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# THE EFFECT OF THE ADRENAL MEDULLA IN THE DEVELOPMENT OF DIET-INDUCED HYPERTENSION

BY

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## ABSTRACT

THE EFFCT OF THE ADRENAL MEDULLA IN THE DEVELOPMENT OF DIET-INDUCED HYPERTENSION

BY

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Male Sprague-Dawley rats fed either a high-fat or glucose-enriched diet for ten weeks developed higher blood pressure (BP) and higher urinary excretion of norepinephrine and epinephrine than rats fed a control diet. Rats fed the high-fat diet also developed obesity and fasting hyperinsulinemia. Adrenal demedullation had no effect on body weight or plasma insulin levels of rats within any diet treatment. Urinary epinephrine excretion was very low or undetectable in adrenal-demedullated rats. In addition, adrenal demedullation attenuated norepinephrine excretion and reduced BP in rats fed the high-fat or glucose diet. Only rats with an intact adrenal medulla responded to the high-fat or glucose diet by developing higher catecholamine excretion and higher BP than rats fed the control diet. This suggests that adrenal medullary catecholamines play a role in the BP response to dietary fat or glucose.

TO MY PARENTS

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# CHAPTER 1 INTRODUCTION AND THESIS RESEARCH OBJECTIVES

Previous studies in this laboratory have shown that male Sprague-Dawley rats fed either a high-fat diet or a glucose-enriched diet developed higher blood pressure than rats fed a control diet (Kaufman et al., 1989b). Blood pressure responses to dietary macronutrients may be mediated by changes in insulin levels, sympathetic nervous system (SNS) activity, and/or renal sodium handling. Dietary fat and carbohydrate can enhance SNS activity (Krieger and Landsberg,1988; Schwartz et al., 1983; Young and Landsberg, 1981) demonstrating that SNS activity is sensitive to the macronutrient composition of the diet. The SNS, in turn, may raise blood pressure through effects on various tissues, including the heart, the blood vessels, and the kidney (Krieger and Landsberg, 1988). The central neural mechanisms coordinating changes in the functional state of sympathetic nerves with changes in dietary intake are unknown. However, neurons in the ventromedial hypothalamus (VMH) are involved in regulation of feeding behavior and may also play a role in mediating the effects of nutrients on sympathetic outflow (Anand et al., 1964; Frohman, 1971; Niijima,1981; Opsahl, 1977; Young and Landsberg, 1980).

Higher urinary epinephrine excretion in the hypertensive rats suggests enhanced adrenal medullary activity. Development of sustained hypertension has been induced in rats by prolonged intravenous infusion of epinephrine at physiological concentrations

(Majewski et al., 1981b). Epinephrine may increase blood pressure by increasing cardiac output due to a ß-adrenoceptor mediated increase in cardiac contractility. At high concentrations, epinephrine may produce vasoconstriction via its effects on vascular alpha-adrenoceptors. However, epinephrine may also exert indirect effects on blood pressure. Catecholamines may alter norepinephrine release from sympathetic nerves through activation of either inhibitory prejunctional alpha-adrenoceptors or facilitatory prejunctional ß-adrenoceptors (Langer, 1977). A facilitatory prejunctional ß-adrenoceptor system has been described and may be a mechanism whereby epinephrine can enhance norepinephrine release from sympathetic nerves (Langer et al., 1977, 1981; Majewski, 1981a, 1986; Nezu et al., 1985; Stjarne, 1975). Norepinephrine, the adrenergic neurotransmitter, is synthesized and stored in sympathetic nerve endings. It plays a role in the control of blood pressure and has been implicated in the development of human essential hypertension (Kopin, 1981; Tarazi, 1983). In animal models, epinephrine has been shown to activate prejunctional ß-adrenoceptors, thereby increasing norepinephrine release. It is postulated that epinephrine may produce an increase in blood pressure (Majewski and Rand, 1986) via this mechanism. The removal of circulating epinephrine by adrenal demedullation may attenuate blood pressure responses in spontaneously hypertensive rats (Borkowski and Quinn, 1984).

The purpose of this thesis research is to determine whether the adrenal medulla contributes to the hypertensive responses to

dietary lard or dietary glucose. The following hypotheses will be tested: (1) an intact adrenal medulla is required for the development of the elevated urinary excretion of norepinephrine observed in hypertensive rats (those fed either the high-fat or glucose-enriched diet); (2) diet-induced hypertension will not develop in rats lacking an adrenal medulla. If absence of the adrenal medulla interferes with the hypertensive response to dietary fat or dietary glucose, attempts to prevent chronic diet-induced hypertension in rats through methods such as adrenal demedullation or blockade of epinephrine by ß-adrenoceptor blockers may provide us with further information about the pathophysiology of diet-induced hypertension.

# CHAPTER 2 LITERATURE REVIEW

## 2.1 SYMPATHETIC NERVOUS SYSTEM AND DIETARY INTAKE: A GENERAL REVIEW

### 2.1.1 Response of the Sympathetic Nervous System to Dietary Intake

Fasting or food restriction suppresses SNS (SNS) activity (Landsberg and Young, 1978; Rappaport et al., 1982a; Young and Landsberg, 1977c), while sucrose feeding stimulates the SNS in the rats (Landsberg and Young, 1978; Young and Landsberg, 1977a) and glucose ingestion increases plasma norepinephrine concentrations in man (Welle et al., 1980). The SNS response is nutrient-specific: dietary fat or carbohydrate stimulate the SNS, while dietary protein has little effect (Kaufman et al., 1986). Although SNS activity and adrenal medullary activity are positively correlated under most conditions, these two components of the sympathoadrenal system are capable of independent responses: during combined fasting and cold exposure, SNS activity is suppressed (in response to fasting) while adrenal medullary secretion of catecholamines is enhanced (Young et al., 1984).

### 2.1.2 Mechanism of Sympathetic Nervous System Activity Changes Resulting from Diet

Since activity of the SNS is regulated from central neurons, it is obvious that the central nervous system must possess mechanisms capable of assessing and responding to alterations in nutritional state. Insulin is a major signal that relates dietary intake to sympathetic activity. Insulin-mediated glucose metabolism within critical neurons related to the ventromedial portion of the hypothalamus appears to be one of the important mechanisms that couples dietary intake and sympathetic nervous activity (Landsberg, 1986; Landsberg and Young, 1985).

Insulin affects the central nervous system, particularly in the region of the hypothalamus (Anand et al., 1964; Frohman,1971; Frohman and Bernardis, 1971; Opsahl, 1977; Van Houton and Posner, 1981). The ventromedial hypothalamus (VMH) is considered to be a "satiety center." Destruction of the VMH results in hyperphagia, hyperinsulinemia, and obesity (Brobeck, 1946; Opsahl, 1977; Young and Landsberg, 1980), whereas feeding behavior is inhibited when this region is electrically stimulated (Frohman and Bernardis, 1971). Neuronal activity in the VMH is responsive to changes in cirulating levels of glucose and insulin (Anand et al., 1964; Frohman 1971; Opsahl, 1977). The VMH can also influence the activity of sympathetic centers in the brainstem, thereby regulating the amount of central sympathetic outflow (Ban, 1975). Furthermore, gold-thioglucose, a compound that destroys neurons in the VMH, impairs

the ability of the SNS to respond to fasting (Young and Landsberg, 1980). Such experiments suggest that the VMH is involved in mediating the effects of nutrients on the SNS and that glucose and insulin may serve as signals for the hypothalamus.

Based on the model established by Landsberg and Young (Landsberg and Young 1981a), sucrose feeding increase sympathetic outflow by suppressing inhibitory pathways from the VMH to sympathetic centers in the brainstem. Nevertheless, the idea that the VMH normally inhibits sympathetic outflow is still in debate (Bunag, 1983; Landsberg and Young, 1981a). An alternative explanation is that sucrose-feeding may enhance sympathetic activity by altering hypothalamic sensitivity to stimulation. Either increased facilitatory or decreased inhibitory inputs on the sympathetic pathway descend from the hypothalamus through synapses in the medulla, spinal cord, or thoracolumbar chains (Bunag, 1983).

### 2.1.3 The Link Between Dietary Intake and Sympathetic Nervous System Function

The link between dietary intake and SNS activity might be effected by either glucose or insulin. Infusion of insulin and glucose leads to stimulation of the SNS (Rowe et al., 1981). The insulin-induced elevations of plasma norepinephrine noted in this study (in the absence of hypoglycemia) indicate activation of the SNS, as the clearance of norepinephrine is not changed by insulin infusion.

Furthermore, the pattern of cardiovascular stimulation induced by insulin is consistent with primary sympathetic stimulation (Rowe et al., 1981). Thus, increases in circulating insulin, in association with normal or elevated levels of glucose, result in stimulation of SNS activity.

