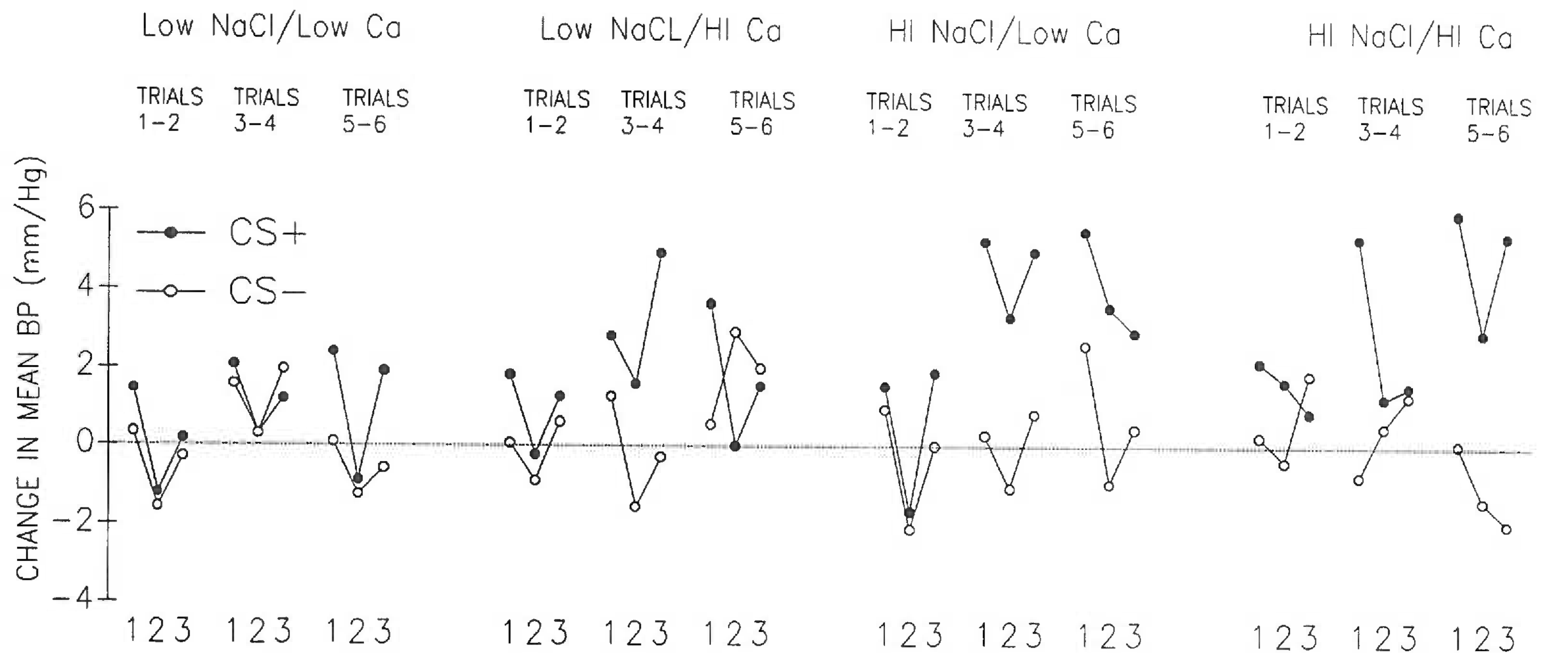


TWO SECOND COUNTING PERIOD IN EACH TRIAL BLOCK

strain was responsible for the interaction.

Also significant was a NaCl by Calcium by CS type by Trials by CP interaction ($F\{4,120\}=3.25$, $p=0.014$). Figure 32 shows the CRs to both reinforced and unreinforced stimuli for each diet group collapsed across strains. Follow-up analyses looking at Calcium by CS type by Trial Blocks by CP interactions at both high and low NaCl levels indicated a significant interaction within the low NaCl groups ($F\{4,68\}=2.55$, $p=0.047$). Further division of the low NaCl data into high and low calcium levels indicated a significant CS type by Trial Blocks by CP interaction among rats fed the low NaCl/high calcium diet ($F\{4,32\}=3.27$, $p=0.023$). When the data were further partitioned into the separate CS-type responses and analyzed individually, no significant interaction involving Trial Blocks was found for the CS+ response as might be expected in a discriminative learning paradigm. Instead, a Trial Blocks by CP interaction was found in the CS- data ($F\{4,32\}=3.31$, $p=0.022$). It seems likely that both the Strain by Calcium by CS type by Trials Blocks interaction described earlier, as well as the NaCl by Calcium by CS type by Trials Blocks by CP interaction presented here were derived from the unusually large magnitude of the CS- pressor response of the WKY animals maintained on the low NaCl/high calcium diet during the third trial block of the early trials. This unique response, best illustrated in Figure 26, appeared to

Figure 32. Conditioned blood pressure responses during the early trial blocks for each diet group collapsed across strain. Data are shown for each CP averaged across two trials into three-trial blocks. Values are expressed as change in mean blood pressure.


