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The Role of the Hypothalamic Paraventricular Nucleus in Stress-Induced Renin and Corticosterone Secretion

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THE ROLE OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS
IN STRESS-INDUCED RENIN AND CORTICOSTERONE SECRETION

by

Kathy Richardson Morton

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
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DEDICATION

This dissertation is dedicated to my parents, Virgil and Audrey Bullington, for their constant support and encouragement, and to my daughter, Lindsay.
VITA

The author, Kathy Richardson Morton, was born on January 26, 1957, in Knoxville, Tennessee. She completed her secondary education at West High School in Knoxville, Tennessee in June, 1975. In September, 1975, Ms. Morton entered the University of Tennessee, where she graduated magna cum laude with a Bachelor of Arts degree in Biology in June, 1978. Following graduation, she was employed as an Associate Chemist for the Bristol-Myers Company in Cincinnati, Ohio. In 1982, Ms. Morton did a summer rotation in the laboratory of Dr. Robert Ten Eick at Northwestern University.

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Ms. Morton has accepted a post-doctoral position in the laboratory of Dr. Neil MacLusky in the Department of Reproductive Science at the University of Toronto and Toronto General Hospital. She has been awarded a post-doctoral fellowship from the Medical Research Council of Canada.
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CHAPTER I

INTRODUCTION

Stress has profound effects on neuroendocrine function, increasing plasma corticosterone and prolactin levels, and plasma renin activity (PRA). Neurons in the hypothalamic paraventricular nucleus (PVN) play an important role as integrators of endocrine, autonomic and behavioral functions. The purpose of this dissertation research project was to investigate the role of neurons in, and afferents to, the PVN in mediating stress-induced neuroendocrine responses, specifically increased PRA, plasma renin concentration (PRC), and corticosterone levels. Male Sprague-Dawley rats were used for these experiments. The stressor consisted of a conditioned emotional (fear) response paradigm, which has been shown to cause increases in renin and corticosterone secretion. In this paradigm, each rat receives a footshock 10 minutes following placement in a chamber, and then was returned to his home cage. This procedure is repeated for three consecutive days. On the fourth day, instead of receiving shock 10 minutes after being placed in the test chamber, the rat is removed and sacrificed by decapitation. Trunk blood was collected for hormone assays. The experimental control rats were treated identically to rats in the treatment group with the exception that shock was not administered at any time.
1. **Effect of Electrolytic PVN Lesions on Stress-Induced Renin and Corticosterone Secretion.** Bilateral electrolytic lesions in the PVN prevented the stress-induced increase in PRA, PRC and corticosterone levels. In contrast, electrolytic lesions in the thalamic nucleus reuniens, dorsal and caudal to the PVN, did not prevent the stress-induced increase in either PRA, PRC or corticosterone levels. Since the majority of the diencephalic corticotropin releasing factor (CRF) neurons are located in the parvocellular subnuclei of the PVN, it is not surprising that electrolytic PVN lesions prevented the stress-induced increase in corticosterone secretion. However, the finding that the stress-induced increases in PRA and PRC were also prevented was unexpected.

2. **Effect of Ibotenic Acid Lesions of Cells in the PVN on Stress-Induced Renin and Corticosterone Secretion.** Ibotenic acid is a selective neurotoxin that destroys cell bodies while leaving fibers of passage intact. To determine whether cell bodies in the PVN or fibers of passage mediate stress-induced increases in PRA, PRC and corticosterone levels, the cells in the PVN were selectively destroyed by injection of ibotenic acid. The corticosterone, PRA and PRC responses to stress were blocked by ibotenic acid injection. These observations suggest that neuronal cell bodies in the PVN mediate the stress-induced increase in PRA, PRC and corticosterone.

3. **Effect of 6-Hydroxydopamine-Induced Lesions in the PVN on Stress-Induced Renin and Corticosterone Secretion.** Since the data indicated that cell bodies in the PVN mediate stress-induced renin and
corticosterone secretion, and the PVN is known to have an extensive catecholaminergic innervation, the next approach was to determine if catecholaminergic innervation of PVN neurons plays an important role in the neuroendocrine response to stress. This was accomplished by injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the PVN. 6-OHDA is taken up by the catecholaminergic nerve endings resulting in degeneration of these nerve terminals. Lesion placement and damage to noradrenergic nerve terminals was verified by immunocytochemical methods, using an antibody against dopamine β-hydroxylase. The 6-OHDA injections prevented the stress-induced increase in PRA, PRC and corticosterone levels, suggesting that intact catecholaminergic afferents to the PVN are critical for the stress-induced increase in renin and corticosterone secretion.

4. Effect of Microinjection of the β-Adrenoceptor Antagonist Sotalol into the PVN on Stress-Induced Renin and Corticosterone Secretion. The results from the experiment with 6-OHDA suggest that catecholaminergic nerve terminals in the PVN are involved in mediating the effect of stress on renin and corticosterone secretion. In previous studies, the β-adrenoceptor antagonist propranolol attenuated the stress-induced increase in PRA. Since propranolol is known to cross the blood-brain barrier, it is possible that β-receptors, located in the PVN, mediate the effect of stress on renin and corticosterone secretion. To test this hypothesis, the β-adrenoceptor antagonist sotalol was microinjected into the PVN. Sotalol prevented the stress-induced increase in corticosterone levels, but did not attenuate the stress-induced increase in PRA or PRC. The results suggest that a catecholaminergic input to cells in the PVN mediate
the effect of stress on renin and corticosterone secretion. Furthermore, stress-induced increases in corticosterone levels are mediated via β-receptors, whereas stress-induced increases in PRA and PRC are mediated by different receptors.
CHAPTER II

LITERATURE REVIEW

A. RENIN

1. Introduction

The renin-angiotensin system plays a role in the maintenance of sodium balance (Parfrey et al., 1981) and the regulation of blood pressure and volume (Fujii and Vatner, 1985). Renin is the enzyme responsible for the conversion of angiotensinogen to angiotensin I (AI), a decapeptide. AI is further converted to the octapeptide angiotensin II (AII) by a peptidyl dipeptidase, converting enzyme. This reaction is slow in vitro, but rapid in vivo due to the activity of tissue-bound converting enzyme present on the luminal aspect of vascular endothelial cells throughout the body (Caldwell et al., 1976). Renin is the rate-limiting enzyme in the synthesis of AII. AII is the physiologically active component of the system and a potent pressor agent. AII causes vasoconstriction by a direct action on vascular smooth muscle (Fujii and Vatner, 1985) and indirectly via the sympathetic nervous system (Peach et al., 1971).

2. Description of Renin

Renin is a glycoprotein with a molecular weight of about 40,000 (Galen et al., 1979). Specifically, it is an aspartyl proteinase (Dzau and Pratt, 1986) that cleaves a leucine-leucine peptide bond between
residues 10 and 11 of angiotensinogen. Renin has a circulation half-life of 4-15 minutes (Michelakis and Mizukoshia, 1971; Gordon and Sullivan, 1969; Gutman et al., 1973; Oates et al., 1974; Oparil et al., 1970; De Vito et al., 1977) and is metabolized by the liver and kidneys. Inactive forms of renin (prorenin and activation intermediates) have been found in the plasma of humans (Leckie, 1981), dogs (Wilczynski and Osmond, 1983) and rats (Osmond et al., 1982). Prorenin is a circulating, high molecular weight form of renin with no intrinsic enzymatic activity (Sealey et al., 1979; Inagami and Murakami, 1980). Prorenin release following bilateral nephrectomy was reported to be under β-receptor regulation (Wilczynski and Osmond, 1986). Nielsen and Poulsen (1988) reported that the kidney is the main source of inactive renin.

3. Physiological Aspects of the Angiotensins

a. Angiotensinogen (renin substrate)

The glycoprotein angiotensinogen (approximate molecular weight 60,000) is the substrate for renin. The main source of angiotensinogen is the liver. Immunocytochemical studies (Richoux et al., 1983) and Northern Blot analysis with complementary mRNA sequences for angiotensinogen (Ingelfinger et al., 1986) have shown that the liver contains and synthesizes angiotensinogen. Angiotensinogen is widely distributed in the blood and other extracellular fluids (Reid and Ramsay, 1975; Horky et al., 1971; Keeton and Campbell, 1980).

Plasma levels of angiotensinogen can be decreased by adrenalectomy (Carretero and Gross, 1967), hypophysectomy (Goodwin et al., 1970) and
diseases that impair liver function (Ayers, 1967). The administration of glucocorticoids, mineralocorticoids and sodium replacement can prevent the decrease in angiotensinogen levels produced by adrenalectomy (Nasjletti and Masson, 1969). Nephrectomy increases plasma levels of angiotensinogen (Hiwada et al., 1976; Carretero and Gross, 1967; Bing and Poulsen, 1969). This increase is thought to be mediated by adrenocortical steroids. Eggena and Barrett (1988) demonstrated an additive effect of nephrectomy and dexamethasone on the stimulation of renin substrate secretion. Freeman and Rostorfer (1972) observed that adrenalectomy caused a 70% reduction of the increase in plasma angiotensinogen caused by nephrectomy. Surgical stress, which stimulates the release of adrenocortical steroids, also increases plasma levels of angiotensinogen for several days in rabbits (Campbell et al., 1973; Romero et al., 1970; Morris et al., 1977). These studies suggest that an intact pituitary-adrenal axis is necessary for adequate substrate production.

b. Angiotensin I (AI)

The amino terminal decapeptide AI is cleaved from angiotensinogen by renin in the blood. Angiotensin I has been reported to act in a similar manner to AII with respect to the adrenal medulla (stimulating the release of catecholamines) and the central nervous system (inducing thirst), but with lower potency (Fitzsimons, 1971; Peach, 1974).

c. Angiotensin II (AII)

AII has many physiological effects, all of which are involved in the maintenance of blood pressure and plasma volume. AII is a potent
pressor agent, acting directly on the vascular smooth muscle to cause vasoconstriction (Skeggs et al., 1956) and indirectly, via the sympathetic nervous system (Peach et al., 1971; Scroop et al., 1971). Similarly, AII increases cardiac contractility in vitro by a direct action on the myocardium and indirectly via norepinephrine release from the cardioaccelerator nerves (Blumberg et al., 1975). AII acts on the peripheral arterioles to maintain arterial pressure in low cardiac output states (Davis and Freeman, 1976).

AII stimulates the secretion of aldosterone by a direct action on the zona glomerulosa of the adrenal cortex (Bartter et al., 1961; Biron et al., 1961). Blockade of AII receptors decreases aldosterone production to nondetectable levels (Davis and Freeman, 1976). AII also stimulates vasopressin secretion when administered intraventricularly in rats and dogs (Share, 1979; Keil et al., 1975) or intravenously in conscious dogs (Reid et al., 1982; Bonjour and Malvin, 1970), conscious rats (Kneple and Meyer, 1980) and humans (Uhlich et al., 1975; Padfield and Morton, 1977). Aldosterone and vasopressin act at the distal tubules of the kidney to increase sodium and water reabsorption, respectively. Together, they act to restore blood pressure and volume in response to the physiological stress of hypotension, hemorrhage and/or hypovolemia.

AII inhibits renin release when it binds to the juxtaglomerular (JG) cells (Dzau and Pratt, 1986), and increases the concentration of angiotensinogen in the plasma of dogs (Blair-West et al., 1974; Beaty et al., 1976) and rats (Nasjletti and Masson, 1973; Khayyall et al., 1973). AII also stimulates secretion of angiotensinogen in vitro from rat hepatocytes (Klett and Hackenthal, 1987). It is likely that the
stimulation of angiotensinogen by AII occurs via enhanced glucocorticoid secretion. AII stimulates glucocorticoid secretion by a direct action on the adrenal cortex (Kaplan, 1965) and indirectly by potentiating the effect of CRF on ACTH secretion (Keller-Wood et al., 1986; Maran and Yates, 1977; Spinedi and Negro-Vilar, 1983). Reid et al. (1978) suggested that AII stimulates angiotensinogen production to provide a positive feedback mechanism to prevent lack of substrate supply during periods of elevated renin secretion.

4. Regulation of Renin Secretion
   a. Introduction

Renin secretion from the kidney is regulated by a number of neural, chemical and mechanical factors. In general, factors that cause a decrease in either renal perfusion pressure, blood volume or plasma sodium concentration will stimulate renin secretion.

Renin is secreted by the granular juxtaglomerular cells located in the afferent arterioles of the kidney (Taugner et al., 1979; Taugner et al., 1984). The juxtaglomerular cells are in close proximity to the macula densa, a specialized portion of the distal tubule that is sensitive to changes in sodium reabsorption across the tubular epithelium. The macula densa monitors the ionic environment in the tubular fluid and relays the message to either increase or decrease renin secretion. Studies by Itoh et al. (1985) suggest that adenosine, via the activation of A1 receptors, may be a signal from the macula densa to the juxtaglomerular cells to inhibit renin release. It is reported that a decrease in the amount of sodium (Churchill et al., 1978), potassium (Shade et al.,
or chloride (Rostand et al., 1985) that crosses the macula densa cells will stimulate renin secretion.

Renin secretion is also stimulated by a decrease in renal arterial pressure. The idea that a "pressor substance" (later to be known as renin) was secreted in response to a decrease in renal blood flow was first postulated by Goldblatt et al. (1934). This idea was further developed by Tobian et al. (1959) who suggested the existence of a renal baroreceptor that was responsible for the inverse relationship between blood pressure and renin secretion.