Administration of 2-deoxyglucose, a glucose analogue, reduces SNS activity in rats (Rappaport, 1982b). This finding supports a role for glucose metabolism in mediating the effect of insulin on the SNS. Since alterations in sympathetic outflow originate from the central nervous system, so changes in glucose metabolism within a small location of the brain, specifically in the hypothalamus (Landsberg, 1981b) may relate sympathetic responses to changes in glucose metabolism and may serve as a link between insulin and sympathetic activation.

### 2.1.4 Effect of Dietary Macronutrients on the Sympathetic Nervous System

Changes in nutrient composition have been shown to induce changes in SNS activity (Jung et al., 1979). Sucrose or fat enhance SNS activity (Fournier et al., 1986; Rappaport, 1982a; Schwartz et al., 1983; Young and Landsberg, 1977a), while dietary protein does not stimulate the SNS (Kaufman et al., 1986). This implies that the effect of increased energy intake on the SNS is dependent upon diet composition, not caloric intake alone (Ernsberger and Nelson, 1988; Kaufman et al., 1986; Ueda, 1973; Vander Tuig and Romsos, 1984).

### 2.1.5 Effect of Carbohydrates on the Sympathetic Nervous System

Intravenous injection or oral administration of glucose is known to increase plasma catecholamine concentrations in normal human subjects (Robertson, 1974; Rowe et al., 1981; Young et al., 1979). In addition, norepinephrine turnover rate is increased in the heart, pancreas, liver, and brown adipose tissue of rats fed sucrose supplements (Young and Landsberg, 1977, 1979a and 1982). These findings confirm that SNS activity is incresed by dietary carbohydrates. Various carbohydrate sources (glucose, starch, and others) appear to be equally potent stimulators of SNS activity when they are fed in isocaloric quantities (Walgren et al., 1987). The mechanisms involved in stimulation of SNS activity by carbohydrate are not clear. Evidence in favor of increased central nervous system glucose metabolism activated by insulin has been advanced to explain the relationship between dietary carbohydrate intake and SNS activity (Landsberg and Young, 1981b; Rowe, 1981).

### 2.1.6 Effect of Dietary Fat on the Sympathetic Nervous System

Overfeeding mixed high-caloric diets with substantial fat content increases norepinephrine turnover rate in pancreas, liver, heart, and interscapular brown adipose tissue (Schwartz et al., 1983; Young et al., 1982; Young and Landsberg, 1979a). Urinary catecholamine excretion, another index of sympathoadrenal activity, is increased during fat feeding (Schwartz et al., 1983). The

mechanisms involved in stimulation of the SNS by fat are not clear. Plasma insulin but not plasma glucose concentrations are significantly elevated in high-fat-fed rats, suggesting that a state of insulin resistance exists (Grundleger and Thenen, 1982). High fat feeding results in (1) decrease insulin receptor number, (2) decrease in insulin-stimulated glucose transport, and (3) pathway-specific changes in basal and insulin stimulated glucose metabolism (Grundleger and Thenen, 1982). Thus, it is possible that insulin may serve as a signal within the central nervous system in mediating the effects of dietary fat on the SNS.

## 2.2 EFFECT OF DIET ON BLOOD PRESSURE

### 2.2.1 Obesity and Hypertension

Studies have shown that hypertension commonly exists in obese individuals (Chiang, 1969; Kannel and Brand, 1967; Stamler et al., 1978) and weight loss in obese humans leads to a decrease in blood pressure (Tuck et al., 1981; Young et al., 1981; Young and Landsberg, 1982). Several mechanisms have been proposed to explain the relationship between body weight and blood pressure. Reductions in blood pressure in association with caloric restriction in obese animals may result from reduced SNS activity as well as secondary effects of reduced adrenergic activity on renal sodium excretion and the renin-angiotensin-aldosterone axis (Christlieb, 1973; Christlieb et al., 1976; Sowers et al., 1982). Insulin may be involved in obesity-related hypertension (Christlieb, 1985; Krieger and Landsberg, 1988; Landsberg, 1987; Manicardi et al., 1986; Modan et al., 1985). Insulin-induced renal reabsorption of sodium can result in sodium-dependent hypertension (Baum 1987; Dahl, 1972; 1958; De Fronzo et al., 1975; Luft et al., 1982; MacGregor, 1983). Another possibility is that a sodium-replete vasculature may respond to sympathetic stimulation by increased peripheral vascular resistance, thereby leading to hypertension. Insulin has been proposed as a hypertensive hormone because of its enhancement of sodium retention and stimulation of the SNS (Rowe et al., 1981; Young and Landsberg, 1982). Insulin may be involved in obesity-related hypertension (Christlieb, 1985; Krieger and Landsberg, 1988;

Landsberg, 1987; Manicardi et al., 1986; Modan et al., 1985), since obesity is associated with hyperinsulinemia and insulin stimulation of the SNS is maintained even in states of insulin resistance (O'Hare et al., 1985).

### 2.2.2 Effects of Macronutrients on Blood Pressure

High sucrose content in the diet results in a rapid rise of blood pressure in rats (Ahrens, 1974, 1980; Beebe, 1976; Bunag, 1983; Fournier, 1986; Michaelis, 1981; Preuss and Preuss, 1980; Smith-Barbaro, 1980; Young and Landsberg, 1981) and human subjects (Ahrens, 1975). The mechanism involved in the development of higher blood pressure by sucrose ingestion may be by enhancing the activity of the SNS (Bunag et al., 1983; Preuss and Fournier, 1982; Young and Landsberg, 1977a). Fructose feeding can also produce hypertension (Hwang, et al., 1987). In addition to hypertension, hyperinsulinemia and insulin resistance can be induced in rats by feeding diets high in fructose (Hwang et al., 1987) or sucrose (Reaven et al., 1979, Wright et al., 1983). Insulin resistance and hyperinsulinemia associated with either fructose or sucrose feeding may be involved in mediating carbohydrate-induced hypertension.

In addition to high salt and high carbohydrate intake, elevations in blood pressure may result from other dietary factors, such as the level and type of fat consumed. Saturated fatty acids may increse blood pressure

(Kaufman and Peterson, 1988; Kaufman et al., 1989; Smith-Barbaro, 1983). However , the effect of dietary fats on blood pressure remains controversial. Saturated fat has been reported to cause a decrease or no changes in blood pressure in strains of rats that do not overeat high-fat diets (Beebe, 1976; Young and Landsberg, 1981; Wexler 1981). Rao et al.(Rao et al., 1981) reported that polyunsaturated fats may serve as "hypotensive agents" when compared to diets with a comparable amount of saturated fat. However, polyunsaturated fats have also been shown to increase blood pressure when compared to a low fat high carbohydrate control diet (Kaufman et al., 1989a).

## 2.3 SYMPATHOADRENAL SYSTEM AND HYPERTENSION

### 2.3.1 Decoupling of Sympathetic Nervous System and Adrenal Medullary Responses

To define the role played by catecholamines in the regulation of physiologic processes, it is important to accurately assess sympathoadrenal activity. The sympathoadrenal system consists of the SNS and the adrenal medulla (Young and Landsberg, 1979b). Norepinephrine (NE) released from sympathetic nerves exerts its effects within the immediate vicinity of the nerve terminal, whereas norepinephrine and epinephrine (EPI) from the adrenal medulla provide their effects through the circulation. Under usual circumstances, the circulating levels of norepinephrine are not sufficiently high to activate adrenergic receptors. Thus,

norepinephrine is generally considered a neurotransmitter, whereas epinephrine acts as a hormone. Catecholamine release from both the peripheral sympathetic nerve endings and the adrenal medulla is a direct consequence of a descending flow of impulses from sympathetic centers in the brainstem and hypothalamus. Since the vast majority of circulating epinephrine is derived from the adrenal medulla, measurement of plasma epinephrine concentration or urinary epinephrine excretion provides an index of the functional state of the adrenal medulla. On the other hand, circulating norepinephrine may originate either from the adrenergic synapses or from the adrenal medulla. So the plasma norepinephrine level or urinary norepinephrine excretion serves as an index of overall sympathoadrenal activity. In experimental animals, it is possible to assess sympathetic activity by measuring norepinephrine turnover rate in individual sympathetically innervated organs (Young and Landsberg, 1979a and 1982). This technique permits precise distinction between the activity of sympathetic nerves and adrenal medulla, and has the potential to recognize differences in sympathetic outflow to specific organs. Although the technique of norepinephrine turnover is a more specific index of sympathoadrenal activity, it requires that the animal be sacrificed. Urinary catecholamines may be measured repeatedly without sacrificing or disturbing the animals. Therefore urinary catecholamine measurement offers some advantage over measurement of plasma catecholamine levels or norepinephrine turnover when assessment of sympathoadrenal activity is needed in long-term studies.

The two branches of the sympathoadrenal system may operate independently in a reciprocal fashion under certain circumstances such as concomitant fasting and cold exposure (Young et al., 1984; Young and Landsberg, 1977b). Furthermore, certain physiological processes such as thermogenesis and insulin secretion may be affected differently by neurally released norepinephrine than they are by catecholamines of adrenal medullary origin (Young et al., 1984).