CR BP RESPONSE DURING FIRST SIX TRIALS AVERAGED OVER STRAINS
Low NaCl/Low Ca
Low NaCL/HI Ca
HI NaCl/Low Ca
HI NaCl/HI Ca
TRIALS 1-2
TRIALS 3-4
TRIALS 5-6
CS+
CS-
CHANGE IN MEAN BP (mm/Hg)
6
4
2
0
-2
-4
1 2 3
TWO SECOND COUNTING PERIODS IN EACH TRIAL BLOCK

be an isolated event that probably did not reflect any dietary effect on learning.

In sum, examination of the overall 28-trial conditioning session indicated that learned responses may have developed within the first trial block in both strains. Analyses of the early trials confirmed that discriminative learning did occur. Furthermore, both strains were influenced by the dietary manipulation. Hypertensive animals fed the high NaCl diets demonstrated greater blood pressure reactivity to the reinforced stimulus then did SHRs fed the low NaCl diets. In the normotensive strain, only subjects fed the high NaCl diets showed consistent evidence of discriminative learning. Dietary calcium appeared to have only a limited influence on blood pressure reactivity. SHRs maintained on the high calcium diets showed progressive increases in response to the stimulus onset while hypertensive animals fed the low calcium diet showed little change over trials. WKYs showed only a minor influence of dietary calcium which was isolated to a peculiar reversal in the expected discriminative response between the two CS types. There was relatively little interaction between NaCl and calcium. Such interactions were limited to the paradoxical reversals in CS type responding found among WKYs on the low NaCl/high calcium diet and probably did not indicate a reproducible dietary influence.

Brain Electrolytes

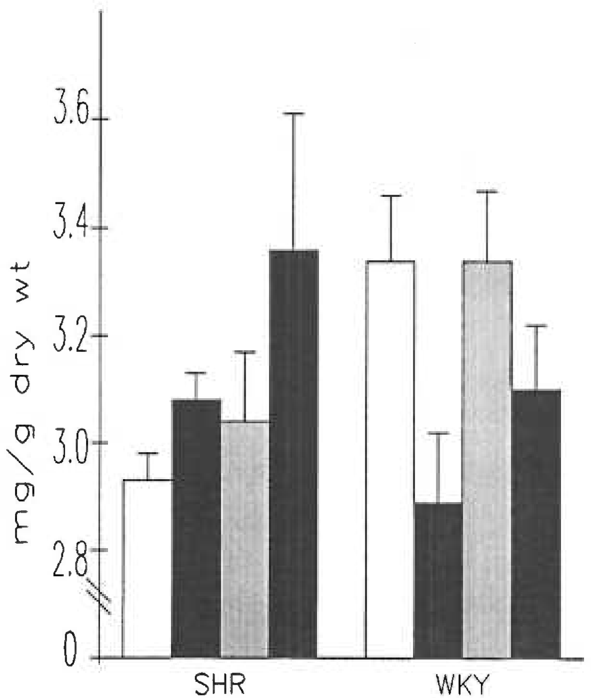
Calcium, phosphorus, magnesium, potassium and sodium levels were evaluated in cortex, brainstem and cerebellum in order to elucidate strain and dietary influences on brain electrolyte concentrations. Significant findings revealed by three-way ANOVAs using Strain, NaCl and Calcium as between group factors are shown in Figure 33.

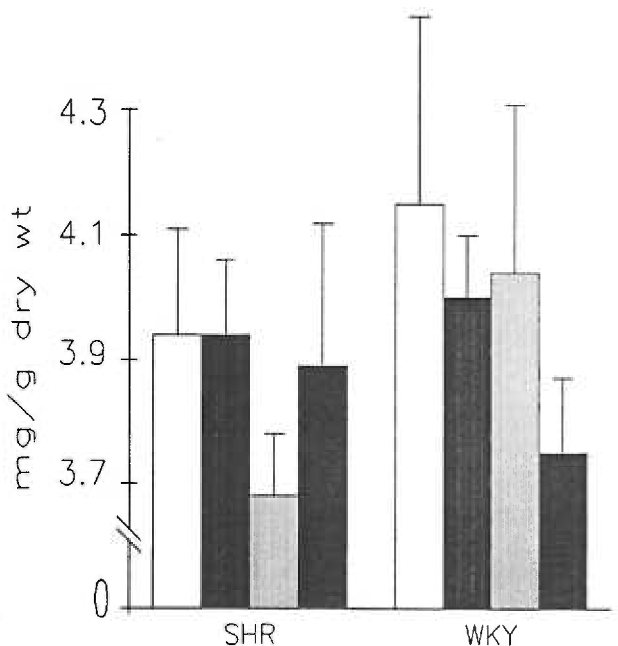
A significant Strain x Calcium interaction was found for potassium in the brainstem ($F\{1,59\}=9.75$, $p=0.003$). Follow-up analyses demonstrated a significant strain difference in brainstem phosphorus among animals fed the low calcium ($F\{1,33\}=11.21$, $p=0.002$), but not the high calcium diet. This was due to a lower amount of potassium in brainstems of the hypertensive animals compared to normotensive animals fed the low calcium diets. Further analyses showed the WKY strain had significantly different levels of brainstem potassium when fed different amounts of calcium ($F\{1,36\}=7.83$, $p=0.008$), i.e., normotensive animals on the low calcium diet showed greater brainstem phosphorus levels than did animals with a high calcium intake. In contrast, the SHR showed no difference in brainstem phosphorus with calcium intake.