Studies by Fahri et al. (1982) have shown that stimulation of renin secretion following a decrease in renal arterial pressure occurs via an intrarenal baroreceptor interacting with a renal \( \beta \)-receptor. Their studies have demonstrated that elevating plasma epinephrine concentration without changing blood pressure does not increase plasma renin activity. However, if epinephrine secretion accompanies a stimulus (e.g., hemorrhage) which also lowers blood pressure, then renin secretion will be increased. This system allows for plasma renin activity to be either stimulated or unaffected by high physiological levels of circulating epinephrine, depending on whether or not a hypotensive threat is involved.

b. Role of the Sympathetic Nervous System in Renin Secretion

The sympathetic innervation of the rat kidney consists of sympathetic cell bodies in paravertebral (thoracic T6 through lumbar L4) and prevertebral (renal, greater splanchnic, and celiac) ganglia (Sripairojthikoon and Wyss, 1987). Stimulation of the renal nerves
increases renin secretion (Di Bona, 1985; Blair et al., 1985). This stimulatory effect is mediated, at least in part, via norepinephrine released from the endings of the postganglionic sympathetic neurons that innervate the kidneys (Johnson et al., 1971; Taher et al., 1976). Circulating epinephrine can also stimulate renin secretion. The effect of stimulation of the renal nerves can be enhanced by activating beta (β) receptors (Vander, 1965). Catecholamines are believed to stimulate renin secretion via a β-adrenergic mechanism since β-antagonists have been shown to inhibit their effect both in vivo (Loeffler et al., 1972; Pettinger and Keeton, 1975) and in vitro (Weinberger et al., 1975; Vandongen et al., 1973). The renin stimulating potency profile is isoproterenol > epinephrine > norepinephrine according to studies in conscious dogs by Johnson et al. (1979). Studies by Healy et al. (1985) and Osborn et al. (1981) suggest that the sympathetic nervous system stimulates renin release by activating β₁ receptors on the juxtaglomerular cells.

c. Role of Extra-Renal Beta Receptors in Renin Secretion

Although there is evidence supporting intrarenal β receptor regulation of renin secretion, this does not exclude a role of extrarenal β-adrenergic receptors that provide a powerful stimulatory effect on renin secretion. For example, Reid et al. (1972) observed increases in plasma renin activity and renin secretion following isoproterenol infusion into the femoral artery of dogs. Renal perfusion pressure was kept constant with an aortic clamp. However, infusion of the same doses of isoproterenol into the renal artery had no effect on plasma renin activity or renin secretion, suggesting that an extrarenal β receptor mechanism is involved
in regulating renin secretion. Johnson et al. (1979) obtained similar results with infusion of epinephrine and norepinephrine into the inferior vena cava (IVC) and renal artery. Epinephrine infusion into the IVC resulted in a 3.5-fold increase in plasma renin activity while IVC infusion of norepinephrine resulted in a 1.5-fold increase in plasma renin activity. Infusion of epinephrine or norepinephrine directly into the renal artery to achieve similar arterial concentrations resulted in no increases in plasma renin activity. Fahri et al. (1982) suggested that the effect of epinephrine in the intact conscious dog can be explained as the combination of an extrarenal and intrarenal action. The extrarenal event is a generalized vasodilation that results in a drop in blood pressure which is sensed by the renal baroreceptor. The intrarenal action is mediated through intrarenal $\beta$ receptors which affect the stimulus-response curve of the renal baroreceptor. At the present time the location of the extrarenal $\beta$ receptor has not been established.

d. Role of Brain Serotonin (5-HT) Neurons in Renin Secretion

In 1980, Zimmermann and Ganong demonstrated that injection of the 5-HT precursors 5-hydroxytryptophan (5-HTP) or tryptophan to anesthetized dogs increased plasma renin activity. In humans, the 5-HT precursor L-tryptophan was reported to increase renin secretion (Modlinger et al., 1979), and the 5-HT antagonist cyproheptadine inhibited the secretion of renin induced by furosemide (Epstein and Hamilton, 1977). In rats, the 5-HT releasers p-chloroamphetamine (PCA) and fenfluramine dose-dependently increased plasma renin activity via release of 5-HT from serotonergic nerve terminals (Van de Kar et al., 1981; 1985). The 5-HT$_2$ antagonist
LY53857 dose-dependently inhibited the increase in plasma renin activity and plasma renin concentration following administration of fenfluramine and MK-212, a 5-HT agonist, suggesting that stimulation of 5-HT$_2$ receptors enhances renin release (Lorens and Van de Kar, 1987).

The effect of PCA on plasma renin activity was prevented by either pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) or by a chemical lesion of the dorsal raphe nucleus with the 5-HT neurotoxin, 5,7-dihydroxytryptamine (Van de Kar et al., 1982). A mechanical lesion in the mediobasal hypothalamus, as well as posterolateral deafferentation of the hypothalamus (separating the hypothalamus from the midbrain) also blocked the effect of PCA on plasma renin activity (Karteszi et al., 1982). Anterolateral section through the retrochiasmatic area was ineffective in blocking the effect of PCA on plasma renin activity (Karteszi et al., 1982). These results suggest that a serotonergic pathway, with cells in the dorsal raphe nucleus in the midbrain and terminals in the mediobasal hypothalamus, stimulates renin secretion.

1. Role of Beta Receptors in the Serotonergic Stimulation of Renin Secretion

There is evidence for $\beta$ receptor involvement in mediating the serotonergic stimulation of renin secretion. Van de Kar and Richardson Morton (1986) demonstrated that the $\beta_1$ and $\beta_2$ antagonist sotalol and the $\beta_1$ antagonist atenolol prevented the increase in plasma renin activity after the injection of PCA and fenfluramine. These findings supported those of Alper and Ganong (1984) who demonstrated that pretreatment with the sotalol and propranolol prevented the increase in plasma renin
activity produced by PCA. These studies suggested a possible role for the sympathetic nervous system in mediating the increase in renin secretion following the administration of 5-HT releasers.

However, in rats which were adrenal medullectomized and sympathectomized by chronic injections of 6-OHDA, there was no inhibition of the PCA- and fenfluramine-induced increase in plasma renin activity. Renal norepinephrine content was reduced to undetectable levels, suggesting that it is not likely that the serotonergic stimulation of renin secretion is mediated via the sympathetic nervous system or adrenal catecholamines (Van de Kar and Richardson Morton, 1986). These results seem to be in contradiction with the fact that the \( \beta \)-adrenergic receptor antagonists sotalol and atenolol prevented the PCA- or fenfluramine-induced increase in plasma renin activity. However studies by Johnson (1984) and Johnson et al. (1979), as described earlier, provide evidence that \( \beta \) receptors that mediate the increase in plasma renin activity are located outside the kidney, suggesting an entirely different mechanism of stimulating renin secretion. In addition, many beta receptor antagonists are known to bind to 5-HT receptors (Middlemiss et al., 1977) and it is possible that sotalol and atenolol are present in sufficient concentrations in the brain to block the 5-HT receptors and inhibit the serotonin-mediated increase in plasma renin activity (Lemmer et al., 1985; Garvey and Ram, 1975). To summarize, the data suggest that brain 5-HT neurons regulate renin secretion via a mechanism that is not related to the autonomic sympathetic nervous system or adrenal catecholamines.
e. Role of the Hypothalamic Paraventricular Nucleus in Renin Secretion

The PVN plays a role in neurogenic and genetic models of hypertension (Ciriello et al., 1984; Zhang and Ciriello, 1985). The PVN is a logical central site to influence renin secretion as it receives afferent information from the baroreceptor region as well as having efferent projections to the sympathetic areas of the spinal cord (Sawchenko and Swanson, 1982).

Electrolytic lesions in the PVN have been shown to prevent the increase in plasma renin activity produced by the 5-HT releasing drug PCA as well as preventing the increase in plasma renin activity that follows immobilization stress (Gotoh et al., 1987). Previous studies (Gotoh et al., 1985) demonstrated that bilateral knife cuts behind the hypothalamic paraventricular nucleus (PVN) reduced the renin response to immobilization and the gravitational stress of head-up tilting, suggesting a role for the PVN in both stress-induced and 5-HT-stimulated renin secretion.

Electrical stimulation of the PVN in conscious rats increased plasma renin activity in a frequency-related manner. There were no significant changes reported in heart rate, mean arterial pressure or renal vascular resistance at any of the frequencies that produced an increase in plasma renin activity (Porter, 1986). These results suggest that neurons in the PVN stimulate renin secretion.

Additional studies by Porter (1988) demonstrated the relationship between renal perfusion pressure and plasma renin activity during continuous low-level stimulation of the PVN. He found that for any given
decrease in renal perfusion pressure, the plasma renin activity increase was greater during the ongoing PVN stimulation. The effect of stimulation of the PVN was prevented by both renal denervation and pretreatment with propranolol. These studies suggest that stimulation of the PVN elicits increased responsiveness of the kidneys to decreases in renal perfusion pressure, and that this effect is mediated via the sympathetic nervous system.

f. Other Hypothalamic Areas Involved in Renin Secretion

The lateral and posterior hypothalamic areas have long been known as sites where pressor responses can be elicited with electrical stimulation (Brody et al., 1986; Ciriello and Calaresu, 1977). Therefore it is not surprising that increases in plasma renin activity are also observed following electrical stimulation of the lateral (Zanchetti and Stella, 1975) and posterior (Natcheff et al., 1977) hypothalamus. Unlike the studies by Porter (1986) involving the PVN, these increases in plasma renin activity were accompanied by increases in blood pressure and required intact renal nerves. It is likely that the lateral and posterior hypothalamus exert their cardiovascular effects through long descending neuronal projections to the NTS (Kuypers et al., 1973) or ventrolateral medulla (Brody et al., 1986).

Gotoh et al. (1987) reported that bilateral electrolytic lesions of the ventromedial nucleus prevented the increase in plasma renin activity produced by each of the following stimuli: the 5-HT-releaser PCA, immobilization, head-up tilt under inactin anesthesia, and a low-sodium diet. The authors hypothesize that the stimuli that increase plasma renin
activity via the ventromedial nucleus act by increasing sympathetic activity since the $\beta$-adrenergic receptor antagonist propranolol blocks the renin response to PCA, immobilization and head-up tilt. The PCA response is also abolished by another $\beta$-adrenergic receptor antagonist, sotalol, and by ganglionic blockade (Alper and Ganong, 1984). In addition, stimulation of the ventromedial nucleus increases blood pressure (Bunag and Inoue, 1985), while bilateral lesions of this nucleus prevent the development of experimental hypertension (Brody and Johnson, 1980).

g. Extrahypothalamic Pathways Involved in Renin Secretion

Other central nervous system sites play a role in renin release. Electrical stimulation of the ventrolateral medulla (Richardson et al., 1974), dorsal medulla (Passo et al., 1971) and mesencephalic dorsal periaqueductal grey (Ueda et al., 1967) produced increases in plasma renin activity that were mediated via the renal nerves.

h. Renin and Angiotensin in the Brain

Administration of renin or AII into the cerebral ventricles stimulates vasopressin secretion (Keil et al., 1975), increases drinking behavior and increases blood pressure (Reid and Ramsay, 1975). The anterior forebrain and nuclei of the anteroventral third cerebral ventricle (AV3V) play a role in the AII-induced stimulation of drinking behavior, vasopressin release and increased arterial pressure. These brain areas are richly innervated by catecholaminergic fibers arising from cell bodies in the $A_2$ area of the nucleus tractus solitarius, and other brainstem regions involved with central cardiovascular regulation (Saper
et al., 1983; Palkovits et al., 1974; Meldrum et al., 1984). Bellin et al. (1987) have shown that rats with 6-OHDA injections into the lateral cerebral ventricle had significantly attenuated drinking and blood pressure responses following both central and systemic AII administration. Rats pretreated with desmethylimipramine (DMI) prior to injection of 6-OHDA (to protect noradrenergic terminals) had no thirst or pressor response deficits following AII administration. These results suggest a role for norepinephrine in mediating AII-induced drinking and blood pressure responses.

The distributions of angiotensin I and II, angiotensinogen, angiotensin-converting enzyme (ACE) and renin in the brain have been studied by various biochemical and immunocytochemical techniques (Brownfield et al., 1982; Changaris et al., 1977; Fuxe et al., 1976; Fuxe et al., 1980; Basso et al., 1982; Fischer-Ferraro et al., 1971), although the physiological role for these substances is not clear. Ganong et al. (1979) suggested that AII-like immunoreactive material in the brain may be involved in modulating the release and action of brain catecholamines.

The presence of a brain renin-angiotensin system is highly debatable. Fuxe et al. (1980) demonstrated renin-like immunoreactivity in the paraventricular, periventricular and supraoptic nuclei in the rat and mouse. Dabsys et al. (1988) demonstrated an increase in angiotensin-converting enzyme activity levels in the amygdaloid complex following bilateral destruction of the ventral noradrenergic pathway with 6-OHDA. There was a concomitant increase in arterial blood pressure that significantly correlated with the ACE activity levels in the amygdala. Studies in nephrectomized animals revealed AII-like immunoreactivity in
brain tissue (Phillips and Stenstrom, 1983) and significant elevations in angiotensinogen content in the hypothalamus and midbrain (Gregory et al., 1982). However, the AII-like material from rat brain has been shown to be different from authentic AII by both gel filtration (Meyer et al., 1982) and anion exchange HPLC techniques (Pohl et al., 1988). These studies indicated that the AII-like material has a molecular weight of 5000 - 7000 (compared to 1046 for AII) and is more polar but less positively charged than AII.

Although indirect studies suggest the presence of each of the components of the renin-angiotensin system in the brain (Printz et al., 1982) there is no evidence to support a functional brain renin-angiotensin system. Brownfield et al. (1982) have demonstrated that although angiotensin and converting enzyme immunoreactivities are present in the brain, they are not co-distributed, suggesting that there is no brain pathway for the formation of angiotensin.

1. Role of Brain Catecholamines in Renin Secretion

Norepinephrine in the brain was proposed to inhibit secretion of renin from the kidney through changes in sympathetic activity and secretion of vasopressin (Blair et al., 1977; Reid et al., 1978). The antihypertensive drug clonidine is an alpha (α)2-agonist with a biphasic effect on blood pressure: a transient increase followed by a prolonged decrease. Intracerebroventricular injection of clonidine lowers plasma renin activity at a dose which is ineffective when given intravenously (Reid et al., 1975). Blair et al. (1977) have demonstrated that administration of L-DOPA to dogs pretreated with the peripheral decar-
boxylase inhibitor carbidopa resulted in a drop in blood pressure and an inhibition of renin secretion. Since this treatment also increases brain catecholamine content, this study suggests that catecholamines formed from L-DOPA can act within the central nervous system to cause a decrease in renin secretion. In this study the decrease in plasma renin activity was dependent upon the presence of the renal nerves.

The 5-HT releaser fenfluramine has a biphasic effect on plasma renin activity: an initial increase followed by a sustained decrease. Studies by Van de Kar et al. (1985) suggested that the initial increase in plasma renin activity following fenfluramine administration is mediated via release of serotonin, while the delayed, long-lasting suppression of plasma renin activity is mediated via a catecholaminergic mechanism. However, the long-term suppression of plasma renin activity following fenfluramine administration is not dependent on the peripheral sympathetic nervous system, as it was not prevented by adrenal enucleation combined with 6-OHDA-induced sympathectomy (Van de Kar et al., 1988). To determine whether $\alpha_2$-adrenoceptors mediate the suppressive effects of fenfluramine, the $\alpha_2$-antagonists yohimbine and rauwolscine were injected prior to fenfluramine administration. Neither $\alpha_2$-antagonist prevented the long-term suppressive effect of fenfluramine on plasma renin activity or plasma renin concentration. The data suggest that fenfluramine does not inhibit renin secretion by activating $\alpha_2$-adrenoceptors in the CNS. This is in contrast to clonidine and $\alpha$-methyl DOPA, which inhibit sympathetic outflow to the kidneys through activation of central $\alpha_2$-adrenoceptors.

Privitera et al. (1979) have shown that low doses of intracisternally-injected propranolol produced dose-dependent decreases in plasma
renin activity and mean arterial pressure (MAP), whereas identical doses given intravenously had no significant effect on plasma renin activity or MAP. A concomitant reduction in circulating norepinephrine levels was also seen. This study suggests that propranolol could be acting at a central $\beta$-adrenergic site to suppress renin release.

It is clear that central catecholamines play a role in mediating the suppression of plasma renin activity, but the site(s) of action and type of receptors involved have yet to be identified.