### 2.3.2 Role of Catecholamines in Hypertension

Scince the heart and blood vessels contain specific receptors for catecholamines, these structures can be influenced either by norepinephrine liberated from the sympathetic nerve endings or by catecholamines secreted by the adrenal medulla into the general circulation. The increased vascular reactivity to catecholamines which was reported in certain forms of experimental and human hypertension was first interpreted as indirect evidence that the SNS might be involved in the etiology and maintence of hypertension (De Champlain, 1972; 1977; Goldstein, 1983). However, other studies have failed to demonstrate elevated catecholamine levels in patients with established hypertension, and the exact significance of these changes in the vascular reactivity has not yet been elucidated.

Epinephrine could produce a rise in blood pressure in several ways. First, the effect could be due to stimulation of cardiac ß-

adrenoceptors. This may lead to an increase in cardiac output due to a ß-adrenoceptor-mediated increase in cardiac contractility. At high concentrations, epinephrine may produce vasoconstriction via its effects on vascular alpha-adrenergic receptors. Another possibility is that epinephrine acts indirectly by enhancing the release of catecholamines from sympathetic nerves and/or the adrenal medulla. Activation of ß-adrenoceptors in the adrenal medulla by epinephrine results in enhanced release of catecholamines, whereas ß-adrenoreceptor blocking drugs prevent this action of epinephrine (Majewski et al.,1981a).

### 2.3.3 Dual Adrenoceptor-Mediated Control of Norepinephrine Secretion

The central nervous system governs the amount and rate of delivery of nerve impulses and provides the general control of norepinephrine release from sympathetic nerves. Catecholamines may alter norepinephrine release from sympathetic nerves through activation of either inhibitory prejunctional alpha-adrenoceptors or facilitatory prejunctional ß-adrenoceptors (Langer, 1977,1981). Others have suggested that a positive feedback mechanism mediated by presynaptic ß-adrenoceptors is activated during norepinephrine release until the transmitter in the synaptic gap reaches the threshold concentration that triggers a negative feedback mechanism mediated by presynaptic alpha-receptors resulting in inhibition of release. Thus, norepinephrine release by nerve stimulation may be modulated by different presynaptic mechanisms.

The concentrations of norepinephrinre required to activate presynaptic alpha-adrenoceptors in general are at least 30 to 100 times higher than those necessary for activation of the presynaptic ß-adrenoceptors. Thus, the first mechanism, controlled by ß-adrenoceptors, would be activated by low concentrations of the transmitter, leading to an increase in the release of norepinephrine. The second one, controlled via alpha-adrenoceptors, would be triggered when higher concentrations of norepinephrine are reached in the synaptic gap, leading to inhibition of the transmitter release (Alder-Graschinsby and Landsberg, 1975; Langer 1977; Stjarne and Brundin, 1975).

The facilitatory prejunctional ß-adrenoceptor system is a possible mechanism whereby epinephrine may enhance norepinephrine release from sympathetic nerves. Isoproterenol, a ß-agonist, increases the stimulation-evoked release of norepinephrine from the sympathetic nerves of guina-pig isolated atria (Alder-Graschinsky and Langer, 1975). This observation has been confirmed in many other sympathetically innervated tissues from a number of species, using different ß-adrenoceptor agonists, such as salbutamol, terbutaline, epinephrine, and isoproterenol. For example, both epinephrine and ß-adrenoceptor agnoists can facilitate release of [$^3$H]-norepinephrine from human omental arteries; this effect is antagonized by infusion of ß-adrenoceptor antagonist propranolol (Stjarne and Brundin, 1975). These effects are most likely due to specific activation of the ß-adrenoceptors

since they are blockd by ß-adrenoceptor antagonists (Majewski, 1986).

It has also been suggested that norepinephrine released from sympathetic nerves could activate prejunctional ß-adrenoceptors and enhance the subsequent release of norepinephrine during a series of nerve impulses, thus forming a "positive feedback loop". However this mechanism has not been confirmed by *in vivo* experiments. If it is true that neurally released norepinephrine can activate prejunctional ß-adrenoceptors, the ß-adrenoceptor blocker mentioned above should inhibit norepinephrine release from nerve endings. The ß2-subtype adrenoceptor may be involved in the facilitatory mechanism and epinephrine may activate such prejunctional ß-adrenoceptors to stimulate norepinephrine release (Dahlof et al., 1978; Stjarne and Brundin, 1975).

Other studies show that circulating epinephrine is taken up into the sympathetic endings to be stored and released as a cotransmitter (Majewski et al., 1981b). Such synaptically released epinephrine may also activate the facilitatory presynaptic ß-adrenergic receptor (Majewski, 1981a; Nezu et al., 1985). This process may have several important consequences. First, by accumulating epinephrine, the nerves can concentrate the very low levels of epinephrine found in plasma. By this mechanism, occasional bursts of epinephrine secretion from the adrenal medullas can lead to accumulation of epinephrine in sympathetic nerves and have a prolonged effect on sympathetic

neurotransmission. Release of epinephrine as a cotransmitter may produce a more intense and persistent facilitation of norepinephrine release than can be achived by circulating epinephrine alone (Majewski and Rand, 1986).

ß-adrenergic blockers may produce their hypotensive effects via several mechanisms (De Champlain, 1977). ß-blockers act to reduce cardiac contractility and cardiac output. They may also inhibit sympathetically-mediated renin secretion (Christlieb, 1973). Several lines of evidence suggest a central mechanism for the hypotensive action of ß-blockers (Majewski and Rand, 1986). Finally, ß-blockers might produce a hypotensive effect by interfering with the positive feedback mechanism for norepinephrine release mediated by presynaptic sympathetic ß-adrenergic receptors (De Champlain et al., 1977; Langer, 1977; Yamaguchi et al., 1977). Blockade of these presynaptic ß-adrenergic receptors would result in a decreased release of norepinephrine at the synapse.

## 2.4 CONTRIBUTION OF THE ADRENAL MEDULLA TO HYPERTENSION

### 2.4.1 Effect of Adrenalectomy on Blood Pressure

Previous experiments have demonstrated that adrenalectomy prevents the development of hypertension or decreases blood pressure in already hypertensive rats (Louis, 1969; Ozaki, 1966). Such adrenalectomy studies do not permit one to differentiate

between the effects of loss of adrenal cortical steroids and loss of adrenal medullary catecholamines.

One might predict that blood pressure could be reduced by a deficiency of adrenal cortical steroids since these hormones are known to influence sodium balance. In fact, such adrenalectomized rats must be maintained by subsituting saline for drinking water. These difficulties with surgical adrenalectomy can be overcome by selective surgical removal of the adrenal medulla (Booth, 1970).

### 2.4.2 Effect of Adrenal Demedullation on Blood Pressure

Sustained hypertension has been induced in rats by prolonged intravenous infusion of epinephrine at physiological concentrations (Majewski et al., 1981b). As described above, circulating epinephrine could facilitate the neurotransmission from sympathetic nerve endings by stimulating prejunctional ß-adrenoceptors.

Recently findings in pithed anesthetized spontaneously hypertensive rats confirmed that adrenal demedullation results in attenuated blood pressure responses to sympathetic stimulation. Furthermore, the blood pressure responses to sympathetic nerve stimulation were restored by infusion of epinephrine, salbutamol, or procaterol (Borkowski and Quinn, 1983; 1984). These results suggest that prejunctional ß2-adrenoceptors were involved in the blood pressure responses to sympathetic stimulation. If epinephrine

is involved in the development of raised blood pressure, the antagonism of prejunctional facilitatory ß2-adrenoceptors or depletion of epinephrine might present mechanisms to suppress the development of hypertension.

Surgical adrenal demedullation may attenuate development of hypertension by : (1) a reduced vasoconstrictor effect due to depletion of plasma catecholamines of adrenal origin (Borkowski and Quinn, 1983) and /or (2) a reduced epinephrine-mediated facilitation of sympathetic neurotransmitter release.

# CHAPTER 3 EFFECTS OF THE ADRENAL MEDULLA IN DIET-INDUCED HYPERTENSION

## 3.1 INTRODUCTION

Previous studies in our laboratory have shown that blood pressure increases when Sprague-Dawley rats are fed diets high in fat or glucose (Kaufman et al., 1988). Norepinephrine excretion was increased in hypertensive rats (those consuming fat or glucose), suggesting that SNS activity was enhanced in these animals (Kaufman et al., 1989). Dietary intake is known to have a marked effect on SNS activity in the rat. Fasting suppresses sympathetic activity while overfeeding with sucrose or lard results in SNS stimulation. Thus, diet-induced changes in SNS activity may provide an important link between diet and blood pressure. Insulin may be a major link between changes in dietary intake and SNS activity. The SNS then may increase blood pressure through influences on tissues, such as the heart, the blood vessels, and the kidneys (Krieger and Landsberg, 1988).