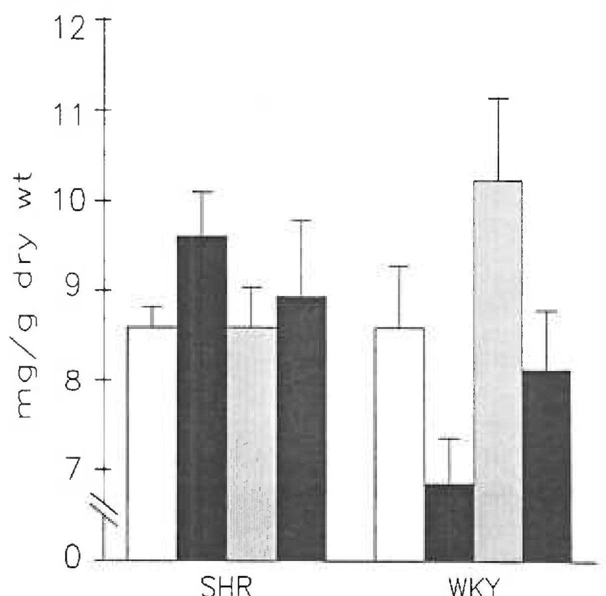
Phosphorus levels in the cerebellum differed in a manner similar to brainstem phosphorus. A significant Strain by Calcium interaction ($F\{1,53\}=6.16$, $p=0.016$) was found to result from a significantly reduced level of

Figure 33. Significant findings of brain electrolyte determinations. Values are expressed as groups means + SEM. Subject n's for diet groups 1, 2, 3 and 4 were 8, 8, 7 and 6 for the SHR strain and 8, 9, 9 and 9 for the WKY strain respectively.

BRAIN ELECTROLYTES







phosphorus in the cerebellum of SHRs fed a low calcium diet compared to WKYs on the same diet ($F\{1,29\}=9.96$, $p=0.004$). No strain difference was found at higher levels of calcium intake. Further analyses showed a main effect for calcium in cerebellar phosphorus levels of the WKY ($F\{1,32\}=10.45$, $p=0.003$). As observed with brainstem phosphorus levels, WKYs fed the low calcium diet had greater cerebellar phosphorus levels than did rats fed the high calcium diet. SHRs did not show any effect of diet on cerebellar phosphorus levels. A significant main effect for strain ($F\{1,53\}=5.03$, $p=0.029$) was also observed in the overall analysis. This was due to the larger concentration of cerebellar phosphorus in the normotensive strain when assessed across all diet groups.

A significant Strain by Calcium interaction was also found in brainstem potassium levels ($F\{1,60\}=4.61$, $p=0.036$). A significant strain difference was found among animals fed the high ($F\{1,31\}=9.74$, $p=0.004$) but not the low calcium diets. This strain difference was due to greater brainstem potassium concentrations in the hypertensive strain. A main effect for strain was also found ($F\{1,60\}=4.96$, $p=0.03$) with SHRs having a higher concentration of brainstem potassium regardless of diet.

All other comparisons of strain and diet associated differences in brain electrolyte concentrations were found to be insignificant.

Discussion

Dietary manipulations of sodium chloride and calcium influenced a number of variables in the current study including: resting and stressed blood pressure, vascular reactivity, learned discriminative blood pressure responses, locomotor activity as well as serum and brain electrolyte concentrations. Increased dietary NaCl had a hypertensive effect on both resting and stress-induced blood pressure in the spontaneously hypertensive rat (SHR). Calcium reduced blood pressure in both the SHR strain and its normotensive control strain, the Wistar-Kyoto rat (WKY). In addition, calcium supplementation was able to reduce stress-induced, but not baseline NaCl-potentiated blood pressure in these animals. In contrast, calcium had no effect on NaCl-dependent increases in vascular reactivity to norepinephrine (NE) injections in either strain. Likewise, calcium did not significantly alter NaCl-induced elevations in circulating epinephrine (EPI) levels. The high NaCl diets were also found to modify learned blood pressure responses in both strains and locomotor activity in hypertensive animals. Such responses were not modified by calcium.

Though the mechanisms by which diet influences blood

pressure and other behaviors are not understood, observations of diet-induced changes in both serum and brain electrolytes in the present study suggest that altered electrolyte availability may influence cellular functions in the periphery, as well as within the central nervous system (CNS). Dietary alterations in blood pressure (McCarron et al., 1985), vascular reactivity (Hatton et al., 1989; Gradin, Elam & Persson, 1985) and sympathetic reactivity (Koepke & DiBona, 1985), as well as serum (Hatton et al., 1987, 1989) and brain electrolyte concentrations (Harris, Canres & Forte, 1981) have been demonstrated in other studies. However, no study, to date, has observed the interrelationship among these variables. Furthermore, the interaction of dietary NaCl and calcium in modifying the above mentioned variables has only recently been investigated.