B. CORTICOSTERONE

1. Introduction

In 1856 Brown-Sequard discovered that the adrenal glands are essential to life. By the 1930's it became clear that the adrenal cortex is the source of two vital classes of hormones: one, the glucocorticoids, regulate carbohydrate and protein metabolism, and the ability to tolerate prolonged stress (Selye, 1946; Selye, 1943; Cori and Cori, 1927; Britton and Silvette, 1931; Long et al., 1940). The second class is the mineralocorticoids, which control water and electrolyte metabolism and the ability of the kidney to reabsorb sodium (Loeb et al., 1933; Harrop et al., 1933). Aldosterone is the primary salt-retaining hormone of the adrenal cortex and is synthesized in the glomerulosa layer of cortical cells. Cortisol (in humans) and corticosterone (in rats) are the main glucocorticoids secreted by the adrenal gland and are synthesized in the fasciculata layer. The fasciculata layer of cells in the adrenal cortex is stimulated by adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary gland (Li et al., 1943; Sayers et al., 1943). Ingle et
al., (1938) found that ACTH secretion from the anterior pituitary is inhibited by secretions of the adrenal cortex. The role of the hypothalamus in integrating stimulatory and inhibitory inputs to the adenohypophysis was studied by Harris (1948) and Rasmussen (1938). Characterization of the hypothalamic factor that stimulates corticotropin secretion (CRF) was completed by Vale et al. (1981).

2. Corticotropin-Releasing Factor (CRF)

CRF from ovine hypothalamic tissue is a 41-amino acid peptide that releases ACTH and β-endorphin from anterior pituitary cells (Vale et al., 1981; Plotsky, 1985; Rivier et al., 1983; Rivier and Vale, 1983). This peptide is 5-10 times more potent in releasing ACTH and β-endorphin than vasopressin, which was originally thought to act as a corticotropin releasing factor (Saffran and Schally, 1977). Swanson et al. (1983) described the localization of CRF immunoreactive cells and fibers in three distinct systems in the rat brain. The first group is in the hypothalamic paraventricular nucleus (PVN). Approximately 2,000 CRF-stained cells are found in each of the eight subdivisions of the PVN, although most (80%) of the cells are concentrated in the parvocellular division (especially the periventricular and medial parts), and about 15% are found in the magnocellular division where oxytocinergic cells predominate. These CRF-containing neurons are the only ones that demonstrated increased staining intensity following adrenalectomy. The second group of CRF-containing neurons are involved in mediating autonomic responses. They include the central nucleus of the amygdala, bed nucleus of the stria terminalis, substantia innominata, lateral hypothalamic area, medial and lateral
preoptic areas, central periaqueductal grey, laterodorsal tegmental nucleus, locus coeruleus, dorsal vagal complex, parabrachial nucleus and regions containing the A1 and A5 catecholamine cell groups. The third group of CRF-immunoreactive cells are found in the cerebral cortex, especially layers II and III of the neocortex, and limbic regions such as the cingulate gyrus, prefrontal areas and areas bordering the rhinal fissure (Swanson et al., 1983). CRF-like immunoreactive neurons were also observed in the cerebellum and spinal cord (Olschowka et al., 1982). Outside the central nervous system, CRF-like immunoreactivity has been found in endocrine cells of the pancreas and gastrointestinal system, also in the liver, pituitary, adrenal, lung and placenta (Petrusz et al., 1985).

The parvocellular neurons of the PVN contain CRF (Merchenthaler et al., 1982; Liposits et al., 1983), which is transported along axons to the median eminence where it is released into the hypophyseal portal vessels. Makara et al. (1981) have shown that the CRF fibers in the stalk-median eminence region either originate from or run through the PVN. In the pituitary gland, CRF is then bound to specific membrane receptors of ACTH-containing cells (Wynn et al., 1984; Leroux and Pelletier, 1984). ACTH release is stimulated which, in turn, increases steroid hormone production in the adrenal cortex. The glucocorticoids then exert negative feedback effects on both the hypothalamus (Yates and Urquhart, 1962) and anterior pituitary (Russell et al., 1969; Mulder and Smelik, 1977) via glucocorticoid receptors (Gustafsson et al., 1983). ACTH immunoreactive fibers are found in most parts of the parvocellular division of the PVN and in the oxytocinergic cells of the magnocellular division (Sawchenko et al.,
Afferent connections to CRF neurons include presynaptic boutons containing immunoreactive ACTH 1-39 (Liposits et al., 1985) and CRF (Liposits et al., 1985), suggesting an autoregulatory process in the control of CRF release. Parvocellular CRF neurons also receive input from magnocellular neurons in the same nucleus (Leranth et al., 1983) and send processes to these magnocellular elements (Liposits et al., 1985). CRF neurons have been shown to have angiotensin II reactivity in adrenalectomized, colchicine-treated rats (Lind et al., 1985).

Rivier and Vale (1983) suggested that vasopressin may act synergistically with CRF under certain physiological conditions to promote ACTH secretion. In addition, in vitro studies have shown that vasopressin acts directly on the anterior pituitary corticotropes to stimulate ACTH secretion (Vale et al., 1983). Together, these results suggest that vasopressin may act synergistically with CRF under certain physiological conditions to promote ACTH secretion.

Sawchenko (1987) demonstrated that adrenalectomy (ADX) enhanced both CRF and vasopressin immunoreactivity suggesting that they may be colocalized in parvocellular neurosecretory neurons of the PVN. Other studies support this observation (Tramu et al., 1983; Kiss et al., 1984; Sawchenko et al., 1984; Piekut and Joseph, 1985). Dual immunocytochemical studies have shown the co-localization of CRF and vasopressin in the parvocellular neuronal perikarya in the PVN. In general, CRF immunostained cells are concentrated in the medial parvocellular part of the PVN, while the vasopressin neurons are predominantly in the magnocellular part of the PVN. However, in adrenalectomized, colchicine-treated rats, a
dense accumulation of vasopressin-immunoreactive cells were seen in the medial parvocellular part of the PVN, in a similar distribution to that seen for CRF-containing cell bodies (Piekut and Joseph, 1986). Extra-hypothalamic sites of ADX-enhanced CRF immunoreactivity included the cerebral cortex, amygdala and the bed nucleus of the stria terminalis. These extra-hypothalamic sites contained no vasopressin immunoreactivity colocalized with the CRF (Sawchenko, 1987). Hypophysectomy as well as ADX has been shown to enhance CRF immunostaining in PVN cell bodies (Bugnon et al., 1983; Merchenthaler et al., 1983; Paull and Gibbs, 1983; Swanson et al., 1983). Sawchenko (1987) reported that hypophysectomy produced results comparable to ADX-enhanced CRF and vasopressin staining in the PVN. Low doses of dexamethasone attenuated and high doses prevented the ADX-induced enhancement of CRF and vasopressin immunoreactivity, with the adrenal steroid potency profile as dexamethasone > corticosterone > deoxycorticosterone > aldosterone. These results suggest that adrenal steroids, particularly glucocorticoids, play a primary role as regulators of peptide expression.

3. Other Factors Affecting ACTH Release

Other neuropeptides that stimulate the release of ACTH include angiotensin II (AII) (Ramsay et al., 1978; Steele et al., 1981) and oxytocin (Beny and Baertschi, 1980; Pearlmutter et al., 1974). In vitro dose-response studies by Spinedi and Negro-Vilar (1983) ranked the corticotropin-releasing activity as follows: CRF > vasopressin > oxytocin > AII. The stimulatory effects of AII may involve the stimulation of AII receptors in the anterior pituitary (Hauger et al., 1982; Mukherjee et
al., 1982) or the hypothalamic PVN (Saavedra et al., 1986). The density of the AII binding sites in the PVN have been shown to increase following repeated immobilization stress (Castren and Saavedra, 1988). It is possible that AII receptors in the PVN play a role in modulating stress-induced corticosterone secretion.

4. Role of Brain Catecholamines in Corticosterone Secretion

a. Introduction

In general, the ascending noradrenergic pathways appear to relay afferent visceral information to the forebrain (Cunningham and Sawchenko, 1988). There has been considerable controversy as to whether catecholamines play an excitatory or inhibitory role in corticosterone secretion. Norepinephrine and/or epinephrine have been implicated as stimulators of the secretion of virtually every anterior pituitary hormone (Sawchenko and Swanson, 1982). With respect to ACTH secretion, brain catecholamines were originally postulated to have an inhibitory role (van Loon et al., 1971; Weiner and Ganong, 1978; Cuello et al., 1973; Scapagnini et al., 1970). However, in recent years more accurate and reliable anatomical and pharmacological studies suggest a stimulatory role for catecholamines in corticosterone secretion (Szafarczyk et al., 1985; Feldman et al., 1988; Alonso et al., 1986; Guillaume et al., 1987).

b. Anatomical Evidence

The predominantly noradrenergic (Sawchenko and Swanson, 1981) nucleus of the solitary tract (NTS) sends fiber projections to the parvocellular PVN, substantia innominata, central nucleus of the amygdala
and bed nucleus of the stria terminalis (Ricardo and Koh, 1978), all of which contain CRF-stained cells (Swanson et al., 1983). Swanson et al. (1983) suggested that the nucleus of the solitary tract, which receives direct vagal and glossopharyngeal afferents (Beckstead and Norgren, 1979) could serve as a major relay point of visceral sensory information to CRF cell bodies in the basal forebrain. Likewise the parabrachial nucleus, which receives a large input from the NTS (Norgren, 1978), and the locus coeruleus also project to many of the sites that contain CRF-immunoreactive cell bodies, including the parvocellular PVN, the bed nucleus of the stria terminalis and the central nucleus of the amygdala. These nuclei may also be involved in the relay of visceral sensory information to CRF cell bodies (Swanson et al., 1983).

Another fiber projection is described from the predominantly noradrenergic cells in the Al region of the ventrolateral medulla (which receives an input from the NTS) to the magnocellular (vasopressinergic) and parvocellular PVN. Reciprocal connections have also been described from the parvocellular PVN to the dorsal vagal complex and spinal cord (Hosoya and Matsushita, 1979; Swanson and Kuypers, 1980). Electrophysiological studies by Kannan and Yamashita (1985) have also demonstrated reciprocal connections between neurons in the NTS region and the PVN. Studies by Skofitsch et al. (1985) have demonstrated binding sites for CRF in sensory areas of the rat hindbrain and spinal cord, specifically in the posterior part of the NTS, the substantia gelatinosa nervi trigemini and laminae I and II of the spinal cord. Since these areas are rich in nerve endings of primary sensory neurons, this observation suggests a possible role for CRF in mediating peripheral sensory processes.
Cunningham and Sawchenko (1988) used an immunofluorescence double-labeling procedure to determine which anterogradely labeled fibers and terminals in the PVN also displayed dopamine $\beta$-hydroxylase immunoreactivity, indicating the presence of catecholaminergic neurons. Specifically, projections from the A1 (caudal ventrolateral medulla) region were found to synapse on magnocellular vasopressinergic neurons in the PVN, while projections from the A2 (medial part of the NTS) region were located primarily throughout the parvicellular division of the PVN. The projections were the most dense in the dorsal medial parvicellular region, which is known to contain a large population of CRF-immunoreactive neurons. A less-dense projection was found in the magnocellular division of the PVN. The A6 (locus coeruleus) projections were found almost exclusively in the parvicellular division of the PVN, specifically in the periventricular zone, which contains dopamine, somatostatin and thyrotropin-releasing hormone-containing neurons. These findings provide anatomical evidence that the noradrenergic cells in the medial NTS innervate CRF-immunoreactive cells in the PVN.

Liposits et al. (1986) showed that PNMT-immunoreactive axon terminals have synaptic connections with dendrites, somata and spinous structures of CRF-immunoreactive neurons. The parvocellular subnuclei demonstrated a more intense adrenergic innervation than the magnocellular subnuclei. The mammalian anterior pituitary does not receive catecholaminergic innervation, unlike the neural and intermediate lobes which receive noradrenergic and dopaminergic innervation (Bjorklund et al., 1967; Saavedra et al., 1975).
c. Role of the Ventral Noradrenergic Bundle in Corticosterone Secretion

The ventral noradrenergic bundle (VNAB) originates from the medullary A1 and A2 cell groups, and the pontine locus coeruleus. It extensively innervates the hypothalamus, conveying most of the catecholaminergic innervation to the PVN (Moore and Bloom, 1979; Palkovits, 1981; Swanson and Sawchenko, 1980). The VNAB is thought to be the source for the noradrenergic modulation of ACTH secretion (Szafarczyk et al., 1985; Feldman et al., 1988). Also, afferents from the epinephrine-containing cell groups (C1-C3) reach the hypothalamus via the VNAB (Ungerstedt, 1971).

Injections of 6-OHDA into the VNAB significantly reduced CRF levels in hypophysial portal blood (HPB) as well as producing significant depletion of hypothalamic norepinephrine and epinephrine (Guillaume et al., 1987). Similar results were obtained by Eckland et al. (1988), who found that 6-OHDA injections into the VNAB or the lateral ventricles caused a reduction of HPB CRF concentration. However, HPB norepinephrine was reduced only with the i.c.v. 6-OHDA injections and was unchanged in the VNAB-lesioned group.

Further support for a stimulatory role of norepinephrine in CRF secretion is provided by Plotsky (1987). The increase in hypophysial-portal immunoreactive CRF (irCRF) following electrical stimulation of the VNAB was prevented by i.c.v. pretreatment with the α₁-adrenoceptor antagonist corynanthine, but not by the β-adrenergic antagonist propranolol. Corynanthine also blocked the dose-dependent increases in irCRF
following icv administration of norepinephrine (0.1-5.0 nmol). In contrast, 5 nmol or greater doses of norepinephrine resulted in a dose-dependent inhibition of irCRF release that could be blocked by propranolol, but was unaffected by corynanthine. This suggests that low doses of norepinephrine stimulate CRF release via an α-receptor mechanism, whereas higher doses of norepinephrine inhibit CRF release via a β-receptor.

d. Stimulation of CRF/Corticosterone Secretion by Epinephrine and Norepinephrine

Microinjections of norepinephrine (40 nmoles) into the PVN significantly increased serum corticosterone levels. Epinephrine (40 nmoles) microinjections into the PVN were even more efficacious than norepinephrine in increasing serum corticosterone levels, whereas dopamine (40 nmoles) had no effect (Leibowitz et al., 1986).

Intravenous infusion of either l-epinephrine or the β-adrenoceptor agonist l-isoproterenol into pentobarbital anesthetized rats resulted in a dose-dependent increase in plasma corticosterone levels. The effect of epinephrine and isoproterenol on plasma corticosterone levels was associated with a parallel and dose-related increase in plasma ACTH (Berkenbosch et al., 1983).