It also has been suggested in our laboratory that enhanced adrenal medullary activity may be involved in the development of diet-induced hypertension by finding that urinary epinephrine excretion was slightly higher in hypertensive rats than in normotensive rats. Others have suggested that epinephrine may play a role in the development of hypertension (Majewski, 1981a; 1981b; 1986; Nezu, 1985). The effect of epinephrine on blood

pressure may be direct via increased cardiac contractility (ß-adrenergic effect) or vasoconstriction (alpha-adrenergic effect). Epinephrine may also raise blood pressure indirectly through a stimulatory presynaptic regulation of norepinephrine release by facilitatory prejunctional ß-adrenoceptors (Alder, 1975; Borkowski, 1984; Langer , 1977, 1981; Majewski, 1981a, 1986; ).

It has been suggested that refined carbohydrate may raise blood pressure through effects on the SNS based on the insulin-hypertension model established by Landsberg (Landsberg and Young, 1981b). However the mechanism by which dietary fat affects blood pressure is not well understood. Since the Western diet is composed of more that 40% of calories as fat, it becomes interesting and important to investigate the mechanism by which high fat feeding increases blood pressure. Therefore, the present studies were undertaken to define the role of the adrenal medulla in the hypertensive response to dietary fat or dietary glucose.

In order to isolate the effects of dietary fat and carbohydrate, semisynthetic diets were formulated to provide similar quantities of all other components, including protein, minerals, vitamins, and fiber (see Table 1). Rats were fed one of three semisynthetic diets (high fat, glucose, or control), and half of them in each group underwent adrenal demedullation prior to the beginning of diet treatment. Blood pressure responses were monitored throughout the experiment.

Table 1

COMPOSITION OF EXPERIMENTAL DIETS

| Diet | High-Fat | Glucose | Control |
|---|---|---|---|
| Caloric Density (kcal/g) | 6.09 | 4.02 | 4.20 |
| Methionine (g/kg) | 4.0 | 3.0 | 3.0 |
| AIN-76 Vit. Mix* (g/kg) | 13.5 | 10.0 | 10.0 |
| AIN-76 Mineral (g/kg) | 47.3 | 35.0 | 35.0 |
| Choline Chloride (g/kg) | 2.7 | 2.0 | 2.0 |
| Cellulose (g/kg) | 67.5 | 50.0 | 50.0 |
| Casein** (g/kg) | 317.0 | 210.0 | 224.0 |
| Corn Starch (g/kg) | 151.0 | 0.0 | 625.0 |
| Glucose (g/kg) | 0.0 | 642.0 | 0.0 |
| Corn Oil (g/kg) | 73.0 | 48.0 | 51.0 |
| Lard (g/kg) | 324.0 | 0.0 | 0.0 |
| Protein (% of energy) | 21.5 | 21.5 | 21.5 |
| Carbohydrate (% of energy) | 12.0 | 66.4 | 66.5 |
| Fat (% of energy ) | 66.5 | 12.1 | 12.0 |

* Because of differences in caloric density and anticipated food intake, the High-Fat diet contained 3.49 mg NaCl and 7.03 mg elemental calcium per gram, while the Control and Glucose diets contained 2.58 mg NaCl and 5.20 mg elemental calcium per gram.

**Vitamin Free Casein, ICN Nutritional Biochemicals.

The present studies were undertaken to define the role of the adrenal medulla in the hypertensive response to dietary fat or dietary glucose. It is hoped that Information derived from these studies may ultimately be applicable to the understanding of certain forms of human hypertension.

## 3.2 METHODS AND MATERIALS

### Animals and Experimental Diets

Male Sprague-Dawley rats (8 rats/group) (Harlan, Indiananpolis, IN) were housed individually in hanging stainless steel cages in a room temperature of $20^{o}\pm2$ C with a 12h light/dark cycle. At ten to eleven weeks of age the rats were offered one of three semisynthetic diets (see Table 1). Diets were formulated to provide equal quantities of protein, vitamins, and minerals. The high-fat diet provides 66.4% of total energy as fat (primarily lard) and 12.1% as corn starch. The glucose diet provides 66.4% of energy as glucose and 12.1% as fat. The control diet provides 66.5% of energy as corn starch and 12.0% as fat. Animals were allowed ad libitum access to water and food.

There were six groups of rats (8 rats/group) in this experiment. Two groups were assigned to a particular diet treatment. Rats in each diet treatment underwent either bilateral adrenal demedullation or sham operation as described below. Body weight was monitored throughout the experiment. Food intake was

monitored throughout the experiment and corrections were made for spillage. Diets were analyzed for gross energy content.

Surgery

At 7-8 weeks of age, bilateral adrenal demedullation was performed in half of the rats in each diet treatment. Under pentobarbital anesthesia, bilateral adrenal demedullation was performed by clamping the suprarenal fatty tissue, making a 180 slit in the adrenal cortex and pressing the medulla out as one piece by passing the adrenal through practically closed forceps (Booth, 1970). Sham operation was performed in control rats by identifying the adrenals bilaterally without incising them.

Rats were allowed to recover from surgery for 1 week prior to beginning blood pressure recording. The completeness of demedullation was confirmed histologically by demonstrating the absence of chromaffin tissue (Figure 1a,1b) and biochemically by demonstrating very low or undectable urinary epinephrine excretion. Sham-operated adrenals appeared normal histologically. Viable adrenal cortical tissue was identified histologically in demedullated rats (Figure 1a, 1b ). Plasma corticosterone content was measured to confirm the intact function of the adrenal cortex, and did not differ among demedullated and sham-operated groups.

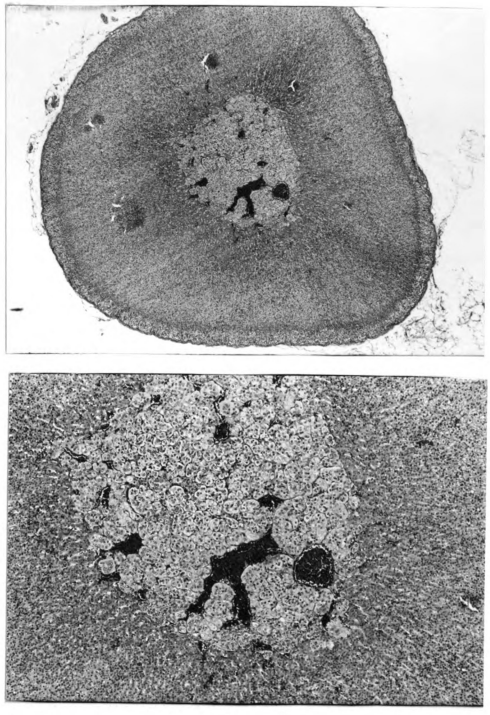

Figure 1a The intact adrenal gland with chromaffin tissue (upper, 4X; lower, 10X).

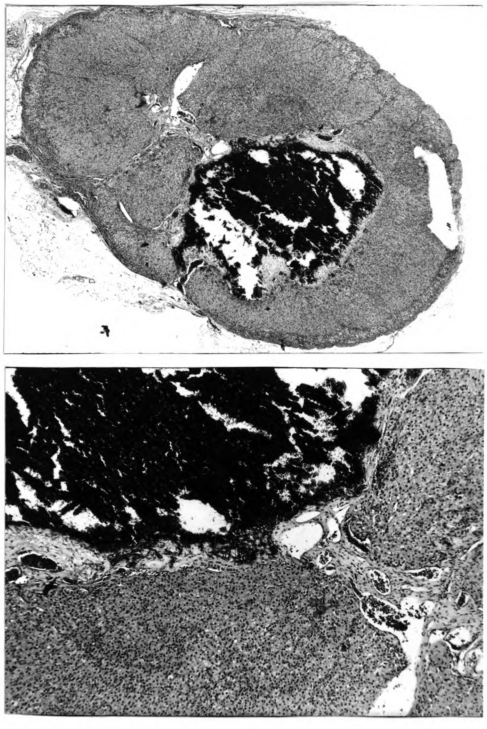

Figure 1b The demedullated adrenal gland (upper, 4X; lower, 10X).

## Blood Pressure Measurement

Blood pressure was measured on concious restrained rats using a photoelectric sensor (IITC, Inc., Woodland Hills, CA) and tail cuff sphygmomanometer. This indirect measurement provides results that correlate closely with directly measured arterial blood pressure (Bunag, 1982; Pfeffer et al., 1971). Rats were acclimated to the procedure repeatedly prior to the beginning of data collection. Blood pressures were recorded weekly between the hours of 1200 and 1700 during feeding of experimental diets. Pressures recorded during five successive inflation-deflation cycles were averaged to obtain a single weekly BP reading.