In the present study, 8 weeks of high NaCl intake raised blood pressure during stressed and non-stressed conditions in the SHR. This confirmed results showing a similar exacerbation of hypertension in SHRs fed a high NaCl diet for just two weeks (Wyss et al., 1987; Chen, Meng, Jin & Oparil, 1988). Moreover, elevations in dietary calcium prevented the blood pressure rise in unrestrained SHRs given short term exposure to a high NaCl diet (Wyss et al., 1989). Similar hypotensive calcium effects were found in the present study, but only during restraint-stress. Calcium

did not significantly effect NaCl-induced hypertension during baseline blood pressure as it did in the 2-week dietary manipulation reported by Wyss et al. (1989). It is possible that longer exposure to the high NaCl diet may have overshadowed calcium's ability to attenuate the effects of NaCl. If so, then calcium associated reductions in NaCl-potentiated blood pressure might only be found during the extremes of restraint stress.

In contrast, McCarron et al. (1985) found that 20 weeks of exposure to a high NaCl diet did not increase blood pressure unless coupled with low calcium intake. Spontaneously hypertensive rats fed a low calcium diet had higher blood pressure than animals fed a control diet, regardless of NaCl content. Conversely, calcium supplementation significantly lowered blood pressure below that of control fed SHRs. Elevated NaCl intake reduced blood pressure in animals fed either a high or low calcium diet, indicating that increased NaCl intake potentiated calcium's hypotensive effect. The conflicting data found in McCarron's study probably reflects the difference in NaCl-sensitivity of SHRs obtained from Charles River and those from Taconic Farms breeders. The Charles River SHR has not consistently shown a hypertensive effect of dietary NaCl (Chen et al., 1988) while more recent reports have demonstrated a unique NaCl-sensitivity in the Taconic Farm population (Chen et al., 1988; Wyss et al., 1987, 1989).

Alternatively, the lower levels of dietary NaCl (1.0%) used in McCarron's study (1985) may have had a hypotensive effect in contrast to the 8.0% NaCl diets used in the current report and in Wyss' studies.

Any of several mechanisms might have been responsible for the NaCl-associated increases in baseline and stress-induced blood pressure found in the present study. Increased NaCl intake may have elevated blood volume, thereby increasing cardiac output and blood pressure. Normally, elevated NaCl intake causes a transient increase in blood volume which is subsequently reduced by a compensatory increase in renal sodium excretion (Langston, 1963). This has been verified in the SHR by Ely, Friberg, Nilsson & Folkow (1985), who found that animals maintained on low, normal and high NaCl diets had similar cardiac outputs, blood volumes and serum Na+ electrolyte values across diets. Furthermore, they found that excess sodium and water consumed by the high NaCl group was excreted within 45 minutes of intake eliminating any sustained volume changes induced by the different diets. However, recent findings suggest that Taconic Farm NaCl-sensitive SHRs may have blunted cardiopulmonary volume receptor activity. As a result, these animals may not sufficiently correct NaCl-induced volume increases (Thornton, Wyss & Oparil, 1989).

An alternative theory suggests that NaCl-induced hypertension results from alterations in sympathetic nervous

system activity. Dietz, Schomig, Rascher, Strasser & Kubler (1980) have shown NaCl-enhanced plasma EPI concentrations during stress in the SHR. Both human and animal studies have found NaCl to potentiate the release of circulating NE (Dietz et al., 1980; Winternitz & Oparil, 1982; Wyss et al., 1989). In addition, NaCl-induced elevations in renal sympathetic nerve activity have been demonstrated by Koepke and DiBona (1985). Consistent with these reports were findings of increased plasma EPI during both rest and restraint among SHRs fed high NaCl diets in the present study. However, there was no dietary effect on either baseline or stressed levels of circulating NE. The literature contains several reports demonstrating NaCl-induced increases in circulating NE. However, in these studies, catecholamines were only assessed after two to three weeks of NaCl loading (Dietz et al., 1980; Winternitz & Oparil, 1982; Wyss et al, 1989). Longer periods of high NaCl intake have not shown alterations in circulating NE (Gradin et al., 1986).

Diet-related adrenal medullary responses observed in the present study were interesting in that they directly paralleled blood pressure changes observed with diet. While NaCl elevated both blood pressure and circulating EPI levels, supplemental calcium tended to reduce the magnitude of both variables. This suggests the possibility that both nutrients influenced blood pressure by altering the

regulation of adrenal medullary outflow. This was supported by findings that blood pressure and EPI were correlated both at rest and during stress among SHRs maintained on all the diets.

The effects of NaCl on sympathoadrenal activity have been well documented, but calcium's role in altering CNS sympathetic responses has not been thoroughly characterized. Luft et al. (1988) have shown a significant reduction of resting EPI levels in stroke-prone SHRs fed a high calcium diet. In contrast, Hatton et al. (1989) were unable to show a significant diet-induced difference in circulating catecholamines, but they did demonstrate a trend for reduced EPI levels with increasing calcium intake in the SHR. Other studies have shown similar trends in adrenal medullary activity with dietary calcium manipulations (Stern et al., 1984, Kageyama, Suzuki, Hayashi & Saruta, 1986; Luft et al., 1988). The results suggest that alone, calcium's sympathoadrenal effects may be minor, but under conditions of NaCl-enhanced circulating EPI, increased calcium intake may be sufficient to reduce blood pressure in the SHR.