Tilders et al. (1985) found that intravenous infusion of epinephrine increased plasma ACTH and corticosterone to levels that are comparable to those induced by stress. The stimulation of ACTH secretion by epinephrine appears to be mediated via CRF and β-adrenergic receptors since it was prevented by i.p. injections of propranolol or administration
In vitro studies with incubated rat hypothalami demonstrated that norepinephrine produced a dose-dependent stimulation of CRF release which was prevented by the $\beta$-adrenoceptor antagonists propranolol and timolol. The norepinephrine-induced CRF release was not affected by the $\alpha_1$-adrenoceptor antagonists thymoxamine, prazosin or corynanthine or the $\alpha_2$-adrenoceptor antagonist idazoxan (Tsagarakis et al., 1988). This study suggests a stimulatory role for norepinephrine on CRF release that is mediated by $\beta$ receptors.

e. Role of Dopamine Neurons in Corticosterone Secretion

The PVN is uniformly innervated with dopaminergic neurons. The dopaminergic innervation of the PVN originates from cells of the arcuate nucleus (Al2), zona incerta (Al3) and periventricular nucleus (Al4) (Hokfelt et al., 1984). Immunocytochemical studies indicated that both dopamine and norepinephrine terminals synaptically contact magnocellular neurons on their dendrites or cell bodies (Decavel et al., 1987). In addition, tyrosine hydroxylase immunoreactivity has been reported in both fibers and neurons of the PVN (Swanson et al., 1981; Liposits et al., 1986).

Dopamine has been shown to stimulate corticosterone secretion. Injection (i.p.) of the dopamine agonist pergolide caused a dose-dependent increase in plasma corticosterone levels, which was blocked by pretreatment with the dopamine antagonists spiperone and haloperidol. Spiperone also partially inhibited the increase in plasma corticosterone levels following administration of quipazine, a serotonin agonist, although much higher
doses were required than for pergolide. In addition, the peripheral
dopamine antagonist domperidone did not prevent the increase in plasma
corticosterone levels by pergolide (Fuller and Snoddy, 1981). These
results support a role for central dopamine receptors in corticosterone
secretion. Similar results were obtained by Jezova et al (1985), who
found that subcutaneous injection of the dopamine agonist apomorphine
increased plasma ACTH and corticosterone levels. Haloperidol pretreatment
completely inhibited the apomorphine-induced stimulation in plasma ACTH.

5. Role of 5-HT Neurons in Corticosterone Secretion

Many studies support the hypothesis that stimulation of serotonergic
receptors leads to increased corticosterone secretion in unstressed
animals (Buckingham and Hodges, 1979; Fuller and Snoddy, 1980; Fuller,
reported that administration of the 5-HT uptake inhibitor fluoxetine to
anesthetized rats caused increased release of CRF-41 into hypophyseal
portal blood. Lorens and Van de Kar (1987) demonstrated that plasma
corticosterone levels were increased by the 5-HT agonist MK-212, the 5-
HT_{1A} agonists ipsapirone and 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-
DPAT), and the serotonin releaser fenfluramine. The 5-HT_{2} antagonist
LY53857 did not prevent the effect of MK-212 and fenfluramine on
corticosterone secretion. These data suggest a possible role for 5-HT_{1A}
receptors in the stimulation of corticosterone secretion. This conclusion
was confirmed by Koenig et al. (1987) and Gilbert et al. (1988).

The dorsal and median raphe nuclei in the midbrain are known to be
the major sources of serotonergic pathways to the hypothalamus (Azmitia
and Segal, 1978; Parent et al., 1981; Van de Kar et al., 1980). In particular, the median raphe nuclei are the primary source of 5-HT fibers to the suprachiasmatic nucleus, anterior hypothalamic area and medial preoptic area, while the anterolateral hypothalamic area and arcuate nucleus receive 5-HT inputs from both the dorsal and median raphe nuclei (Van de Kar and Lorens, 1979).

With respect to the PVN, a relatively light serotonergic innervation has been described (Aghajanian et al., 1973; Steinbusch, 1981). Immunocytochemical studies by Sawchenko et al. (1983) found that the major source of ascending serotonergic projections to the PVN were from the dorsal and median raphe nuclei and the medial lemniscus. A significant decrease in PVN serotonin was reported following surgical destruction of the dorsal raphe nucleus (Palkovits et al., 1977).

Immunocytochemical studies by Liposits (1987) demonstrated a moderate serotonergic innervation to the PVN, with a prominent distribution to the parvocellular subnuclei. Serotonergic axons were found to overlap with CRF-immunoreactive axons. Further ultrastructural examination revealed that serotonin-containing terminals formed axo-dendritic and axo-somatic synapses with CRF-immunoreactive neurons. This study suggests that serotonin can influence CRF-containing neurons via synaptic transmission.

Midline injections of 5,7-dihydroxytryptamine (5,7-DHT) into the raphe nuclei produced significant depletions of 5-HT in the hypothalamus, and prevented the increase in corticosterone following stimulation of cortical and limbic areas (dorsal hippocampus, basolateral amygdala, mesencephalic reticular formation and medial septal nucleus). The ether-
induced increase in corticosterone levels was not affected by this lesion. Injection of 5,7-DHT into the PVN also prevented the adrenocortical response to dorsal hippocampal and photic stimulation. These results suggest that 5-HT in the PVN mediates the stimulation of corticosterone secretion following either peripheral or central stimuli (Feldman et al., 1987).

In contrast, Van de Kar et al. (1982) demonstrated that the PCA-induced stimulation of corticosterone secretion was not mediated by either the dorsal or median raphe nucleus. In addition, posterolateral cuts which interrupted caudal inputs to the hypothalamus did not block the serotonergic stimulation of corticosterone secretion by the serotonin-releasing drug PCA (Van de Kar et al., 1985).

Serotonin has been shown to play a role in mediating the diurnal variation in basal plasma corticosterone in rats. Plasma corticosterone levels peak at the onset of the dark cycle in the rat (Krieger and Hauser, 1978; Wilkinson et al., 1979). Depletion of brain serotonin with p-chlorophenylalanine (PCPA) raised the morning low and prevented the evening increase in plasma corticosterone (Scapagnini et al., 1971; Vernikos-Danellis et al., 1973). Similar results were observed with rats fed a tryptophan-free diet for 21 days (Vernikos-Danellis et al., 1977). In humans, plasma cortisol and ACTH levels also follow a diurnal rhythm, except that the highest levels are found in the morning (0600h), with the lowest values at night (2200h) (Watabe et al., 1987). This study demonstrated a similar diurnal pattern for plasma CRF, with peak values at 0600h and significantly lower levels at 1800h and 2200h. These results suggest that the diurnal rhythm in CRF secretion plays a role in
regulating the diurnal rhythm in cortisol and ACTH secretion.

In conclusion, the studies presented in this section demonstrate the presence of CRF cell bodies in the PVN, and a role for brain catecholamines in the stimulation of CRF secretion.

C. STRESS

1. Introduction

   a. Definition

   Stress has been defined by Hans Selye (1973) as the "non-specific response of the organism to any demand made upon it" (Selye, 1973). By this definition, the word "stress" describes a response, but it is often used interchangeably with the stimulus or "stressor" that elicits the response (Mills, 1985). The stress response can occur following a wide variety of stimuli. Stressors can be classified as physiological, environmental or psychological. Physiological stressors include surgery, injection of foreign substances such as proteins, diseases, anesthesia, loss of blood, exercise and trauma. Environmental stressors include prolonged exposure to cold or heat, noise, radiation, pollutants and chemicals. Psychological stressors include a threatening predator, intense competition among members of the same species, prolonged conflict, learning how to avoid a painful stimulus (such as electric shock), conditions characterized by novelty, anticipation, fear, unpredictability and change (Kidman, 1984; Gray, 1971; Mills, 1985).

   b. Cannon's Alarm Reaction

   Cannon (1914) described the role of the sympathetic nervous system
in the "fight or flight" emergency or "alarm reaction". This reaction permits the body to perform more strenuous muscle activity than normally possible. Cannon noted that when animals underwent stressful situations there was a marked increase in the activity of sympathetic nerves. This stimulation of the autonomic nervous system mobilizes the body's resources to deal with the stressful situation. The alarm reaction is thought to be transmitted from the hypothalamus through the reticular formation to the spinal cord to evoke massive catecholamine release from the sympathetic nerve endings (Guyton, 1981).

c. Selye's General Adaptation Syndrome

Selye (1936) was the first to describe a biological stress syndrome produced by diverse noxious agents. His description subsequently became known as the "general adaptation syndrome", a three-stage process that describes the long-term adjustments elicited by prolonged stress. The first stage is the "alarm reaction", when the body is exposed to diverse stimuli to which it has not adapted. Following the initial alarm reaction, a "stage of resistance" occurs in which resistance to the original stressor (which continues to exist) is increased, but resistance to other stressors is decreased. If the stressor continues for a long enough time, the stage of resistance develops into a final "stage of exhaustion", with a catastrophic decline in resistance to all other types of stressors. If the stress reaction continues undiminished, death ensues (Selye, 1976).

In cases of prolonged stress, Selye (1946) described a major role for glucocorticoids during the alarm reaction. Glucocorticoids increase
sugar deposition in the liver and facilitate the conversion of proteins and fats into sugars. They enhance the responsiveness of the blood vessels to epinephrine and norepinephrine. This will provide the body with energy sources to be rapidly utilized in the event of sudden strenuous activity. Glucocorticoids also lower the body's resistance to infection and have anti-inflammatory properties, including decreased numbers of lymphocytes and eosinophils, decreased thymus weight and inhibition of antibody formation. Stress appears to play a major role as a suppressor of immune function.

Other physiological changes induced by prolonged stress include a depression of thyroid activity, inhibition of body growth, and suppression of sexual and reproductive behavior (Gray, 1971). In simple terms, the body copes with prolonged stress by closing down any process that detracts from immediate energy mobilization.

d. Sympathetic and Endocrine Stress Responses

The biological stress response is believed to be mediated by the stimulation of two systems (Kidman, 1984). One, the pituitary-adrenal axis, is activated by sensory nerves which relay information regarding external stressors to the hypothalamus. Hypothalamic neurons contain CRF and release it into the portal circulation, stimulating the anterior pituitary gland to produce and release ACTH. In response, the adrenal cortex produces corticosteroids. The glucocorticoids liberated into the bloodstream inhibit the synthesis and/or release of ACTH from the pituitary by a negative feedback mechanism.

The second system is the sympathetic adrenal medullary axis, which
plays an important role in the alarm reaction described by Cannon. In this system, once the sensory nerves relay the information regarding the external stressors to the hypothalamus, it relays a message through the reticular formation to the spinal cord to evoke massive catecholamine release from the sympathetic nerve endings and adrenal medulla.

e. Physiological Changes in Response to Stress

Selye (1950) emphasized the fact that the physiological changes (primarily in the adrenal cortex) in response to stressful stimuli were the same irrespective of the stimulus used. This suggests that whether the stressor is physiological, environmental or psychological, the biological stress response would always be the same. This general hypothesis allows for comparison of physiological responses following a variety of different stressors.

Since stress is abstractly defined, it is helpful to have quantifiable parameters that are known to change when animals or humans are exposed to a stressor. In the experiments presented in this dissertation, the neuroendocrine responses to stress were measured with respect to plasma renin activity and plasma corticosterone levels. The stressor was a behavioral paradigm known as conditioned fear, or conditioned emotional response (CER), designed to produce psychological stress.

B. The Conditioned Fear Paradigm

a. Description

Each rat was transported from his home cage to the stress chamber.
Ten minutes following placement in the chamber, the experimental (stressed) rat received an inescapable foot shock and was returned to his home cage. This procedure was repeated for three consecutive days. In previous experiments it was apparent that the stressed rats had learned that their placement in the chamber will be followed by a foot shock. By the third day, in contrast to the control animals, the stressed rats defecated, urinated and demonstrated freezing (no movement) behavior. On the fourth day, after 10 minutes in the test chamber, instead of receiving shock the rat was removed, transported to a third area outside the stress room and immediately sacrificed by decapitation. The control rats were treated identically except that shock was not administered at any time. A more detailed description of this procedure can be found in the Methods section.

b. Pavlov's Dog: Classical Conditioning

Ivan Pavlov (1927) presented studies on classical conditioning in which a dog was repeatedly exposed to a situation where an initially neutral stimulus (e.g., a tone or light flash) was followed directly by a stimulus of biological significance to the animal (food). The latter "unconditioned stimulus" (UCS) elicited a range of "unconditioned responses" (UCR). As the pairings of stimuli were continued, the originally neutral stimulus became a "conditioned stimulus" (CS), capable of eliciting some part of the responses formerly elicited exclusively by the UCS. This "conditioned response" (CR) in Pavlov's dogs was salivation.
c. Definition of Fear

Fear can be defined as an emotional response to an aversive stimulus. In this respect, it is classified as an emotion. The common element that emotions share is that they represent a reaction to a "reinforcing event" or signals of reinforcing events. Reinforcing events include both rewards and punishments. An example of a punishment would be the removal of a reward, or the failure of an expected reward to occur. The emotional responses will depend on the type of reinforcing event and the animal's knowledge of these events (Gray, 1971).

d. Measures of Fear

Hall (1941) established the link between the study of fearfulness and the use of defecation as a measure of fear. He developed a standard test (the Open Field) for the measurement of emotional defecation. Parker (1939) demonstrated that defecation occurred in response to a variety of situations which one would expect to elicit fear. He recorded the amount of defecation elicited by six different situations: the Open Field test, a buzzer, sudden dropping, a tilting box that caused the rat to slide down an inclined plane, immobilization and forced swimming. High positive correlations were obtained between the scores on every test. Further factor analysis indicated a general factor (presumably fear) which all the tests were measuring. These results support the statement by Selye (1950) that physiological changes in response to stressful stimuli are the same irrespective of the alarming stimulus used.
e. Emotional Factors

There are physiological events that occur within an animal to influence its behavior in a given environment. In 1890, William James and Carl Lange independently expressed a theory for the interrelationship between physiological events and emotional responses. The James-Lange theory, as it is known, defines emotions as the perception of the physiological changes that occurred in response to a stimulus. For example, the feeling of being frightened would consist solely of feeling your heart pound, your breathing become faster, and so on. Unfortunately, it is not possible to isolate specific physiological functions and correlate them exclusively with a particular emotional state. However, the role of emotional factors should not be overlooked. Studies by Mason et al. (1976) recognize the sensitivity of the pituitary-adrenal cortical system to psychological influences. Departing from Selye’s non-specific physiological response hypothesis (1950), Mason et al. (1976) proposed that there are similar psychological reactions to various external stimuli. Factors such as heat, cold, fasting or exercise do not produce an adrenal cortical response if emotional arousal is carefully avoided (Mason et al., 1976). To illustrate, on the first day of starvation, food-deprived monkeys that could watch other monkeys being fed in nearby cages had an elevation of blood corticosteroids that occurred too early to be attributed to a physiological response due to a lack of food. However when the monkeys were carefully adapted to their surroundings, kept in a soundproof room, and maintained on regular "feeding" (non-caloric, fruit-flavored pellets) schedules, there were none of the usual
changes in glucocorticosteroids associated with fasting periods. Mason concluded that there is a considerable degree of emotional disturbance, due to discomfort, inherent in the laboratory setting. Kidman (1984) expanded this idea to a "cognitive theory of stress" which says that, in spite of our best efforts to control hunger, temperature and other environmental pressures, we still become frustrated over perceived threats to our welfare and security. Thus, the stress response consists of a variety of physiological and psychological factors. These factors are most likely integrated through the central nervous system, in particular the hypothalamus, as it coordinates the interaction between the autonomic nervous system, the endocrine system and many forms of emotional behavior.