## Urine Collection

Urine was collected for catecholamine analysis over a period of 72 hours while rats were housed in individual metabolic cages (Nalge Rochester, NY). Rats were acclimated to metabolic cages for 36-48 hours prior to beginning urine collection. Urine was collected in the presence of 4 ml of 3N HCL. pH was adjusted between 2-3 and specimens were frozen for subsequent analysis of catecholamine concentrations. Urine collection was performed initially during weeks 3-5 and again during weeks 7-9 after beginning experimental diets.

## Catecholamine Analysis

Catecholamine analysis was performed by liquid chromatography with electrochemical detection (BAS LCEC Application Note 15, 1981) using a modification of the method of Riggin and Kissinger (Riggin and Kissinger, 1977). After preliminary purification using a cation exchange resin (Bio Rex 70, 100-200 mesh, Bio-Rad Laboratories, Richmond, CA), catecholamines were concentrated by passage over alumina (Bioanalytical Systems Inc., West Lafayette, IN.) Then catecholamines were analyzed using a reverse phase column (Bio-Sil, ODS-5S, 250x4 mm; Bio-Rad Lasboratories, Richmond, CA) and an electrochemical detector (LC-4A, Bioanalytical Systems Inc.) with glassy carbon electrode. Specimens were analyzed in duplicate and the results averaged. For norepinephrine, the interassay coefficient of variation was 7% and the intraassay variation was 5%. For epinephrine, the interassay coefficient of variation was 10% and the intraassay variation was 8%.

## Plasma Analysis

At the end of the experiment, plasma was collected for analysis of glucose (glucose oxidase method; Beckman Glucose Analyzer), as well as insulin (radioimmunoassay kit using rat insulin as the standard; Incstar, Stillwater, MN), and corticosterone (radioimmunoassay kit; Endocrine Sciences, Tarzana, CA).

## Body Composition Analysis

At the end of the experiment, the liver, heart, and hindlimb muscles (gastrocnemius, soleus, and plantaris) were dissected and weighed.

## Calculation and Statistics

Data are presented as means$\pm$ SEM. Multiple comparisons were made by analysis of variance, and treatment means were compared using the Bon—ferroni t test (Gill, 1978; Steel and Torrie, 1960). All differences were considered significant at $p<0.05$.

## 3.3 RESULTS

### Effects of Experimental Diets on Blood Pressure in Adrenal Demedullated and Sham-operated Rats

Initially blood pressure measurements were made during the week before starting the experimental diets (week 0) while all rats were fed a laboratory chow diet. No differences in blood pressure were observed during this baseline period among sham-operated and demedullated groups that were later assigned to different diet treatments. The changes in blood pressure are shown in Table 2. Systolic blood presures of sham-operated rats fed the high-fat diet were higher than sham-operated rats fed the control diet during weeks 4 through 10 (Table 2 and Figure 2). The systolic pressures of sham-operated rats fed the glucose diet were significantly higher than sham-operated rats fed the control diet during weeks 5,7,9 and10 of diet treatment (Table 2 and Figure 2). Systolic blood pressures showed no significant difference among demedullated rats fed either fat, glucose, or starch diets. There was a significant interaction between diet and surgical treatment during weeks 5,7,9 and 10. Sham-operated rats fed fat or glucose diets had significantly higher blood pressures than demedullated rats fed the same diet. There was no siginificant difference between sham-operated and demedullated rats fed the control diet with the exception of week 8.

Table 2

Systolic Blood Pressure (mm Hg) Response to Experimental Diets in Adrenal Demedullated (D) and Sham-operated (S) Rats

| | High-Fat | | Glucose | | Control | |
|---|---|---|---|---|---|---|
| | S | D | S | D | S | D |
| Week 0 | 149±2 | 153±2 | 154±4 | 150±6 | 152±4 | 154±2 |
| Week 2 | 154±3 | 158±2 | 154±2 | 145±3 | 154±2 | 154±2 |
| Week 3 | 158±3 | 150±3 | 151±3 | 151±3 | 153±4 | 151±2 |
| Week 4 | 165±3$^{b}$ | 150±3* | 155±3$^{a}$ | 147±2 | 151±3$^{a}$ | 150±2 |
| Week 5 | 158±3$^{b}$ | 146±2* | 159±2$^{b}$ | 143±3* | 144±3$^{a}$ | 149±2 |
| Week 6 | 162±4$^{b}$ | 152±2* | 153±3 | 151±2 | 149±4$^{a}$ | 144±4 |
| Week 7 | 155±3$^{b}$ | 135±4* | 153±1$^{b}$ | 135±3* | 141±3$^{a}$ | 139±2 |
| Week 8 | 155±2$^{b}$ | 137±2* | 150±1 | 136±2* | 146±2$^{a}$ | 136±3* |
| Week 9 | 163±4$^{b}$ | 142±4* | 156±2$^{b}$ | 143±3* | 147±3$^{a}$ | 143±3 |
| Week 10 | 164±3$^{b}$ | 144±3* | 156±2$^{b}$ | 144±3* | 145±4$^{a}$ | 142±3 |

Values are means ± SEM for 7-8 rats in each group.

Asterisks indicate a significant difference ($p<0.05$) from sham-operated rats within the same diet treatment.

Different superscript letters indicate a significant difference ($p<0.05$) among groups within a surgical treatment. For example, during week 6, within sham-operated animals, blood pressure was significantly higher in rats fed the high-fat diet (superscript b) than in rats fed the control diet (superscript a ). Blood pressure of sham-operated rats fed the glucose diet (no superscript) did not differ significantly from that of sham-operated rats fed either the high-fat diet or the control diet.

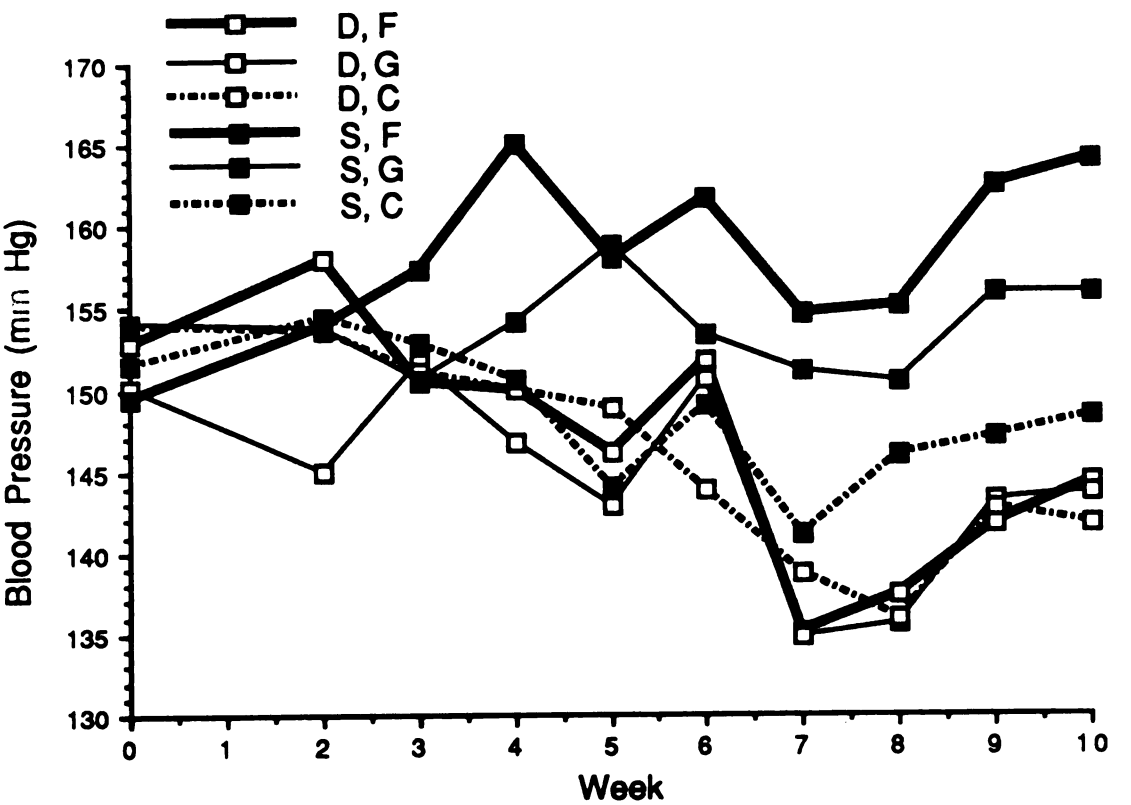


**Figure 2** Systolic blood pressure response to experimental diets in demedullated (D) and sham-operated (S) rats: high-fat (F), glucose (G), and control (C). Data are presented as means for all animals in each group. Within the fat-fed groups, adrenal demedullation significantly ($p<0.05$) lowered BP during weeks 4-10. Within the glucose fed groups, adrenal demedullation significantly ($p<0.05$) lowered BP during weeks 5,7,8,9,10. Within the sham-operated groups, BP was significantly higher ($p<0.05$) in fat-fed rats than in control rats during weeks 4-10.