Diet-mediated changes in stress reactivity might also have resulted from altered vascular sensitivity to sympathetic activity. Numerous studies have documented NaCl-induced changes in vasoconstriction among hypertensive patients and animals (Gradin et al., 1985; Rankin, Luft, Henry, Gibbs & Weinberger, 1981). Rankin et al. (1981)

demonstrated larger pressor responses to infusions of NE among normotensive subjects fed a high NaCl diet. Skrabal et al. (1984) also showed increased pressor reactivity to infusions of NE among NaCl-sensitive, normotensive individuals. Moreover, such increased reactivity was exacerbated by increased dietary NaCl. Gradin et al. (1985, 1986) have repeatedly shown increased pressor responses to i.v. infusions of an α_1-adrenergic agonist in the NaCl loaded SHR. These reports agree with the present study in which NaCl was shown to elevate mean arterial blood pressure responses to i.v. NE injections in the SHR.

Hyperresponsiveness to vasoconstrictor substances in NaCl loaded SHRs might be due to pressure-induced structural changes, increased receptor sensitivity or to pressure-independent mechanisms such as NaCl mediated increases in sympathetic growth factors. It is difficult to reconcile the possibility of increased vascular receptor sensitivity with the notion that NaCl induces increases in sympathetic activity as found in the majority of studies. Theoretically, increased sympathetic tone should cause a compensatory reduction in adrenergic receptor sensitivity and or numbers. In fact, Sripairojthikoon, Oparil and Wyss (1989) were unable to find any difference in α_1-adrenergic receptor binding in NaCl-loaded SHRs.

An alternative explanation is that NaCl-associated increases in EPI release caused a compensatory down

regulation of β-adrenergic receptors. This would presumably diminish β-receptor mediated vasodilation among NaCl-loaded animals, resulting in an exaggerated α-receptor mediated vasopressor response to exogenous NE. However, increased pressor responses to α_1-adrenergic agonists have been observed in conjunction with normal vasodilatory responses to specific β_2-adrenergic agonists in NaCl loaded SHRs (Gradin et al., 1984), indicating that NaCl-related changes in β-receptor activity are not likely responsible for the observed changes in vascular reactivity.

Consistent with Folkow's hypotheses of pressure-induced structural adaptation (1978), NaCl-enhanced vasoconstrictor responses might have been due to vascular restructuring. Theoretically, increased baseline pressure resulting from NaCl loading would provide a stimulus for secondary vascular hypertrophy. Given the resulting reduction in the radius of the vascular lumen, such changes would elevate blood pressure responses to vasoconstrictor agents. If the increased vascular reactivity observed with NaCl loading was due to pressure-induced structural change, it seems likely that calcium supplementation would have attenuated the response. Presumably, the reduced blood pressure associated with increased calcium intake would have attenuated the stimulus for hypertrophy resulting in a concomitant reduction in NaCl-induced vascular reactivity.

Alternatively, rather than potentiating pressure-

induced structural changes, NaCl may have caused vascular restructuring by increasing circulating sympathetic growth factors. Recent work has indicated that both NE and EPI accelerate growth of cultured vascular smooth muscle cells (Yamori et al, 1984; Yamori, Mano, Nara & Horie, 1987). It is possible that the elevations in plasma EPI found with NaCl loading increased the rate of vascular hypertrophy in hypertensive rats. Accordingly, since calcium intake did not significantly alter sympathetic activity, as indicated by its lack of effect on circulating catecholamines, it would not be expected to influence sympathetic-induced, pressure-independent changes in structure or vascular reactivity.

There was no evidence of dietary NaCl influence on blood pressure in the WKY during either handling, rest or restraint. This has been a consistent trend in NaCl supplementation studies of the WKY (Ely et al., 1985; Koepke & DiBona, 1985). Manipulations of calcium have been mildly successful in altering blood pressure in the WKY (McCarron et al., 1985; Hatton et al., 1987; Ayachi, 1979) confirming the hypotensive effects of calcium observed in the present study. Dietary manipulations were not effective in altering either plasma catecholamines or pressor responses in the WKY. Similarly, Doris (1988) found that long-term NaCl loading did not alter vascular reactivity to NE in the normotensive rat. Hatton et al. (1989) were also unable to

alter circulating catecholamines or vascular reactivity in the WKY with dietary calcium. However, Doris (1988) found that after 6 months of increased dietary calcium intake, vasopressor responses to NE were reduced in normotensive rats. The lack of pressor activity among WKYs in the present study may have been due to the relatively short duration of dietary exposure.

Consistent with results from the present study, SHRs showed a greater dietary influence as well as more exaggerated blood pressure responses to novel, conditioned (CS) and unconditioned stimuli (US) during the conditioning procedure. The larger magnitude of the orienting responses observed in the SHR was consistent with other conditioning studies using this strain (Hatton et al., 1981, 1987). Though SHRs were initially more reactive, repeated exposure to the tones attenuated responses to levels comparable to those of the WKY. Moreover, dietary influences during habituation occurred exclusively in the SHR. Specifically, greater calcium intake was able to attenuate the exaggerated orienting responses observed with increased NaCl intake, confirming similar observations made during the stress reactivity experiment.