3. Cardiovascular Changes in Response to Classical Conditioning

Stressful conditions have been implicated in the etiology of cardiovascular disease and hypertension (Hollenberg et al., 1981; Manuck et al., 1981; Schulte et al., 1984). Changes in heart rate and arterial blood pressure can be classically conditioned (Dykman and Gantt, 1956). A neutral stimulus that elicits no cardiovascular response (conditioned stimulus) is paired with another stimulus (unconditioned stimulus) that evokes a vigorous cardiovascular response (unconditioned response). After several pairings of the conditioned and unconditioned stimulus, the previously neutral conditioned stimulus will eventually cause cardiovascular changes in anticipation of the unconditioned stimulus (Cohen and Randall, 1984).

Smith et al. (1984) have demonstrated that the perifornical area of the hypothalamus (referred to as HACER, the Hypothalamic Area Controlling
Emotional Responses) is responsible for the cardiovascular responses that accompany emotional behavior in monkeys. Their operational definition of emotion included cardiovascular responses (elevated arterial blood pressure) and a behavioral response (suppression of lever pressing). When the hypothalamic area (HACER) was electrically stimulated, it produced the same cardiovascular pattern as that associated with the emotional situation. The cardiovascular but not the behavioral portion of the emotional response was lost following ablation of the HACER. Afferents to the HACER include the amygdala and septal area as well as brain stem autonomic areas such as the central grey, nucleus tractus solitarius, and dorsal motor nucleus of the vagus. Efferents include a direct projection to the intermediolateral cell column cells of the thoracic cord, an important site with respect to sympathetic control of cardiovascular function. Other efferent projection sites include the raphe nuclei, the parabrachial complex, the hypothalamic PVN and the periaqueductal grey.

Cardiovascular changes have been observed with the Sidman avoidance behavioral testing (Galosy et al., 1982). The Sidman avoidance paradigm (Sidman, 1953) is used frequently to evaluate cardiovascular changes associated with the postponement of electric shock contingent upon an appropriate response. The paradigm involves delivery of electric shock at regular intervals unless a response (such as lever pressing) occurs to delay the shock. If the animal presses the lever at least once during the interval, it can avoid all shocks (Turkkan et al., 1982).

Anderson and Brady (1971, 1972) observed decreased heart rate and increased pulse and arterial blood pressure during the anticipatory pre-avoidance period. Once the avoidance test was underway, the heart rate
rapidly increased above pre-avoidance levels while pulse and arterial blood pressure remained elevated. Further pharmacological studies indicated that the β-adrenoceptor blocker propranolol partially attenuated the heart rate elevations during avoidance, but did not affect the increases in blood pressure in either avoidance or pre-avoidance periods (Anderson and Brady, 1976). In similar studies, the α-antagonist phenoxybenzamine blocked the elevation in blood pressure during the avoidance period but did not affect pre-avoidance changes in heart rate and arterial blood pressure (Anderson et al., 1976). From these and other studies, Galosy et al. (1982) suggested that reduced heart rate during the pre-avoidance period is dominated by parasympathetic mechanisms, whereas the arterial blood pressure levels may be mediated by peripheral vascular mechanisms which do not involve α receptors, perhaps cholinergically-mediated shifts in blood flow (Anderson et al., 1976). During the avoidance period, a combination of increased sympathetic and decreased parasympathetic activity leads to increased heart rates, while elevated arterial pressure is due to continued sympathetic peripheral vasoconstriction (Galosy et al., 1982).

In humans, the stress response, as elicited by a demanding arithmetic test (Brod et al., 1959), consisted of the following: vasodilatation in skeletal muscle, coronary vessels and brain, while most other resistance vessels and veins underwent a neurogenic vasoconstriction. Increased sympathetic and decreased parasympathetic influences on the heart resulted in tachycardia, increased cardiac output and slightly increased mean arterial pressure. Neuroendocrine responses included increased epinephrine, ACTH, corticosteroids, vasopressin and renin-
angiotensin-aldosterone by means of sympathetic nerve stimulation of the kidney (Brod, 1970). Brod et al. (1962) demonstrated that this circulatory pattern exists in hypertension, especially the early stages. Eliasson (1984) observed that the limbic-hypothalamic pattern of stress responses is nearly identical in animals and man. He proposed that modern humans develop hypertension because they are socially deprived of the acting-out behavior which is a large part of the stress or defense reaction. However, the autonomic and hormonal changes associated with the stress reaction are not suppressible, therefore hypertension results.

4. Neuroendocrine Changes in Response to Stress

a. Stress-Induced Renin Secretion

The physiological response to stress involves the release of catecholamines from the adrenal medulla and sympathetic nerve endings. Stimulation of the renal nerves increases renin secretion (Di Bona, 1985; Blair et al., 1985), and this stimulatory effect is mediated, at least in part, via norepinephrine released at the endings of the postganglionic sympathetic neurons that innervate the kidneys (Davis and Freeman, 1976; Reid et al., 1978; Ganong and Barbieri, 1982). Circulating epinephrine and norepinephrine can also stimulate renin secretion.

Many investigators have reported increases in plasma renin activity in response to a variety of stressors. Clamage et al. (1976) reported increases in plasma renin activity in rats following 30 minutes exposure to either novel "open field" environment or the presence of a hungry cat. Jindra et al. (1980) observed increases in plasma renin activity after acute and repeated immobilization stress. Paris et al. (1987) reported
increased plasma renin activity by the following stressors: 12 minute conditioned fear paradigm; 20 minute immobilization; 20 minute forced swimming in cold water; 2, 12 and 22 minutes of intermittent footshock; and a 3 minute conditioned fear paradigm with 0, 10, 30 and 60 minute recovery. Blair et al. (1976) reported an elevation of plasma renin activity in baboons during avoidance performance. Gotoh et al. (1985) demonstrated that bilateral knife cuts behind the hypothalamic paraventricular nucleus (PVN) reduced the renin response to immobilization and the gravitational stress of head-up tilting. Plasma renin activity clearly can be increased by many different types of stressors.

Sigg et al. (1978) found that restraint causes an increase in plasma renin activity which was not blocked by pretreatment with the β-adrenergic antagonists dl-propranolol or sotalol. However, propranolol pretreatment prevented the increase in plasma renin activity following swim stress and ether stress (Pettinger et al., 1972) and intermittent footshock (Leenen and Shapiro, 1974). Also, Van de Kar et al. (1985) have shown that propranolol significantly inhibits the increase in plasma renin activity following a three-minute conditioned fear paradigm. Evidence for a role of central catecholamines in stress-induced renin secretion was provided by Lightman et al. (1984) who demonstrated that injections of the catecholamine neurotoxin 6-OHDA into the dorsal noradrenergic bundle markedly attenuated the increase in plasma renin activity in response to hemorrhage. Kobayashi et al. (1975) have reported that afferent fibers from the locus coeruleus to the PVN travel in the dorsal noradrenergic bundle. These studies suggest that central catecholaminergic projections to the PVN may be involved in the renin response to stress.
b. Stress-Induced Corticosterone Secretion

1. Introduction

Corticosterone levels have been shown to increase in response to several stressors. For example, Armario et al. (1986) demonstrated that the corticosterone response to stress is directly related to the intensity of the stress stimulus. The stressors used in their study were graded levels of low intensity stressors (either transfer to a novel cage or room, or a loud noise). These results are in agreement with findings by Kant et al. (1983), who demonstrated that increases in plasma corticosterone levels were related to the intensity of footshock. They found that corticosterone secretion was very sensitive not only to an obvious stressor (footshock) but also to any change in the environment accompanying the procedure. Simply placing the rat in the shock box without delivery of any current elicited a strong corticosterone response. These studies suggest that the increase in corticosterone levels in response to stress can represent a sensitive index of the intensity of emotional arousal experienced by the animals.

Dohanics et al. (1986) found that the adrenocortical response to ether stress occurs independently of neurohypophyseal function, and that the full corticosterone and ACTH response to ether or ether plus laparotomy stress requires not only an intact PVN but also an intact neurointermediate lobe.

2. Stress-Induced Secretion of CRF

CRF has been shown to mediate stress-induced ACTH release in rats
(Rivier and Vale, 1983). CRF also has been shown to initiate other biological actions in response to stress. For example, injections of CRF into the cerebral ventricles produced significant increases in plasma concentrations of epinephrine, norepinephrine, glucagon and glucose, elevated mean arterial pressure and heart rate, and inhibited gastric acid secretion. Pretreatment with the ganglionic blocking drug chlorisondamine completely abolished the increases in plasma catecholamines and glucose, heart rate and blood pressure (Lenz et al., 1987). CRF has been shown to activate brain catecholamine systems. Studies by Dunn and Berridge (1987) demonstrated that injections of CRF into the cerebral ventricles of mice resulted in increased dihydroxyphenylacetic acid (DOPAC):dopamine ratios in prefrontal cortex, septum, hypothalamus and brain stem compared to saline controls. The ratio of 3-methoxy-4-hydroxyphenylethleneglycol (MHPG):norepinephrine was increased in the prefrontal cortex and hypothalamus. DOPAC and MHPG are the major catabolites of dopamine and norepinephrine, respectively. CRF release has been measured in conscious, unrestrained rats using a push-pull cannula system implanted in the median eminence (Ixart et al., 1987). Basal CRF release occurs in a pulsatile pattern with peaks about every 45 minutes (peak mean $9.0 \pm 0.7$ pg vs. trough $4.1 \pm 0.3$ pg). Ether elicited an increase in CRF which lasted about 45 minutes with peak values of $54.3 \pm 3.2$ pg.

3. Stress-Induced Secretion of ACTH

Gibbs (1984) found that ACTH secretion increased in response to restraint, cold or ether stress. However, Armario et al. (1985) have found dissociations between ACTH and corticosterone responses: the ACTH
response to restraint stress, unlike the corticosterone response, was the same in control and in chronically stressed rats. This suggests that the increased corticosterone levels from restraint stress are not necessarily due only to increased pituitary ACTH release.

The increase in ACTH following hemorrhage was prevented by PVN lesions, while the responses of renin, epinephrine, norepinephrine, mean arterial blood pressure and heart rate to hemorrhage were not affected (Darlington et al., 1988). However, these lesions consisted of creating an island of the PVN and dorsal hypothalamus by lowering a triangular (Halasz-type) knife into the third ventricle to the level of the PVN and then rotating. This may have disrupted fiber pathways to and from the PVN without actual damage to PVN cell bodies.

Makara et al. (1986) proposed that ACTH hypersecretion following adrenalectomy is driven primarily by CRF- and/or vasopressin-producing neurons in and around the PVN, while either (a) other sources of CRF-41, (b) increased pituitary sensitivity, or (c) other hypothalamic factors may restore the stress-induced ACTH release in the absence of the PVN.

The effect of bilateral lesions of the PVN on circadian rhythm and ether stress responsiveness of plasma ACTH and corticosterone were studied in chronically cannulated female Sprague-Dawley rats. Three weeks after PVN lesions, ACTH values had dropped by at least half, and the diurnal maximum and amplitude for corticosterone were diminished by one third. The peaks for both hormones following ether stress were only half as high as the control peaks (Ixart et al., 1982). These results support the role of the PVN as a mediator of stress-induced ACTH and corticosterone secretion.
4. Role of Vasopressin in Stress-Induced Corticosterone Secretion

Vasopressin promotes the retention of water by the kidney, to maintain plasma volume. There are two major vasopressin systems in the hypothalamus. One system has cell bodies in the PVN and supraoptic nuclei, projecting through the internal zone of the median eminence to the pars neuralis of the pituitary gland. The other system consists of cell bodies in the PVN with nerve terminals in the median eminence (Silverman and Zimmerman, 1983). One difference between these systems is that adrenalectomy resulted in increased staining intensity of vasopressin immunoreactive nerve fibers in the external zone of the median eminence, whereas the fibers of the neural lobe or internal zone of the median eminence were not affected (Zimmerman et al., 1977).

Vasopressin has been shown by Tilders et al. (1985) to have an important role in the stress-induced activation of the pituitary-adrenal system, possibly by potentiating the effects of CRF. Pretreatment of rats with an antiserum to vasopressin inhibited the ACTH response to both restraint and formalin stress. Vasopressin has also been shown to potentiate the activity of CRF (Gillies et al., 1982). Vasopressin secretion is stimulated by hemorrhage (Robertson, 1977), hypoglycemia (Bayliss and Robertson, 1980), electric shock (Husain et al., 1979) and ether (Gibbs, 1984).

Further evidence for the involvement of vasopressin in the ACTH response to stress is that the vasopressin antagonist dpTyr(Me)arginine vasopressin decreases ether stress-induced ACTH release, and administration of both the vasopressin antagonist and CRF antiserum completely
prevented stress-induced ACTH secretion (Rivier and Vale, 1983). However, some studies reported that dpTyr(Me)arginine vasopressin does not block either the intrinsic ACTH-releasing activity or its synergistic effects with CRF (Baertschi et al., 1984; Knepel et al., 1984) and, therefore, should be avoided in studies involving ACTH release (Tilders et al., 1985).

5. Role of Catecholamines in Stress-Induced Corticosterone Secretion

Controversy still exists as to whether catecholamines play an excitatory or inhibitory role in stress-induced corticosterone secretion.

i. Evidence for an Inhibitory Role of Catecholamines

Brain norepinephrine has been postulated to inhibit ACTH secretion (van Loon et al., 1971; Weiner and Ganong, 1978). Eisenberg (1975) administered reserpine (systemically) or phenoxybenzamine (centrally) and found an augmentation of the plasma corticosterone response to ether. This suggests that norepinephrine may function in the central nervous system to inhibit the stress response. However, reserpine is not a selective noradrenergic depletor as it will also deplete serotonin stores (Bugnon et al., 1983).

Suemaru et al. (1985) observed significant increases in serum ACTH and corticosteorne levels 15 minutes after the onset of either ether-laparotomy or cold-restraint stress. The hypothalamic CRF concentration showed a rapid increase 2.5 minutes following ether-laparotomy stress, followed by a significant decrease after 15 minutes, and a subsequent
increase. Norepinephrine concentration in the hypothalamus was reduced, whereas dopamine concentration was increased. Epinephrine concentrations did not show a significant change throughout the stress procedure. From these observations they concluded that norepinephrine in the hypothalamus may not be involved in stimulating hypothalamic CRF secretion in the early phase of acute stress.