Effects of Experimental Diets on Body Weight, Organ Weight and Food Intake in Adrenal Demedullated and Sham-operated Rats

After ten weeks of feeding, body weights of sham-operated rats were 431±13, 394±8, and 377±6g in groups fed the high-fat, glucose, and control diets, respectively. Body weight of fat-fed rats was approximately 15% higher ($p<0.05$) than that of rats fed the control diet and 10% higher ($p<0.05$) than the weight of rats fed the glucose diet (see Table 3, Figure 3). Body weight of sham-operated rats fed the glucose diet did not differ from sham-operated rats fed the control diet.

There were no differences in weight between sham-operated and adrenal demedullated rats within the same diet treatment. Final body weights of the demedullated rats were 418±8, 374±11, and 379±5g in rats fed the high-fat, glucose, and control diets, respectively. Body weight of fat-fed rats was approximately 10% higher ($p<0.05$) than that of rats fed the control diet and 12% higher ($p<0.05$) than the weight of rats fed the glucose diet. Body weight of demedullated rats fed the glucose diet did not differ from demedullated rats fed the control diet.

The change in weight during the 10 week feeding period was also calculated for each animal, in order to correct for differences in initial body weight.

Table 3

Body Weight (g) Response to Experimental Diets in Adrenal Demedullated (D) and Sham-operated (S) Rats

| | High-Fat | | Glucose | | Control | |
|---|---|---|---|---|---|---|
| | S | D | S | D | S | D |
| Week 0 | 262±3 | 260±5 | 258±5 | 251±4 | 245±3 | 259±3 |
| Week 1 | 273±7 | 267±7 | 270±4 | 263±5 | 258±4 | 274±4 |
| Week 2 | 302±6$^{b}$ | 289±7 | 289±5 | 277±6 | 274±4$^{a}$ | 288±3 |
| Week 3 | 319±9$^{b}$ | 313±8$^{b}$ | 307±6 | 294±7$^{a}$ | 293±5$^{a}$ | 307±4 |
| Week 4 | 337±9$^{b}$ | 331±8$^{b}$ | 323±6 | 309±8$^{a}$ | 309±6$^{a}$ | 321±4* |
| Week 5 | 355±10$^{b}$ | 348±8$^{b}$ | 337±6 | 322±8$^{a}$ | 323±6$^{a}$ | 334±4 |
| Week 6 | 373±11$^{b}$ | 366±9$^{b}$ | 352±8 | 335±8$^{a}$ | 337±6$^{a}$ | 345±4 |
| Week 7 | 390±12$^{b}$ | 380±9$^{b}$ | 366±8$^{a}$ | 346±9$^{a}$ | 349±6$^{a}$ | 359±4 |
| Week 8 | 400±12$^{b}$ | 389±9$^{b}$ | 372±8$^{a}$ | 353±10$^{a}$ | 357±6$^{a}$ | 363±4$^{a}$ |
| Week 9 | 412±12$^{b}$ | 400±9$^{b}$ | 381±9$^{a}$ | 361±11$^{a}$ | 365±6$^{a}$ | 371±5$^{a}$ |
| Week 10 | 423±13$^{b}$ | 411±9$^{b}$ | 390±8$^{a}$ | 370±11$^{a}$ | 374±6$^{a}$ | 378±5$^{a}$ |
| Final week | 431±13$^{b}$ | 418±8$^{b}$ | 394±8$^{a}$ | 374±11$^{a}$ | 377±6$^{a}$ | 379±5$^{a}$ |

Values are means ± SEM for 7-8 rats in each group.

Different superscript letters indicate significant differences ($p<0.05$) among groups within a surgical treatment (see Table 2 for a detailed explanation).

Asterisk indicates a significant difference ($p<0.05$) from sham-operated rats within the same diet treatment.

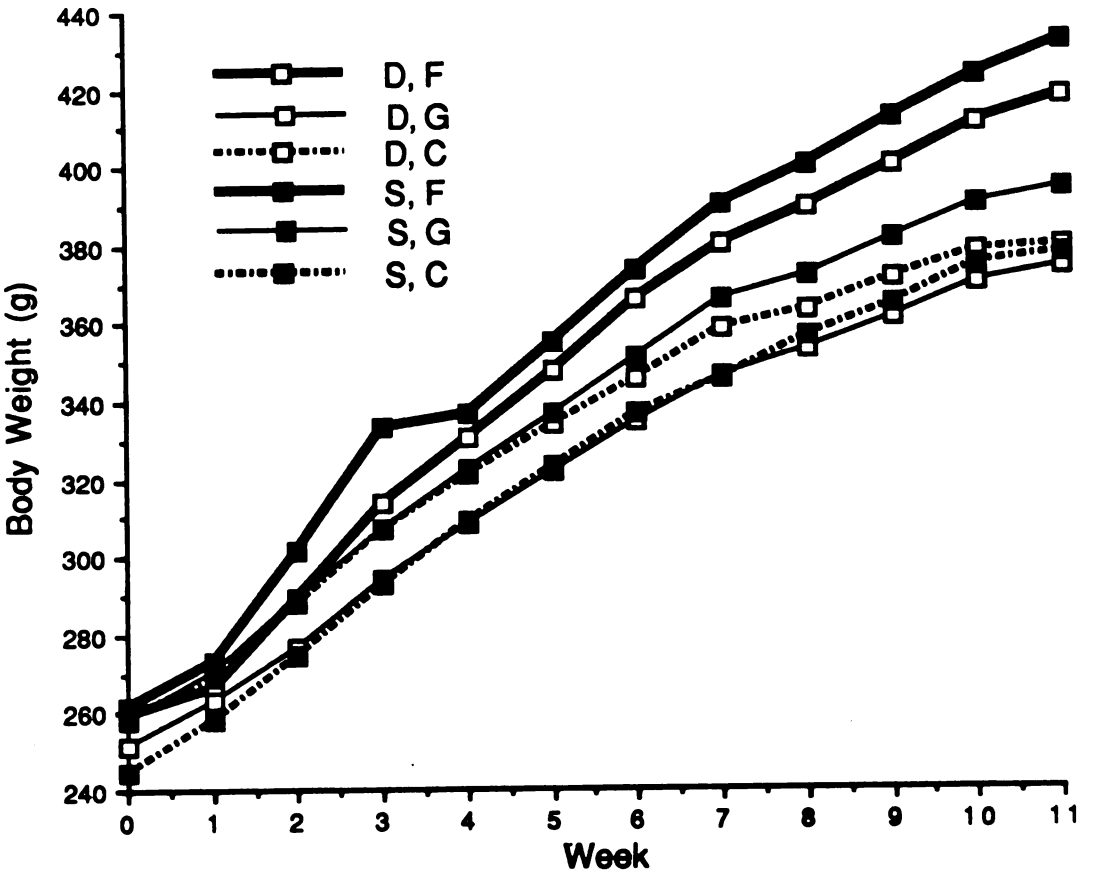


Figure 3 Body weight response to experimental diets in demedullated (D) and sham-operated (S) rats: high-fat (F), glucose (G), and control (C). Data are presented as means for all animals in each group. Body weight of fat-fed rats was significantly higher ($p<0.05$) than that of rats fed the control diet and significantly higher ($p<0.05$) than the weight of rats fed the glucose diet. There was no difference in body weight between rats fed the glucose and control diets. Demedullation had no effect on body weight within the same diet treatment.

There were no differences in cumulative energy intake between sham-operated and adrenal demedullated rats within the same diet treatment. In the sham-operated rats, energy intake was approximately 10% greater ($p<0.05$) in rats fed the high-fat diet than in rats consuming the control diet. In the adrenal demedullated rats, energy intake was approximately 6% greater in rats fed the high-fat diet than in rats consuming the control diet, but this difference was not significant at $p<0.05$. Within each surgical treatment, energy intake of rats fed the glucose diet was intermediate between and not significantly different from that of rats fed the other two diets (see Table 4).

Sham-operated rats fed the high-fat diet had significantly larger liver, heart, and hindlimb muscle than sham-operated rats fed the control diet (Table 5). Previous studies in this laboratory have demonstrated that rats fed the high-fat diet develop a higher energy density of the carcass (an index of obesity) than rats fed the glucose or control diet. However, energy density was not measured as part of the current studies.

## Effects of Experimental Diets on Plasma Insulin Levels in Adrenal Demedullated and Sham-operated Rats

Rats consuming the high-fat diet had significantly higher ($p<0.05$) insulin levels when compared to rats fed the control diet (Figure 4 and Table 6). Demedullation had no effect on plasma insulin levels within the same diet treatment. The relationship between insulin

Table 4

Cumulative Energy Intake (Kcal) in Adrenal Demedullated (D) and Sham-operated (S) Rats

| Surgery | S | D |
|---|---|---|
| High-Fat | 6422±162* | 6155±174 |
| Glucose | 6047±113 | 5850±164 |
| Control | 5815±97 | 5809±120 |

Values are means±SEM for 7-8 rats per group.