Unconditioned stimuli also elicited greater blood pressure responses from the hypertensive strain. The magnitude of responses from both the SHR and WKY strains were consistent with previous observations (Hatton et al.,

1981). In addition, a greater number of trials were required to habituate the SHR's unconditioned response (UR) as was found in Hatton et al.'s (1981) study as well. In contrast to dietary effects observed during habituation, NaCl did not influence pressor responses to the US. Instead, increased calcium intake magnified the UR differences between the two strains. During initial exposures to the US, high calcium SHRs showed significantly greater pressor responses than did normotensive animals fed the high calcium diets. In contrast, URs of SHRs and WKYs fed low calcium diets did not differ. The strain difference observed between the high calcium groups was probably due to the trend for calcium associated attenuation of URs in the normotensive animals. In contrast, SHRs showed no dietary differences in their URs.

The lack of dietary NaCl influence upon URs is difficult to reconcile with observations of NaCl potentiated blood pressure reactivity to handling and restraint. It is possible that the nature of the US overshadowed NaCl's effects on stress-induced blood pressure responses. The shock stimulus may have elicited a maximal pressor response which could have masked NaCl's effects. Repeated exposure to the stimulus would presumable result in habituation and a concomitant attenuation of pressor responses. Such habituation might then reverse the ceiling effect allowing for different response magnitudes between animals on the

different NaCl diets. Careful examination of the data show a trend for larger URs among SHRs fed the high NaCl diets in later trials. Presently, there are no data available which addresses dietary NaCl's effects on responses to shock in hypertensive rats. Therefore, it is difficult to determine whether the lack of observable NaCl influence was due to the conditioning parameters utilized in this study.

Despite the lack of a dietary NaCl effect upon URs, NaCl influenced the performance of learned discriminative blood pressure responses in both strains. High NaCl groups showed discriminative learning while animals fed the low NaCl diets did not show differential responding to the reinforced and neutral stimuli over the duration of the conditioning session. In fact, discriminative responses appeared to diminish with repeated trials among all the diet groups. However, response differences of animals maintained on the high NaCl diet remained significant throughout the 28 trials demonstrating the facilitation of a more robust response with high NaCl intake.

Examination of the first six trials of the conditioning session demonstrated a significant effect of NaCl on learned discriminative responses in the SHR. During the early trials, NaCl potentiated responses to the CS+ but not the CS-, suggesting that the dietary influence was not due to a generalized increase in reactivity.

Though normotensive animals did not show a

discriminative conditioned response (CR) in the overall conditioning session, analyses of the first six trials demonstrated acquisition of the learned response only among WKYs fed the high NaCl diets. Under the low NaCl diet, the magnitude of the pressor response elicited by the reinforced tone was not consistently different from that induced by the neutral tone, providing no evidence that WKYs fed low NaCl diets learned to differentiate between the tones.

There was no consistent influence of calcium upon blood pressure CRs in either strain. This contradicts previous findings by Hatton et al. (1987) who noted larger CRs in Charles River SHRs fed a low calcium diet. The conflicting observations may have resulted from the use of differing calcium levels in the calcium deficient diets. Alternatively, the contradictory results may have been due to the differing calcium sensitivities of the NaCl-sensitive and resistant sub-strains.

The lack of a calcium effect on CRs in the present study was inconsistent with findings of calcium attenuated responses to handling, restraint and orienting stimuli in NaCl-loaded SHRs. Stressors responsive to dietary calcium i.e., handling, restraint and orienting stimuli, differed from the calcium insensitive conditioning stimuli in that they were novel. In contrast, exposure to the conditioned cues occurred repeatedly. Given that calcium's hypotensive effects are more pronounced with stress, it seems plausible

that the novelty of the calcium-sensitive stimuli provided a degree of stress which enhanced calcium's influence. In contrast, repeated conditioning trials habituated blood pressure CRs, and possibly obscured calcium's hypotensive influence on conditioned responses.

The attenuation of differential responding observed with repeated trials is difficult to explain. Despite continuous reinforcement, differential responding to the two CS types diminished over trials. Progressive reductions in the CS+ response magnitude paralleled decreases observed in the UR with repeated exposure to shock. Similar observations of diminished blood pressure CRs with repeated trials have been documented in other reports (Hatton et al., 1981; Hoffman & Fitzgerald, 1978). It is possible that repeated exposure to the US habituates the UR by initiating compensatory responses aimed at maintaining blood pressure with a desired homeostatic range. Repeated pairing of the CS+ and US may initiate similar compensatory responses with reinforced stimulus exposure resulting in a progressive diminution of the blood pressure CR.