Glavin et al. (1985) depleted brain norepinephrine in rats by injecting the dopamine-β-hydroxylase inhibitor FLA-63 (to prevent the conversion of dopamine to norepinephrine) followed by RO4-1284, a reserpine-like vesicular depletor of norepinephrine, dopamine and serotonin. This treatment produced significant depletion of norepinephrine in all brain regions examined. Norepinephrine depletion did not prevent the increase in plasma corticosterone levels following cold-restraint stress. However, in the non-stressed rats, there was no significant difference in plasma corticosterone levels between the norepinephrine-depleted and saline-treated rats. The authors suggest that norepinephrine does not mediate cold restraint stress-induced increases in plasma corticosterone levels, nor does it play an inhibitory role with respect to corticosterone secretion. However, this study combines two non-related stimuli (cold vs. immobilization) as stressors without addressing how this could affect hormonal responses.

Reinstein et al. (1985) have demonstrated that acute, uncontrollable stress (restraint and intermittent footshock for one hour) depleted norepinephrine in the rat brain (increased norepinephrine turnover). Pretreatment with supplemental tyrosine (to prevent norepinephrine depletion) prevented hypothalamic norepinephrine depletion and suppressed
the increase in plasma corticosterone, suggesting an inhibitory role for norepinephrine. However, these corticosterone data were reported as percent of non-stressed control values, which were quite high (37.4 ug/dl). In conclusion, there is weak evidence in these studies to support an inhibitory role for norepinephrine in corticosterone secretion.

ii. Evidence for a Stimulatory Role of Catecholamines in Stress-Induced Corticosterone Secretion

Gibson et al. (1986) found that bilateral injections of 6-OHDA into the PVN prevented the restraint stress-induced increase in plasma corticosterone levels. Pretreatment with the $\alpha_1$-adrenoceptor antagonist prazosin, at a dose which did not influence resting hormone levels, inhibited the increase in plasma corticosterone following restraint stress. The authors suggest an excitatory role for norepinephrine via $\alpha$ receptors in CRF release during acute stress. Similarly, Feldman et al. (1986) injected 6-OHDA into the PVN and found that PVN norepinephrine depletion did not affect basal levels or ether stress-induced increases in corticosterone levels, but it did significantly inhibit the adrenal response to photic and acoustic stimuli and sciatic nerve stimulation, suggesting that noradrenergic terminals in the PVN play a role in the activation of adrenocortical responses following afferent neural stimuli.

Smythe et al. (1983) support the hypothesis that stress-induced ACTH release from the pituitary is stimulated by hypothalamic noradrenergic neuronal pathways. They measured norepinephrine activity in the mediobasal hypothalamus by assessing the ratio of 3,4-dihydroxyphenyl-ethyleneglycol (DHPG)/norepinephrine. Hypothalamic norepinephrine
activity was rapidly increased by cold swim stress in the rat, accompanied by increased ACTH/corticosterone release into the blood. Norepinephrine activity and ACTH release were also stimulated in rats following ether stress. The significant correlation between serum corticosterone and the ratio of hypothalamic DHPG/norepinephrine (shown by linear regression analysis of the combined data for control and stressed rats in their studies) is not evidence, but suggests that hypothalamic norepinephrine neuronal activity may play an important stimulatory role in stress-induced ACTH release.

Studies by Johnston et al. (1985) support the data presented by Smythe et al. (1983). Norepinephrine, dopamine and 5-HT concentrations as well as the concentrations of their respective metabolites MHPG, DOPAC and 5-hydroxyindole-3-acetic acid (5-HIAA) were measured by HPLC. The metabolism of norepinephrine, dopamine and 5-HT in different rat brain nuclei was estimated by the ratio of the metabolite to its respective amine. Ether stress produced large increases in plasma ACTH, accompanied by significant increases in norepinephrine metabolism in the PVN, arcuate and dorsomedial nuclei. Serotonin metabolism was decreased in the PVN, supraoptic nucleus and caudal division of the arcuate nucleus, while significant increases in 5-HT metabolism were detected in the suprachiasomatic nucleus and rostral division of the arcuate nucleus. Dopamine metabolism was selectively increased in the rostral division of the arcuate nucleus. The authors interpret the data as suggesting a stimulatory role for norepinephrine, possibly in the PVN with respect to ACTH secretion.

Studies by Mermet and Gonon (1988) used differential normal-pulse
voltammetry combined with carbon fiber electrodes to measure in vivo extracellular catechols released by noradrenergic nerve terminals in the PVN. In rats pretreated with the monoamine oxidase inhibitor pargyline, ether stress produced an increase in the catechol signal corresponding to extracellular norepinephrine. Electrical stimulation of the ventral noradrenergic bundle also caused an immediate increase in the signal. The increase in norepinephrine release following ether stress was immediate and lasted as long as the stimulus duration. No dopamine release was recorded from either the striatum or the A13 dopaminergic cell body group caudal to the PVN. These data suggest a facilitatory action of norepinephrine on PVN cell bodies which are involved in the hormonal responses to stress.

Immobilization stress (varying from 15 to 180 minutes) significantly increased MHPG-SO$_4$ levels and decreased norepinephrine levels in the following rat brain regions: hypothalamus, amygdala, hippocampus, thalamus, pons, medulla and cerebral cortex, with the most rapid and profound increase in MHPG-SO$_4$ found in the hypothalamus. However, the basal ganglia demonstrated the reverse process- increases in norepinephrine levels with transient decreases in MHPG-SO$_4$ levels. There was a concomitant increase in plasma corticosterone levels following immobilization stress (Tanaka et al., 1982). This study suggests that norepinephrine released in the hypothalamus during immobilization stress may play a role in the stress-induced increase in corticosterone levels.

Beaulieu et al. (1987) demonstrated that bilateral lesions of the central nucleus of the amygdala significantly inhibited the stress-induced ACTH secretion following immobilization. These lesions also reduced the
stress-induced noradrenergic activity in the anterior and lateral hypothalamic areas, the PVN and arcuate nucleus, and the bed nucleus of the stria terminalis. These results suggest that norepinephrine plays a stimulatory role in ACTH secretion and is somehow regulated by neurons in the central nucleus of the amygdala.

As stated earlier, the ventral noradrenergic bundle (VNAB) is thought to be the source for the noradrenergic modulation of ACTH secretion (see CORTICOSTERONE: Role of the VNAB). Szafarczyk et al. (1985) have shown that injection of 6-OHDA into the VNAB obliterates the circadian patterns for ACTH and corticosterone secretion, and also caused an 80% inhibition of the ACTH response to stress.

Injections of 6-OHDA into the VNAB also prevented the increase in corticosterone secretion following exposure to photic and acoustic stimuli and sciatic nerve stimulation (Feldman et al., 1988). However, basal levels of corticosterone and ether-induced increases in corticosterone were not affected. The 6-OHDA-injected rats had average norepinephrine depletions of 72% in the mediobasal hypothalamus and 78% in the paraventricular nucleus when compared to vehicle-injected rats. These data support previous studies (Feldman et al., 1986) in which 6-OHDA injections into the PVN did not affect basal levels or ether-induced increases in corticosterone levels, but did significantly inhibit the adrenal response to photic and acoustic stimulation and sciatic nerve stimulation. These studies suggest that norepinephrine plays a role in the activation of adrenocortical responses following afferent neural stimuli.

In contrast, Velley et al. (1988) have shown that 6-OHDA lesions in the locus coeruleus did not prevent the effect of novel environment
stress on corticosterone, although there was a significant (53%) depletion of hypothalamic norepinephrine. However, this can be explained by the fact that about 90% of the norepinephrine supply to the PVN originates from the lower brainstem A1 and A2 cell groups, while the locus coeruleus projection to the PVN is only about 8% (Sawchenko and Swanson, 1982).

iii. Role of Epinephrine in Stress-Induced Corticosterone Secretion

Szafarczyk et al. (1987) demonstrated a stimulatory role for epinephrine (and norepinephrine) on corticosterone release when intracerebroventricular (icv) injections of epinephrine or norepinephrine induced ACTH release comparable to that occurring under conditions of ether stress. Epinephrine was more efficacious than norepinephrine on an equimolar basis. The stimulating effect of norepinephrine was reversed by icv pretreatment with prazosin, an $\alpha_1$-adrenoceptor antagonist, whereas the effect of epinephrine was inhibited by either prazosin or the $\beta$-blocker propranolol. These results suggest that norepinephrine triggers ACTH release via $\alpha_1$-adrenoceptors, whereas epinephrine acts via both $\alpha_1$- and $\beta$-adrenoceptors. It should be noted that they also administered a combined pretreatment of prazosin and propranolol, and this combined treatment was no more effective than either antagonist alone. They also demonstrated that a unilateral juxta-PVN injection of either epinephrine or norepinephrine produced an increase in plasma ACTH concentration with a time course similar to that for an icv injection, and pretreatment with anti-rCRF-41 serum caused a 50-60% reduction in the ACTH response to either catecholamine. Since the majority of diencephalic CRF neurons are
located in the parvocellular subnuclei of the PVN (Swanson et al., 1983; Liposits et al., 1985), these data suggest a role for $\alpha_1$ and $\beta$ receptors in the PVN in the regulation of corticosterone secretion.

Additional evidence was obtained by Spinedi et al. (1988) who demonstrated that central inhibition of the enzyme that converts norepinephrine to epinephrine, phenylethanolamine-N-methyltransferase (PNMT), produced a selective decrease in hypothalamic epinephrine levels, significantly decreased the plasma ACTH response to ether stress, and at later times also caused a selective decrease in CRF content. In addition, the altered ACTH response to ether stress was not due to changes in arginine vasopressin levels or changes in pituitary responses to CRF. These results support a stimulatory role of central epinephrine neurons on CRF release.

6. Role of Serotonin (5-HT) in Stress-Induced Corticosterone Secretion

The role of serotonin as a mediator of stress-induced corticosterone secretion is not clear. Electrolytic lesions in the dorsal raphe nucleus prevented the stress-induced increase in corticosterone levels as well as increases in plasma renin activity and plasma renin concentration following a ten-minute conditioned fear paradigm (Richardson Morton et al., 1986). Other studies from our laboratory have shown that injection of the neurotoxin 5,7-dihydroxytryptamine into the dorsal and median raphe nucleus did not prevent the stress-induced increase in renin and corticosterone secretion. Furthermore, i.p. injection of the 5-HT$_2$ antagonist LY53857 did not prevent the stress-induced increase in renin
and corticosterone secretion (Richardson Morton et al., 1986), whereas low doses of the 5-HT₁ agonist buspirone and ipsapirone inhibited the stress-induced increase in corticosterone and renin secretion (Van de Kar et al., 1985; Urban et al., 1986; Lorens et al., 1989). These studies suggest that a non-serotonergic (or possibly 5-HT₁) mechanism is involved in stress-induced renin and corticosterone secretion.

Feldman et al. (1987) reported that injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the PVN blocked the adrenocortical responses to cortical and extra-hypothalamic limbic stimulation, but had no effect on the adrenocortical response to ether stress. Immobilization and cold-exposure stress resulted in four-fold increases in ACTH-like immunoreactivity in plasma. This increase was potentiated by i.p. injections of the 5-HT reuptake inhibitor fluoxetine and significantly reduced by the 5-HT antagonists metergoline and cinanserin (Bruni et al., 1982). These results suggest that 5-HT may play a role in stress-induced ACTH release. In contrast, Fuller and Snoddy (1977) found that pretreatment with fluoxetine did not significantly alter the increase in plasma corticosterone levels following swim stress or insulin-induced hypoglycemia. However, fluoxetine did enhance the corticosterone response produced by an injection of L-5-hydroxytryptophan. These results suggest that serotonergic neural pathways are involved in mediating the adrenocortical response to some stressful stimuli (immobilization and cold exposure) but not others (swim stress and insulin-induced hypoglycemia).

Beaulieu et al. (1986) support the studies by Bruni et al. (1982). They found that bilateral lesions of the central nucleus of the amygdala
resulted in significantly decreased immobilization stress-induced ACTH secretion with a concomitant increase in serotonergic activity in the PVN and amygdaloid nuclei. Additional studies by Beaulieu et al. (1987) suggest a serotonergic involvement in immobilization stress. In these studies, bilateral lesions of the central nucleus of the amygdala in unstressed rats increased the serotonergic activity in the PVN, dorsomedial nucleus, anterior hypothalamic area (AHA), cortical and medial amygdaloid nuclei, and the lateral part of the basal amygdaloid nucleus. Immobilization stress applied to lesion rats resulted in a decrease in serotonergic activity to control levels in the PVN, AHA and the dorsomedial nucleus, and below control levels in the ventromedial nucleus. The serotonergic activity remained elevated in each of the amygdaloid areas measured, which are rich in glucocorticoid receptors (Beaulieu et al., 1986). Glucocorticoids have been reported to down-regulate their own receptors in a site-specific manner in the amygdala, septum and hippocampus, but not in the hypothalamus or pituitary (Sapolsky et al., 1984). Also noradrenergic cell bodies (A1-A7), serotonergic cell bodies (B1-B9), and PNMT-immunoreactive nerve cells of the adrenergic cell groups (C1-C3) have been shown to have strong glucocorticoid receptor immunoreactivity (Harfstrand et al., 1986). De Kloet et al. (1986) have shown that corticosterone selectively controls the 5-HT₁ receptor density in the dorsal raphe and dorsal hippocampus. These studies suggest that serotonin may modulate the negative feedback of glucocorticoids on stress-induced ACTH secretion. A moderate to strong nuclear glucocorticoid receptor immunoreactivity has been demonstrated in the majority of CRF-immunoreactive neurons of the parvocellular PVN, septohypothalamic thalamus, bed
nucleus of the stria terminalis, and the medial and central amygdaloid nuclei (Cintra et al., 1987).

C. Effect of Peripheral Sympathectomy on Stress-Induced Renin and Corticosterone Secretion

The physiological response to stress involves the release of catecholamines from the adrenal medulla and sympathetic nerve endings. These catecholamines stimulate the renal nerves to increase renin output. It is generally accepted that the sympathetic nervous system mediates the stress response from the brain to the kidney (Golin et al., 1988; Reid et al., 1978). Studies by Van de Kar et al. (1985) have shown that the β-adrenoceptor antagonist propranolol significantly inhibits but does not completely block the stress-induced increase in plasma renin activity. It should be noted, however, that this effect could be mediated by either central or peripheral β-receptors since propranolol is known to cross the blood brain barrier (Giarcovich et al., 1984; Lemmer et al., 1985). To determine whether stress-induced renin secretion is mediated by the peripheral sympathetic nervous system, rats were adrenal enucleated and given weekly injections of 6-OHDA (i.p.). The adrenal medulla was removed since it does not effectively take up 6-OHDA. The effectiveness of the sympathectomy/adrenal enucleation was verified by determination of plasma epinephrine (less than 1% of control) and norepinephrine (less than 10% of control). The stressor induced a significant increase in plasma renin activity and concentration. Sympathectomized-adrenal enucleated rats showed no inhibition of the stress-induced increase in plasma renin activity/concentration or plasma corticosterone levels (Richardson Morton...
et al., 1988). The data suggest that the sympathetic nervous system and adrenal catecholamines are not the only mediators of the stress-induced increase in renin secretion.