Asterisk indicates a significant difference ($p<0.05$) from sham-operated rats fed the control diet.

Table 5

Organ Weight (g) in Sham-operated (S) and Adrenal Demedullated (D) Rats Fed Experimental Diets

| | Heart | Liver | Hindlimb Muscle¥ |
|---|---|---|---|
| High-Fat (S) | 1.74±0.05[b] | 12.30±0.41[b] | 6.30±0.10[b] |
| High-Fat (D) | 1.59±0.04* | 12.16±0.46[b] | 6.07±0.09[b] |
| Glucose (S) | 1.50±0.03[a] | 11.75±0.52 | 6.07±0.17[b] |
| Glucose (D) | 1.51±0.05 | 10.56±0.56[a] | 5.77±0.12 |
| Control (S) | 1.46±0.02[a] | 10.70±0.27[a] | 5.69±0.08[a] |
| Control (D) | 1.49±0.03 | 10.94±0.32 | 5.72±0.12[a] |

Values are means±SEM for all 7-8 rats per group.

Asterisk indicates a significant difference ($p<0.05$) from sham-operated rats within the same diet treatment.

Different superscript letters indicate significant differences ($P<0.05$) among groups within a surgical treatment (see Table 2 for a detailed explanation).

¥ hindlimb muscle weight represents the sum of right and left leg gastrocnemius, soleus, and plantaris muscles.

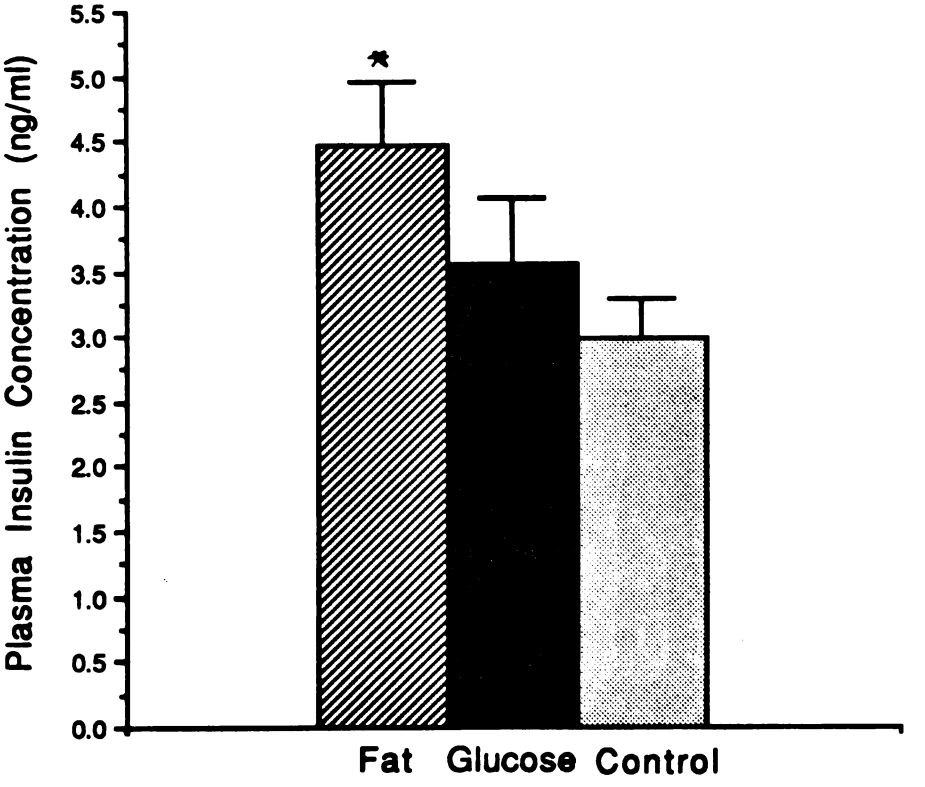


Figure 4 Plasma insulin response to experimental diets. The asterisk indicates a significant difference ($p<0.05$) from the control group.

Table 6

Effects of Experimental Diets on Plasma Insulin Level (ng/ml) in Adrenal Demedullated (D) and Sham-operated (S) rats

| Rats | Plasma Insulin Concentration (ng/ml) | |
|---|---|---|
| High-Fat S | 4.4±0.7 | (7) |
| High-Fat D | 4.5±0.7 | (8) |
| High-Fat S+D | 4.5±0.5* | (15) |
| Glucose S | 3.3±0.4 | (8) |
| Glucose D | 3.8±0.8 | (8) |
| Glucose S+D | 3.6±0.5 | (16) |
| Control S | 3.0±0.5 | (8) |
| Control D | 3.0±0.5 | (8) |
| Control S+D | 3.0±0.3 | (16) |

Values are means± SEM of measurements for each group. Numbers in parentheses indicate number of rats per group.

Within each diet treatment, no differences were observed between adrenal demedullated (D) + sham-operated (S) groups.

Asterisk indicates a significant difference ($p<0.05$) from control S+D rats.

levels and weight gained during ten weeks of diet treatment is shown in Figure 5. Plasma insulin levels were positively correlated with final body weight ($r=0.57$; $p<0.001$) and with gain in body weight during the ten week feeding period ($r=0.61$; $p<0.001$).

Plasma glucose levels were $123\pm5$, $118\pm2$, and $114\pm2$ mg/dl in the sham-operated rats fed the high-fat, glucose, and control diets, respectively. Plasma glucose was significantly higher ($p<0.05$) in sham-operated fat-fed rats compared to sham-operated rats fed the control diet. Plasma glucose levels were $115\pm4$, $109\pm3$, and $109\pm2$ mg/dl in the adrenal-demedullated rats fed the high-fat, glucose, and control diets, respectively (Table 7).

## Effects of Diet and Adrenal-Demedullation on Urinary Catecholamine Excretion

In rats with intact adrenal medullas, an increase in epinephrine excretion was observed as early as 3-5 weeks after beginning the high-fat diet (when compared to epinephrine excretion in rats fed the control diet). Elevated epinephrine excretion was observed during weeks 7-9 of diet treatment in sham-operated rats fed either the high-fat or glucose diets (when compared to epinephrine excretion in rats fed the control diet). Adrenal demedullation dramatically reduced epinephrine excretion of rats consuming each of the three experimental diets (see Table 8). In sham-operated rats, those developing hypertension (rats fed the high-fat or glucose diets) had higher norepinephrine excretion than