Indirect, behavioral indices, as assessed by locomotor activity confirmed findings of increased stress reactivity as well as NaCl-potentiated exacerbation of reactivity in hypertensive animals. The literature suggests that activity is inversely related to freezing behavior and thus is indicative of emotional fear reactivity to a novel

environment (Bouton & Bolles, 1980; LeDoux, Sakaguchi & Reis (1982, 1983). Moreover, the duration of time spent in the center of an open field has also been used as a measure of fear-induced behavior (Sutterer, Stoney & Sanfillipo, 1984). In the present study, hypertensive animals showed less activity with exposure to the novel open field. The SHR also spent more time in the center of the open field. In addition, the decline of center time in successive counting periods was attenuated in SHRs indicating that a more prolonged period of exposure was necessary to habituate to the novel environment. SHRs also showed an interaction between NaCl and Time due to a more rapid decline in center time among hypertensive rats fed a low NaCl diet. This could be interpreted as a more rapid habituation or, a more rapid attenuation of fear in hypertensive animals fed the low NaCl diet. Increased NaCl in the diet was found to reduce total distance in both strains. It may be hypothesized that such decreased activity resulted from fear-induced inhibition of locomotor activity among hypertensive animals as well as in animals fed a high NaCl diet.

Though such an interpretation of locomotor activity confirms evidence of increased stress in the SHR, particularly those fed a high NaCl diet, the data contradict LeDoux et al. (1983) who found that SHRs spent less time in the center, showed greater overall activity and defecated

less in a 3-min exposure to an open field apparatus than did WKYs. Strain differences cannot account for the conflicting activity results found between this and LeDoux's (1983) study, since both used Taconic farm hypertensive rats. The one obvious procedural variation between the two studies was the use of a relatively large (94.5 x 94.5 cm), unisolated, illuminated open field by LeDoux et al. The present study used a smaller (42.0 x 42.0 cm) open field placed in a dark, sound attenuating chamber with automated activity screening. Other studies have shown WKYs to be more responsive to the level of environmental stimulus, such that increasing stimulus intensity reduces activity to a greater extent in the WKY than in the SHR (Sutterer et al., 1984). Moreover, these researchers found that when environmental stimuli were minimized, WKYs showed greater activity than SHRs supporting results from the present study in which animals were exposed to minimal stimulus during the procedure.

It is not clear how changes in blood pressure, stress reactivity and behavior are effected through diet. Recent theory has suggested that genetic defects in electrolyte binding (Postnov et al., 1979) and metabolism (McCarron et al., 1981) coupled with nutritional excesses or deficiencies may interfere with proper vascular and neural control of blood pressure (Hatton, et al., 1987, 1989; Resnick, Muller & Laragh, 1986). Because calcium is important in a number of cellular functions, it is not surprising that alterations

in its metabolism have been implicated in the genesis of hypertension. It has been demonstrated that the level of extracellular calcium concentration mediates membrane permeability. Specifically, increased calcium availability is thought either to increase calcium binding to the cell membrane causing a decrease in membrane conductance, or to inhibit the electrogenic pump, thus inducing a hyperpolarization of the cell. In either case, it is thought that increased extracellular calcium availability increases the threshold of activation of the smooth muscle cell (Webb & Bohr, 1978). It follows that reduced extracellular calcium availability would reduce the cell's ability to regulate ion flux, allowing depolarization and excessive contraction.

McCarron et al. (1981) have suggested that aberrant calcium metabolism may be responsible for both high blood pressure and increased stress reactivity in hypertensive animals (Hatton et al., 1987, 1989). The SHR metabolism is characterized by elevated calcium excretion combined with decreased calcium gut absorption. Aberrant metabolism is further reflected by decreased serum ionized and total calcium (McCarron et al., 1985; Stern et al., 1984). The significance of calcium availability in hypertension has been supported by both human and animal dietary intervention studies in which increased calcium intake concurrently increases serum calcium levels and decreases blood pressure

(McCarron & Morris, 1985; Hatton et al., 1989). Likewise, studies in the SHR show that calcium deprivation decreases calcium availability in association with a potentiation of the hypertensive process (Hatton et al., 1987, 1989). Changes in blood electrolyte content are known to influence the release of circulating factors which effect vascular activity including parathyroid hormone (Gairard, Berthelot, Schleiffer & Pernot, 1982), vitamin D3 metabolites (Resnick, Muller & Laragh, 1986) and renin (Resnick, Nicholson & Laragh, 1986). As such, altered electrolyte availability may provide the stimulus for altered regulation of blood pressure and reactivity indirectly, through the regulation of these factors.

Consistent with numerous other dietary calcium studies (McCarron et al., 1985; Hatton et al., 1987, 1989; Stern et al., 1984), results of the present report indicate that calcium deprivation and supplementation were able to alter serum electrolytes. Other dietary studies have shown increased (Hatton et al., 1989), decreased (Stern et al., 1984) or no difference (Hatton et al., 1987) in serum ionized calcium among calcium supplemented SHRs compared to normotensive controls suggesting that serum ionized calcium may not be an appropriate indication of calcium balance.

The lack of difference observed in serum sodium levels across diets most likely reflects the strong regulation of extracellular sodium. These results were consistent with

Stern et al. (1987) and Hatton et al. (1987) who found no difference in serum sodium levels attributable to either strain or dietary changes. In contrast, McCarron et al. (1985) reported reduced serum sodium levels in animals fed a high calcium/low NaCl diet. Additionally, he found higher sodium levels in hypertensive animals compared to normotensive animals. It is possible that the longer duration of his study facilitated changes in serum sodium which could not be attained within the relatively shorter length of the present experiment.