5. Central Nervous System Pathways Involved in the Stress Response

a. Sensory Afferents

With respect to the role of the central nervous system in the conditioned fear response, it is important to consider how the sensory stimulus (pain) is transmitted to the cerebral cortex for analysis. Specialized nerve fibers found throughout the skin serve as end organs for pain. These fibers enter the spinal cord and ascend through the lateral spinothalamic tract to the posteroventral nucleus of the thalamus. From here the fibers travel to the appropriate loci in the cerebral cortex, depending on the somatic origin of the pain message.

The ascending reticular activating system (ARAS) is a network of interconnective fibers that also travel from the top of the spinal cord to the thalamus, and eventually to the cortex. This tract receives major sensory inputs, and conveys information regarding alertness and excitement.

There appears to be a close connection between pain sensation and the ARAS. The midbrain and brainstem reticular formations receive many pain afferents from the spinothalamic tract. Studies in animals (Olds and Olds, 1965) and humans (Gray, 1971) have suggested that stimulation through electrodes in the periventricular midbrain reticular system generates pain sensation. Other pain reception fibers have been found in
the thalamic portion of the ARAS. Lesions in the thalamic intralaminar nuclei and the thalamic nucleus centrum medianum have been shown to alleviate intractable pain in humans (Gray, 1971).

b. The Limbic System

1. Septo-Hippocampal System

The limbic system, in particular the septo-hippocampal system, appears to play a major role in pain reception. The hippocampus emits a theta wave (4-9 cps rhythm) as a response to a painful stimulus in the rat, and in the emotional states of disappointment and frustration in humans (Gray, 1971). This theta wave can be abolished by lesions in the medial nuclei of the septum, and the thalamic nucleus centrum medianum, suggesting that the pain input route most likely travels from the midbrain reticular formation, through the thalamus, to reach the septum and hippocampus (Gray, 1971).

2. Amygdala

The amygdala is a limbic structure with many interconnections to the hippocampus (Krettek and Price, 1978). The amygdaloid complex has been shown to be involved in the regulation of fear-motivated behavior (Blanchard and Blanchard, 1972; Spevack et al., 1975). In addition, the amygdala appears to play a role in the stress response via the pituitary-adrenal cortical axis. The central nucleus of the amygdala, in particular, contains large numbers of CRF-containing cell bodies and terminals (Swanson et al., 1983; Moga and Gray, 1985). Injection of CRF into the lateral ventricles of rats or dogs elicits autonomic and
behavioral changes that are characteristic of the stress or defense reaction (Brown et al., 1982; Lenz et al., 1987; Britton et al., 1982). Electrical stimulation of the amygdaloid complexes results in an increase in adrenal glucocorticoid secretion in rats (Redgate and Fahringer, 1973), cats (Redgate, 1970) and monkeys (Mason, 1959). The amygdaloid complex is an essential part of the ACTH controlling mechanism in rats (Knigge, 1961; Allen and Allen, 1974). Specifically it has been shown to be necessary for the hypersecretion of ACTH following adrenalectomy (Allen and Allen, 1975). In these studies, lesions which destroyed the direct medial-projecting amgdalo-hypothalamic fibers blocked the hypersecretion of ACTH following adrenalectomy, while lesions of the septal region, stria terminalis and fornices did not.

Lesions in the amygdaloid central nucleus impaired one-way active avoidance learning (Werka et al., 1978), produced marked deficits in passive avoidance performance (McIntyre and Stein, 1973; Grossman et al., 1975), and significantly decreased ACTH secretion in response to immobilization stress (Beaulieu et al., 1987). Axons from cells in the olfactory bulb terminate in the amygdala (Powell et al., 1965) and the medial hypothalamus receives axons from cells in the amygdala (Cowan et al., 1965). Bilateral amygdalectomy significantly reduced the adrenocortical responses to sciatic nerve and olfactory stimulation, without affecting the corticosterone response to ether or to photic and acoustic stimulation (Feldman and Conforti, 1981). This amgdalo-hypothalamic pathway is believed to play a role in the adrenocortical response to a number of somatosensory stimuli (Feldman et al., 1975). The amygdala has also been shown to play an important role in regulating the cardiovascular
system during Sidman avoidance stress testing (Galosy et al., 1982).

3. Hypothalamic Ventromedial Nucleus

With respect to the central neural circuits involved in the fight/flight behavior, de Molina and Hunsperger (1962) described a pathway in cats that descends from the amygdala along the stria terminalis to the ventromedial hypothalamus (VMN) with connections to the midbrain central grey. Stimulation of sites within the hypothalamus, amygdala and midbrain central grey could produce defensive attack while other loci immediately adjacent to those stimulated would produce flight. When the VMN was removed, stimulation of the amygdala no longer produced defensive attack, and removal of the central grey prevented the flight behavior following hypothalamic stimulation.

In addition, Kling et al. (1958) reported that cats made docile by lesions in the amygdala could be made ferocious by subsequent lesions in the VMN. The converse did not apply - cats first made ferocious by lesions in the hypothalamus were not affected by a subsequent amygdalec-tomy.

Based on these and other observations, Gray (1971) suggested that the midbrain central grey is the ultimate controlling area for either flight or defensive behavior. In addition, there is a tonic inhibitory input to the central grey from the VMN. Further modulation of this inhibitory input would come from the amygdala and the septo-hippocampal system. Input from the amygdala would prevent the inhibition, resulting in fight/flight behavior, and the septo-hippocampal input would intensify passive avoidance behavior. The resulting behavior (either passive
avoidance or fight/flight) would depend on which of the inputs to the VMN is stronger under a particular set of environmental conditions. This scheme would provide a basis for the observation that, in times of danger, both freezing and fight/flight patterns may be stimulated simultaneously and alternate rapidly with each other.

Neurons in the VMN have been shown to play a role in stress-induced renin secretion. Gotoh et al. (1988) found that bilateral electrolytic lesions in the VMN prevented the increase in plasma renin activity following immobilization stress. Lesions in the VMN led to excessive levels of food intake and adiposity (Hetherington and Ranson, 1942), and elicited a variety of metabolic and behavioral disturbances termed the VMN syndrome, which has become a model for the study of obesity and motivated behavior (Weingarten et al., 1985; Stellar, 1954).

c. Role of Brain Catecholamines in Stress

A variety of stressors including foot shock (Bliss et al., 1968; Thierry et al., 1968, Iimori et al., 1981), immobilization (Corrodi et al., 1968; Tanaka et al., 1981) and ether stress (Vellucci, 1977) have been shown to decrease norepinephrine concentration and increase norepinephrine turnover in the rat brain.

The metabolism of norepinephrine in the rat brain results in the production of the sulfate conjugates MHPG-SO₄, the major metabolite, and DHPG-SO₄ (Schanberg et al., 1968; Sugden and Eccleston 1971). The functional activity of central noradrenergic neurons has been shown to be directly related to the production of MHPG-SO₄ (Stone, 1975). Levels of MHPG-SO₄ in rat brain represent a useful index for norepinephrine turnover.
in discrete brain regions (Kohno, 1981) as well as in the whole brain (Korf et al., 1973; Stone, 1976).

Rats subjected to a mild psychological stressor (exposure to responses of shocked rats) had decreased norepinephrine and increased MHPG-SO₄ concentrations preferentially in the hypothalamus and amygdala. Rats subjected to footshock stress had decreased norepinephrine and increased MHPG-SO₄ not only in the hypothalamus and amygdala but also the pons, medulla oblongata and cerebral cortex (Iimori et al., 1981). This study suggests that the noradrenergic response to stress varies with the nature or intensity of the stressor.

Brain noradrenergic systems, in particular the locus coeruleus-noradrenergic system, have been shown to play a role in nociception, anxiety and fear (Charney and Redmond, 1983). The locus coeruleus receives innervation from sensory (pain) pathways in the spinal cord, and relays information to the cerebral cortex and limbic structures (hypothalamus, hippocampus, amygdala) (Kuhar, 1986).

Rasmussen and Jacobs (1986) have recorded the electrical activity of noradrenergic neurons in the locus coeruleus of freely moving cats. During CER training, locus coeruleus units showed a large increase in activity in response to a stimulus paired with a noxious air puff, whereas no increase in unit activity is seen in response to a stimulus not paired with the air puff. Also, electrical stimulation of the locus coeruleus in monkeys elicited behavioral responses identical to those seen in experimentally-induced or naturally-occurring fear states (Bertrand, 1969).

In humans, drugs that increase noradrenergic function have been
shown to produce anxiety. The $\alpha_2$-adrenoceptor antagonists yohimbine and piperoxane induced anxiety states in normal subjects (Soffer, 1954; Holmberg and Gershon, 1961). These studies support the role of brain norepinephrine in mediating the response to anxiety and fear.

The Kety hypothesis (Kety, 1970) proposed that forebrain norepinephrine enhances cell firing in neurons that receive afferent environmental information to "affectively important events". Further, norepinephrine would produce a "persistent facilitation" of these inputs which would reinforce the memory process for these events. The extensive innervation of the limbic system (especially the hippocampus) by the locus coeruleus-norepinephrine system suggests that noradrenergic neurons may play a role in the memory or cognitive process of stress.

D. HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN)

A. Introduction

The PVN is a bilateral, triangular-shaped nucleus at the dorsal end of the third ventricle in the hypothalamus. It is a morphologically complex structure, with eight clearly distinguishable subdivisions, which contain as many as thirty different putative neurotransmitters. Three major efferent projection sites, arising from separate neuron groups, include (1) the median eminence, (2) the posterior pituitary and (3) the autonomic centers in the brain stem and spinal cord (Swanson and Sawchenko, 1983; Saper et al., 1976). This suggests that the PVN serves as an integrator of endocrine, autonomic and behavioral functions in a variety of physiological systems.
B. Morphology

The PVN is composed of distinct parvocellular and magnocellular divisions (Krieg, 1932). Swanson and Kuypers (1980) have further characterized the nucleus into eight distinct subdivisions, three magnocellular and five parvocellular. The anterior magnocellular part of the PVN is ventromedial to the descending column of the fornix as it enters the hypothalamus from the septum. The medial magnocellular division is just caudal to the anterior magnocellular level. The largest magnocellular division is the posterior magnocellular part. It first appears in the ventromedial PVN, just caudal to the medial magnocellular division. After dorsolateral expansion it forms a compact cell mass at the lateral margin of the nucleus. Hatton et al., (1976) further subdivide this group by defining two distinct populations of large cells in the medial and lateral halves of the posterior magnocellular region. The lateral group contains cells with multiple nucleoli that are larger and more uniform in orientation, when compared to the medial group. The posterior magnocellular cells number from 1300-2000 on either side of the brain (Olivecrona, 1957; Bodian and Maren, 1951; Swanson and Sawchenko, 1983).

There are five distinct parts of the parvocellular division. The periventricular part, medial to the rest of the PVN, contains small, vertically-oriented fusiform cells. The anterior parvocellular group is not well-defined but consists of small- to medium-sized cells at the level of the anterior and medial magnocellular parts. Extending through the caudal half of the nucleus is the medial parvocellular part, which
consists of densely packed cells. The dorsal parvocellular part is also in the caudal half of the nucleus and consists of horizontally-oriented, medium-sized cells in an elliptical group. The lateral parvocellular part of the PVN is in the caudal third of the nucleus and is the lateral expansion of the parvocellular part (the wings). These cells are horizontally-oriented, medium-sized and fusiform shaped. At its maximum extent the lateral parvocellular PVN extends laterally over the descending column of the fornix, about 750-1000 um from the midline (Swanson and Sawchenko, 1983).

Cells in the parvocellular division project primarily to the external lamina of the median eminence (Wiegand and Price, 1980; Swanson et al., 1980) and to the brainstem (dorsal vagal complex) and spinal cord (Hosoya and Matsushita, 1979; Swanson and Kuypers, 1980). The PVN is also known to project to those segments of the thoracic cord thought to contain sympathetic preganglionic neurons which innervate the kidney (Swanson and Mc Kellar, 1979). Cells in the magnocellular division project primarily to the posterior level of the pituitary gland (Sherlock et al., 1975).

The parvocellular division of the PVN contains about 7000 neurons in the following distribution: anterior 1650; dorsal 600; lateral 1300; medial 2175; periventricular 1275 (Sawchenko and Swanson, 1981).

C. Role of the PVN in Behavior

The PVN plays a major role in the control of feeding. Bilateral damage to the PVN results in increased levels of food intake and excessive weight gain (Leibowitz et al., 1981). This eating behavior can be elicited by hypothalamic injections of norepinephrine and other α-
noradrenergic agonists (Leibowitz, 1975). In particular, injection of the $\alpha_2$-agonist clonidine into the PVN initiates a feeding response in satiated rats (Marino et al., 1983). Goldman et al. (1985) observed a potent feeding response in satiated animals following injection of norepinephrine or clonidine into the PVN. This effect was blocked by the $\alpha$-antagonist phentolamine as well as the $\alpha_2$-antagonists rauwolscine and yohimbine. These results suggest that $\alpha_2$ receptors in the PVN play a role in mediating feeding behavior. Leibowitz et al. (1984) have demonstrated that these $\alpha_2$ noradrenergic receptors fluctuate in density with respect to the circadian cycle, exhibiting a unimodal peak at the onset of the dark cycle, when feeding normally occurs. This shift in $\alpha_2$ receptor density may be correlated with plasma corticosterone levels, which also peak at the onset of the dark cycle (Krieger and Hauser, 1978; Wilkinson et al., 1979). Norepinephrine-stimulated feeding behavior was prevented by adrenalectomy and restored by administration of corticosterone (Leibowitz et al., 1984).

D. Afferent Connections to the PVN

Descending afferent connections to the PVN include a large input from the limbic system structures, especially the lateral septal nucleus and the ventral portion of the subicular cortex, as well as input from the amygdala, in particular, the medial nucleus (Silverman et al., 1981; Tribollet and Dreifuss, 1981). The PVN receives afferent sensory information from the telencephalic limbic system. The bed nucleus of the stria terminalis receives a large input from the amygdala (Krettek and Price, 1978) and smaller projection from the ventral part of the subiculum.
(Swanson and Cowan, 1977). The bed nucleus has been shown to project to the PVN by autoradiographic studies (Swanson and Cowan, 1979). This projection innervates all parts of the parvocellular division and the oxytocinergic parts of the magnocellular division (Sawchenko and Swanson, 1981). Electrophysiological studies have shown that stimulation of the amygdala or septum can have inhibitory (and sometimes excitatory) effects on the PVN magnocellular neurons (Renaud and Arnauld, 1979; Koizumi and Yamashita, 1972; Pittman et al., 1981).