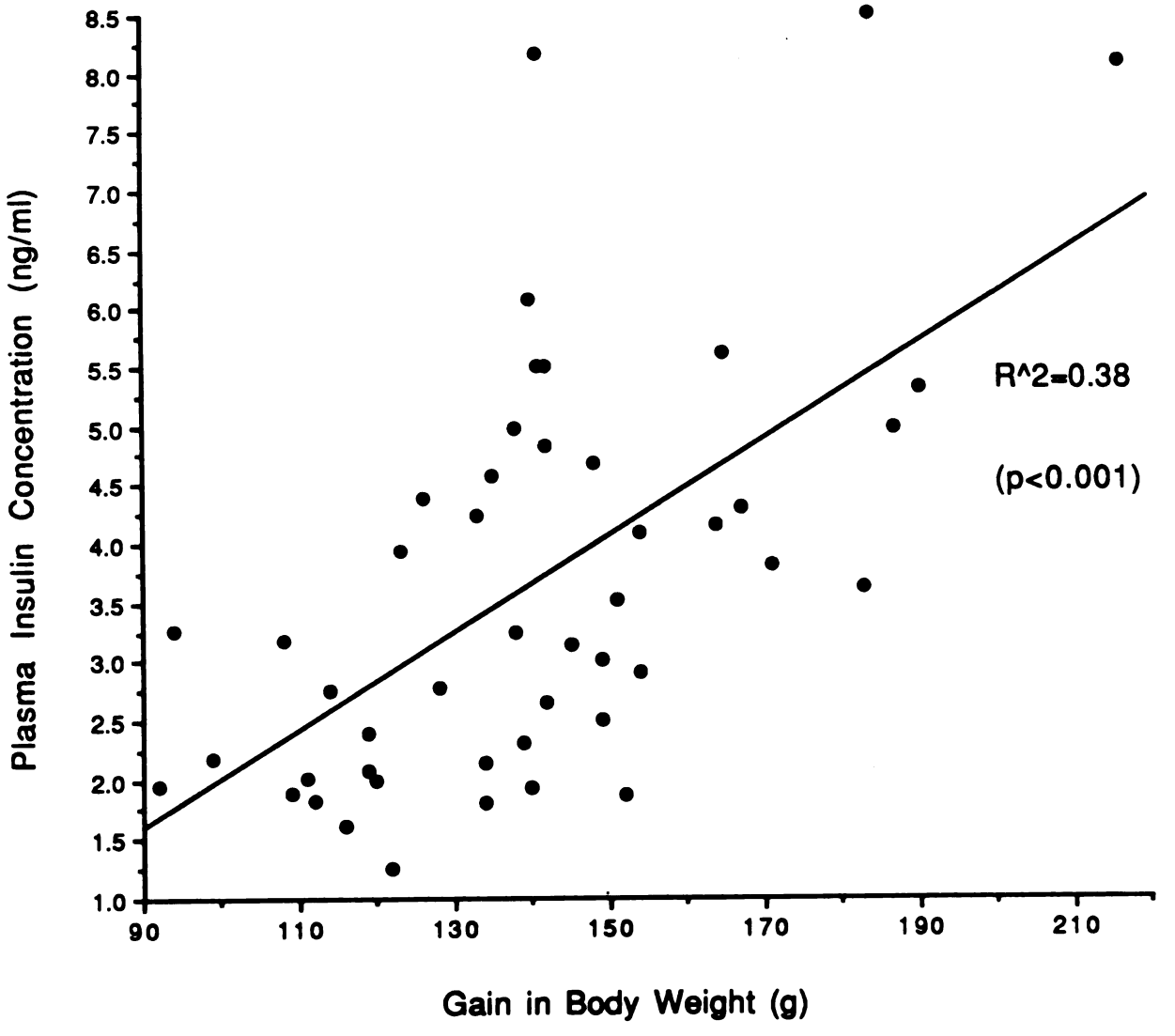


Figure 5 Relationship between plasma insulin level and gain in body weight during the 10 week feeding period. Dots denote individual values for individual rats from each group.

TABLE 7

Effects of Experimental Diets on Plasma Glucose Level (mg/dl) in Adrenal Demedullated (D) and Sham-operated (S) Rats

| DIET | High-Fat | Glucose | Control |
|---|---|---|---|
| S | 123±5* | 118±2 | 114±2 |
| D | 115±4 | 109±3 | 109±2 |

Asterisk indicates a significant difference ($p=0.047$) from sham-operated rats fed the control diet.

Table 8

Catecholamine excretion (ng/day) in Sham-operated (S) and Adrenal Demedullated (D) Rats

| | Weeks 3-5 | | Weeks 5-7 | |
|---|---|---|---|---|
| | NE | E | NE | E |
| High-Fat (S) | 1486±126 | 254±12b | 1720±112b | 269±17b |
| High-Fat (D) | 1362±92 | 15±3* | 1622±101 | 43±5* |
| Glucose (S) | 1437±120 | 231±18 | 1738±121b | 255±20b |
| Glucose (D) | 1339±90 | 10±4* | 1467±47* | 31±2* |
| Control (S) | 1308±60 | 204±14a | 1374±57a | 199±9a |
| Control (D) | 1401±64 | 18±4* | 1588±92 | 25±6* |

Values are means±SEM for 7-8 rats per group.

Asterisks indicate a significant difference ($P<0.05$) from sham-operated rats within the same diet treatment.

Different superscript letters indicate significant difference ($P<0.05$) among groups within a surgical treatment (see Table 2 for a detailed explanation).

NE= norepinephrine
E = epinephrine

normotensive rats (those fed the control diet) during weeks 7-9. This confirms previous findings in our laboratory. Norepinephrine excretion did not differ significantly among sham-operated groups at an earlier stage of diet treatment (during weeks 3-5 of experimental diet feeding).

In adrenal-demedullated rats fed the glucose diet, norepinephrine excretion was significantly lower during weeks 5-7 than in sham-operated rats fed the same diet. In adrenal-demedullated rats fed the high-fat diet, norepinephrine excretion was slightly but not significantly lower during weeks 5-7 when compared to sham-operated rats fed the same diet. Thus, norepinephrine excretion was attenuated significantly by adrenal demedullation in rats fed the glucose diet, with a tendency toward norepinephrine attenuation by adrenal demedullation in rats fed the high-fat diet.

## 3.4 DISCUSSION

The purpose of this study was to investigate the role of the adrenal medulla in the development of diet-induced hypertension. Our objective was to determine whether adrenal demedullation can attenuate the development of hypertension resulting from high-fat or glucose-enriched diets. In order to isolate the effects of fat and glucose, semisynthetic diets were formulated to provide similar quantities of all other components, including vitamins, proteins, fiber, and minerals. Half of the animals in each diet treatment underwent adrenal demedullation while the remaining animals underwent sham operation. Our findings showed that blood pressure was lower in demedullated rats consuming either high-fat or glucose diets than in sham-operated rats consuming the same diets. Data in the present study confirmed previous findings in our laboratory that rats with an intact adrenal medulla fed a high-fat or glucose-enriched diet had higher blood pressure than rats consuming a control diet composed primarily of corn starch.

During the early stage of diet treatment (weeks 3-5), urinary epinephrine excretion was significantly higher in sham-operated rats fed the high-fat diet than in rats fed the control diet. There was no significant difference in norepinephrine excretion among groups. During the late stage of diet treatment (weeks 7-9). The high-fat or glucose diet raised urinary excretion of epinephrine and norepinephrine in sham-operated rats. Sham-operated rats fed the high-fat or glucose diet had higher blood pressure than rats fed the

control diet from week 5. Therefore, we conclude that chronic feeding of diets high in fat or glucose enhanced sympathoadrenal activity (as reflected by higher urinary norepinephrine and epinephrine excretion). In contrast, in the demedullated groups, catecholamine excretion did not differ among rats fed the different diets. As expected, urinary epinephrine excretion was dramatically lower in demedullatd rats than in sham-operated rats. Urinary norepinephrine excretion was reduced by demedullation in rats fed the glucose diet, and was slightly but not significantly reduced in rats fed the high-fat diet. It is possible that the raised blood pressure in sham-operated rats fed high-fat or glucose diets resulted from activation of the sympathetic nervous system, and that this process may have been facilitated by epinephrine released from the adrenal medulla. This explanation seems plausible when we take into consideration that epinephrine may alter norepinephrine release from sympathetic nerve endings through activation of facilitatory ß-adrenoceptors (Majewski, 1986).

Data of rats with intact adrenal glands in this experiment are consistent with previous observations in our laboratry that glucose-induced elevations in blood pressure occur in the absence of obesity, while fat-induced increases in blood pressure are associated with the development of obesity. Previous studies have demonstrated that rats fed the high-fat diet ad libitum develop greater body weight and higher energy density of the carcass (an index of obesity) than rats fed either the glucose or control diet. These findings suggests that different mechanisms may regulate the blood pressure

responses to these two macronutrients. Although fat feeding results in obesity and increased insulin levels in the rats, other hormonal signals related to dietary fat and obesity need further investigation. However, higher urinary catecholamine excretion in both models of diet-induced hypertension suggests that activation of the sympathoadrenal system may be a common pathway mediating the effects of high-fat or glucose diets on blood pressure. The relationship between obesity and development of hypertension is parallel in fat-fed rats, suggesting that elevation in blood pressure depends on the development of hyperphagia, obesity, and hyperinsulinemia. Insulin may be a critical signal involved in the development of hypertension. Effects of insulin might be either direct on renal handling of sodium (De Fronzo, 1985) , or indirect via activation of the sympathetic nervous system (O'Hare et al., 1985, Rowe, 1981).

Based on our findings that fat-fed rats consumed significantly more energy than rats fed the control diet, and glucose-fed rats consumed slightly more energy than control rats, we expected that an enhanced insulin response might exist in fat-fed and glucose-fed rats. In this study, we confirmed that hyperinsulinemia was associated with hypertension in fat-fed rats, while fasting plasma glucose and insulin levels in glucose-fed rats appeared similar to those in control rats. However, it is possible that higher insulin and /or glucose levels may have been present at other times throughout the 24-hour period in rats fed the glucose diet. Thus, enhanced insulin may play a role in glucose-induced hypertension as well as

fat-induced hypertension, and this hypothesis deserves further investigation.

It should be noted that demedullated fat-fed rats became obese and hyperinsulinemic as well as sham-operated fat-fed rats. However, the hypertensive response to fat did not occur in demedullated rats, This suggests that epinephrine plays a major role in the development of diet-induced hypertension and that insulin's effect on blood pressure may be indirect via stimulation of the sympathoadrenal system.

In summary, the present study describes one mechanism linking dietary macronutrient intake to activation of the sympathetic nervous system and changes in blood pressure. We demonstrated that an intact adrenal medulla is required for the development of hypertension in response to dietary fat or dietary glucose. We also found that an intact adrenal medulla is required for the development of raised urinary excretion of norepinephrine observed in hypertensive rats, and diet-induced hypertension will not develop in rats lacking an adrenal medulla. These data suggest that adrenal medullary catecholamines play an important role in the development of certain forms of diet-induced hypertension.

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