Potassium was the only serum electrolyte found to change with dietary NaCl intake. It is likely that this resulted from potassium's involvement in various cellular Na+ transport systems which are also influenced by dietary sodium chloride intake (Giebisch & Stanton, 1979).

Evidence that sodium influences CNS activity has been provided by extensive studies demonstrating that hypertonic saline infused directly into the cerebroventricular system promotes increased sympathetic activity and a concomitant rise in blood pressure (Bunag & Miyajima, 1984). In contrast, brain infusions of calcium facilitate a decrease in blood pressure (Borowitz et al., 1987; Leuson, 1950). If alterations in brain electrolyte availability are able to change blood pressure as indicated in these studies, it follows that dietary changes might provide a means to modify electrolyte availability to brain nuclei involved in

regulation of blood pressure.

The present study demonstrated that brain electrolyte concentrations, and thus electrolyte availability, were influenced by diet. Both potassium and phosphorus levels were found to change with diet. Brain phosphorus alterations were consistent with dietary changes in serum phosphorus. Specifically, reduced calcium intake resulted in increased phosphorus levels. However, such changes were only observed in the normotensive animals. The lack of dietary effect on SHR brain phosphorus levels may reflect a lack of buffering capacity in these animals. It is possible that a normal response to deficient calcium intake would be characterized by an increase in brain phosphorus levels. The absence of such a response in the SHR may be associated with a decreased ability to correct the calcium deficiency induced by the diet.

Alterations in potassium levels with diet have not been consistently observed. The serum electrolyte data from this study demonstrated an increase in potassium with increased NaCl intake. In contrast, brain potassium levels were affected by calcium but not NaCl intake. The significant Strain by Calcium interaction in cerebellar potassium was likely due to the diets inability to influence the SHR strain. Again, the data suggest that the hypertensive strain may be less able to alter electrolyte concentrations in the face of changing dietary intake. Since brain

electrolyte levels for either the SHR or the WKY have not been published, it is difficult to determine the significance of the strain differences observed.

Data addressing the effects of dietary NaCl or calcium on brain electrolytes are limited. Studies have demonstrated reductions in brain calcium content in rats fed a low calcium diet. In these reports it was noted that long-term calcium deprivation was necessary to provoke changes in brain electrolyte concentrations (Harris et al., 1981; Murphy, Smith & Rapoport, 1986). Unfortunately, these studies utilized extremely low calcium levels in order to obtain a deficient calcium state in the animals. However, these studies do provide evidence that brain calcium levels are influenced by parallel changes in serum calcium (Murphy et al., 1986). At present, there is no data addressing brain electrolyte concentration alterations with both NaCl and calcium manipulations.

Observations of reduced weight among animals fed the high NaCl diets suggest that weight may have influenced blood pressure or reactivity. It is likely that the lower palatability and resulting reduction in food intake associated with the high NaCl diet (Wyss et al., 1988) was responsible for the lower weight of these animals. Reports have shown a positive association between blood pressure and body weight in the SHR (McCarron et al., 1985) suggesting that a reduced growth rate, and hence lower body weight, was

probably not responsible for NaCl-associated elevations in blood pressure. However, the possibility that decreased intake resulted in a deficiency of some other electrolyte important in blood pressure and reactivity regulation can not be excluded.

In summary, SHRs showed significant diet-induced changes in reactivity to both novel and conditioned aversive stimuli. In general, a high NaCl diet potentiated hyperreactive responses in the SHR during exposure to novel stressors, while calcium supplementation attenuated these responses. Both strains demonstrated exaggerated CRs associated with a high NaCl intake, though calcium did not influence such responses.

Dietary alterations in cardiovascular and behavior responses were accompanied by changes in both serum and brain electrolytes. Modifications of both NaCl and calcium intake altered serum levels of electrolytes influential in vascular smooth muscle cell function. Altering the availability of these electrolytes peripherally likely influenced the cardiovascular responses to stress. Moreover, changes in brain tissue electrolytes suggests that CNS output to the cardiovascular regulatory system probably also contributed to the difference in stress responses.

Whether alterations in stress reactivity influenced resting blood pressure is difficult to determine. The expected parallel changes in blood pressure and stress

reactivity were only observed in relation to the NaCl diet. Observations of NaCl-enhanced stress reactivity and baseline blood pressure do not indicate whether reactivity increases were derived from increased pressure or vice versa. In contrast, calcium was able to modify NaCl-enhanced stress reactivity, but not baseline blood pressure suggesting either that baseline blood pressure was not significantly influenced by stress reactivity or that calcium's influence was observable only during a cardiovascular challenge.

The results of this study indicate that NaCl potentiates cardiovascular and behavioral responses to both novel and conditioned stressors in the NaCl-sensitive SHR. Calcium appears to modify NaCl's hypertensive effects. How the two dietary components interact physiologically is not known. However, the direct correlation between blood pressure and EPI during restraint supports the notion that dietary blood pressure effects were, in part, mediated by adrenal medullary activity. These results demonstrate calcium's modulating influence on NaCl-exaggerated reactivity and further emphasize the notion that calcium's hypotensive influence is most readily demonstrated during a cardiovascular challenge such as provided by stress (Hatton et al., 1989) or a high NaCl diet.

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