Anterograde autoradiographic techniques were used to describe brain stem afferents to various PVN regions (McKellar and Loewy, 1981). The parabrachial nuclei and locus coeruleus project to the posterior, periventricular, parvocellular and dorsal divisions. The ventral medulla projects to the lateral, medial, posterior, dorsal and parvocellular divisions. The A1 catecholamine cell group projects to the posterior, lateral, medial and dorsal divisions, while the NTS projects to the parvocellular and dorsal divisions. The projections from the A1 and A2 (NTS) catecholaminergic cell groups to the PVN have been further characterized by an immunofluorescence double-labeling procedure to determine which anterogradely labeled fibers and terminals in the PVN also displayed immunoreactive dopamine \( \beta \)-hydroxylase, indicating the presence of catecholaminergic neurons (Cunningham and Sawchenko, 1988). (See Role of Brain Catecholamines in Corticosterone Secretion)

Studies using retrogradely-transported horseradish peroxidase identified cell bodies of neurons that project to the PVN. Labelled cell bodies were found in the mediobasal hypothalamus, the limbic system (lateral septum, posteromedial amygdala and ventral subiculum) and the
brain stem, including the dorsal raphe nucleus, locus coeruleus, parabrachial nuclei, NTS and lateral reticular nucleus (Tribollet and Dreifuss, 1981).

E. Efferent Projections of the PVN

Descending autonomic projections of the PVN were investigated using the anterograde transport technique of Phaseolus vulgaris leuco-agglutinin (Luiten et al., 1985). Two major fiber bundles were described. One (fiber bundle 1) leaves the PVN dorsally and runs caudally in a dorsal periventricular position to reach the midbrain periaqueductal grey, where a number of terminal boutons are observed. This bundle then courses laterally to join the lateral lemniscus. Some projections innervate the dorsal raphe nucleus, the parabrachial nucleus, locus coeruleus and pontine raphe. The second group of fibers (fiber bundle 2) runs medially and caudally from the PVN and travels in a position dorsal to the transition of the internal capsule and medial forebrain bundle. In the midbrain, fiber bundle 2 moves laterally to cover the dorso-lateral pole of the substantia nigra, and has some terminations in the ventral aspects of the reticular formation. At the level of the pons, fiber bundle 2 moves ventrally to join the lateral lemniscus (and bundle 1) to reach the floor of the brainstem. Posterior to the pons, all descending PVN efferents are now in a ventral position adjacent to the pyramidal tracts. While in the medulla, some fibers branch off to terminate in the reticular and medullary raphe nuclei. Nearly all of the bundle fibers traverse the lateral reticular nucleus, nucleus ambiguus, parvocellular reticular nucleus and area postrema, with an especially dense amount of varicosities
and terminal boutons in the NTS and dorsal motor nucleus of the vagus.

F. Catecholaminergic Innervation of the Hypothalamic PVN

There is substantive evidence supporting extensive catecholaminergic innervation of the PVN (Carlsson et al., 1962; Lindvall and Bjorklund, 1974; Hokfelt et al., 1974). Most of the adrenergic and noradrenergic innervation to the PVN is provided by (1) the A1 and C1 cell groups of the ventral medulla, (2) the A2 and C2 cell groups in the medial NTS and (3) the A6 cell group, the locus coeruleus of the pontine central gray. The locus coeruleus receives major fiber projections from the A1 region, and projects to the most medial parts of the parvocellular PVN (Swanson et al., 1981).

G. Neuroactive Substances in the PVN

The colocalization of CRF and vasopressin in the parvocellular PVN has already been presented (see CORTICOSTERONE: CRF). These are only two of many neuroactive substances found in the PVN by immunohistochemical studies. Swanson and Sawchenko (1983) have compiled a thorough listing: Cell Bodies include oxytocin and vasopressin in the magnocellular PVN (Swaab et al., 1975; Vandesande and Dierickx, 1975); somatostatin in the periventricular part of the parvocellular PVN (Dierickx and Vandesande, 1979); dopamine in the periventricular and adjoining parvocellular PVN (Hokfelt et al., 1973); met- and leu-enkephalin in the magnocellular and parvocellular PVN (Rossier et al., 1979; Finley et al., 1981); neurotensin in the medial and periventricular parts of the parvocellular PVN (Kahn et al., 1980); dynorphin coexists with vasopressin in the magnocellular PVN (Watson et al., 1982); substance P in the parvocellular PVN (Ljungdahl et
al., 1978); glucagon in the magnocellular PVN (Tager et al., 1980); renin in the oxytocinergic parts of the magnocellular PVN (Fuxe et al., 1980); and ACTH and $\beta$-endorphin in the magnocellular PVN (Joseph and Sternberger, 1979; Watkins, 1980). The PVN is also the major source of atrial natriuretic peptide (ANF)-containing cells that project to the median eminence (Palkovits et al., 1987). Double-labelling studies with antisera to GAD and CRF in the PVN have indicated that a sub-population of CRF-containing neurons contain the inhibitory neurotransmitter GABA (Meister et al., 1987).

Nerve terminals and fibers in addition to norepinephrine, epinephrine and serotonin (previously described) include "cholinoceptive" acetylcholine neurons (Kimura et al., 1981); ACTH, $\beta$-endorphin and $\alpha$-MSH in the parvocellular and oxytocinergic divisions of the magnocellular PVN, all of which originate in the arcuate nucleus (Joseph, 1980; Bloom et al., 1978; Jacobowitz and O'Donohue, 1978); VIP in the periventricular part of the parvocellular PVN (Sims et al., 1980); GABA (Perez de la Mora et al., 1981); TRH in primarily parvocellular PVN (Hokfelt et al., 1975); and sparse fibers of LHRH (Jennes and Stumpf, 1980); bradykinin (Correa et al., 1979); prolactin (Fuxe et al., 1977) and angiotensin II (Fuxe et al., 1976; Changarlis et al., 1978).

a. Neuropeptide Y

The distribution of neuropeptide Y (NPY) immunoreactivity in the PVN as well as its coexpression in noradrenergic and adrenergic brain stem groups has been determined with the retrograde transport-double immunohistochemical techniques (Sawchenko et al., 1985). Antisera to phenyl-
ethanolamine-N-methyltransferase (PNMT) was used as a marker for adrenergic neurons, and dopamine-ß-hydroxylase (DBH) antisera was used as a marker for adrenergic and noradrenergic neurons. The results indicated a dense NPY input to most parts of the parvocellular PVN in a similar, yet more extensive distribution than that stained by DBH antisera. In the magnocellular division, NPY-stained fibers were evenly distributed throughout areas containing oxytocinergic and vasopressinergic fibers. Catecholaminergic fibers were preferentially associated with vasopressinergic fibers. Combined retrograde transport-double immunohistochemical labeling studies demonstrated that NPY immunoreactivity is extensively contained within the adrenergic C1, C2 and C3 cell groups, with somewhat less immunoreactivity in the noradrenergic cell groups, mostly limited to neurons in the A1 cell group.

Leibowitz et al. (1986) found that NPY injected directly into the PVN increased plasma corticosterone levels within 15 minutes of infusion. The NPY response was similar to that for epinephrine and norepinephrine (See CORTICOSTERONE: Stimulation by Norepinephrine and Epinephrine). Also, NPY injected into the third ventricle results in robust feeding behavior in satiated rats (Allen et al., 1975) in a similar manner to adrenergic neurotransmitters (See PVN: Role in Behavior). Studies by Sahu et al. (1988) suggest that NPY neurons in the brainstem project to the PVN and mediate the increased sensitivity of the feeding response elicited by NPY.
CHAPTER III

METHODS

A. Animals

Male Sprague-Dawley rats (200-250 g) were purchased from Sasco-King Animal Laboratories (Oregon, WI). The rats were housed, two per cage, in a temperature (20-22°C), humidity (50-55%) and illumination (12:12 hour light/dark cycle; lights on at 0700 h) controlled room. Water and food (Wayne Lab Blox, Lab Mills Inc., Chicago, IL) were available ad libitum.

B. Surgery

All surgery was performed under pentobarbital anesthesia (50 mg/kg, i.p.). The rats received atropine sulfate (0.4 mg/kg, i.m.) to reduce respiratory tract secretion and ampicillin (50 mg/kg, i.m.) to prevent infection. Chloramphenicol (1% ophthalmic ointment) was topically applied around the wound margin. The rats were euthanized by decapitation with a guillotine in an area outside the rat room. Trunk blood was collected into centrifuge tubes containing 0.5 ml 0.3 M ethylenediamine tetra acetic acid (EDTA; pH 7.4).
C. Electrolytic Destruction of the PVN

Bilateral electrolytic lesions in the PVN were produced by passing 1.0 mA (D.C.) current for 10 seconds through an intracranial cathode. The anode was clipped to the wound margin. The cathode was a stainless steel pin (0.25 mm diameter) insulated with Epoxylite except for 0.5 mm of its tip. A Kopf stereotaxic apparatus and a Grass DC constant current lesion maker were used. The coordinates for the lesion site were 6.0 mm rostral to lambda, 0.5 mm lateral to the midsagittal suture, and 8.3 mm ventral to the skull surface, with the incisor bar set at -4.0 mm. Control animals were treated identically except that the electrode was not lowered into the skull. The rats were allowed to recuperate from surgery for 14 days, after which time they were subjected to a conditioned emotional (fear) response paradigm (CER). The CER procedure is described in detail below. The rats were sacrificed by decapitation immediately following the last CER procedure. Trunk blood was collected and assayed as described below. The brains were removed and saved for histological verification of the lesion placement. Eight rats were included in the sham/control as well as the sham/stress group, while 12 rats per group were used in both the lesion/control and lesion/stress group.

D. Electrolytic Destruction of the Nucleus Reuniens

Bilateral electrolytic lesions of the nucleus reuniens were produced by passing 1.0 mA current for 5 seconds through an intracranial cathode while the anode was clipped to the wound margin. The coordinates for the lesion site were 5.35 mm rostral to lambda, 0.4 mm lateral to the midsagittal suture and 8.0 mm ventral to the skull surface, with the
incisor bar set at -4.0 mm. Eight rats were included in the sham/control as well as the sham/stress group, 10 rats were used in the lesion/control and 12 rats were used in the lesion/stress group. The rest of the protocol for this experiment was identical to that described in the previous section (Electrolytic Destruction of the PVN).

E. Microinjections of Ibotenic Acid into the PVN

Bilateral stereotaxic microinjections of ibotenic acid (Sigma, St. Louis, MO) were made using a Kopf stereotaxic apparatus with a 5 µl Hamilton syringe and a Trent Wells hydraulic microdrive, to allow for a slow delivery rate. Ibotenic acid (10 µg/µl) was dissolved in 0.01 M phosphosaline buffer (PBS, pH 7.4), and injected in a volume of 0.3 µl per side over five minutes (Fitzsimons and Ciriello, 1986). The injection coordinates (incisor bar at -4.0 mm) were 5.7 mm anterior to lambda, 0.5 mm lateral to the midsagittal suture and 8.5 mm ventral to the skull surface. Control animals received an injection of the vehicle. After completion of the injection (five minutes), the needle was left in situ for an additional five minutes to prevent dorsal diffusion of the neurotoxin along the needle track. The rats were allowed to recuperate from surgery for 14 days, after which time they were subjected to the CER procedure. The rats were sacrificed by decapitation immediately following the CER procedure, and trunk blood was collected and later assayed. Nine rats were included in the vehicle/control and eight rats in the vehicle/stress group, while 12 rats per group were used in both the ibotenic acid/control and ibotenic acid/stress group.
F. Microinjections of 6-Hydroxydopamine into the PVN

Bilateral stereotaxic microinjections of 6-OHDA-HBr (Regis, Morton Grove, IL) were made using a Kopf stereotaxic apparatus with a bilateral cannula constructed of two 26 gauge stainless steel tubes (injectors) with a 1.0 mm distance between centers. The cannula was connected with flexible tubing to two 25 ul Hamilton syringes and a Sage Instruments syringe pump, model 355. 6-OHDA was dissolved in 0.1% ascorbic acid in physiological saline, to prevent autooxidation. In addition, the vials of drug solution were kept on ice and covered with aluminum foil to prevent light exposure. 6-OHDA was injected bilaterally over a five minute period, in a concentration of 8.0 µg free base in 1.5 µl saline containing 0.1% ascorbic acid. Control animals received an injection of the vehicle (0.1% ascorbic acid in physiological saline). After completion of the injection, the needle was left in situ for an additional five minutes to inhibit dorsal diffusion of the neurotoxin along the needle track. The rats were injected with the monoamine oxidase inhibitor pargyline (30 mg/kg, i.p.; Sigma, St. Louis, MO) 30 minutes prior to surgery, to augment the neurotoxic effect of 6-OHDA. The coordinates for the injection site were 5.7 mm rostral to lambda, 0.5 mm lateral to the midsagittal suture and 8.7 mm ventral to the skull surface, with the incisor bar set at -4.0 mm. The rats were allowed to recuperate from surgery for 14 days, after which time they were subjected to the CER paradigm, described below. The rats were sacrificed by decapitation immediately following the last CER procedure. Trunk blood was collected and assayed as described below. The extent of damage to noradrenergic nerve terminals was determined by immunocytochemical techniques (see
Eight rats were included in the vehicle/control as well as the vehicle/stress group, while 12 rats per group were used in both the 6-OHDA/control and 6-OHDA/stress group.

G. Microinjections of Sotalol into the PVN

Rats were placed in a Kopf stereotaxic apparatus and implanted with chronic bilateral guide cannulae (Plastic Products, Roanoke, VA). The guide cannulae were constructed of two 26 gauge stainless steel tubes with a 1.0 mm distance between centers. The inner stylets were constructed of 33 gauge stainless steel wire. The rats were allowed to recuperate from surgery for 14 days, and were handled each day following the surgery to condition them for handling during the injection. At the end of the 14 day period, the rats were subjected to the CER procedure, described below. In this particular study, the rats were transported in their home cage to the CER room, wrapped in a small towel, and held while the dust cap and stylets were removed and replaced with a 33 gauge bilateral injector. The rats received an injection of either vehicle or sotalol, or no injection at all. All injections were performed approximately three minutes before placement in the stress chamber, and 13 minutes before sacrifice. The volume (0.5 µl per side) was injected over a 60 second interval. The injector remained in situ for an additional 60 seconds to help prevent dorsal diffusion of the drug. Sotalol was injected bilaterally (0.5 µl per side), using two 25 µl Hamilton syringes and a CMA microinjection pump. The concentration of sotalol was 2.0 µg in 0.5 µl of physiological saline. Vehicle rats received an injection of saline. The non-injected rats had their dust caps and stylets removed and injectors placed, but no injection occurred. The rats were sacrificed by decapitation immediately
following the CER procedure. Trunk blood was collected and assayed as described below. Fourteen rats were included in the vehicle/control and vehicle/stress groups. The non-injected/control and non-injected/stress groups had six rats each. Sixteen rats were included in the sotalol/-control and eighteen rats were in the sotalol/stress group.

H. Histology

Lesion placement and drug injection sites were verified histologically. The brains were preserved in buffered formalin for 48 hours. The brains were then infiltrated with sucrose by successive four hour intervals in 5% and 10% sucrose solutions, respectively. The brains were stored in a 20% sucrose solution until they were cut on a cryostat (40 micron sections) and stained by the cresyl violet method and inspected by a microscope to verify the position of the tip of the needle or extent of lesion damage.

I. Immunocytochemistry

The brains from the 6-OHDA-treated rats were fixed for five hours in a solution containing 4% paraformaldehyde-0.05% glutaraldehyde in 0.10 M phosphate buffer, pH 7.5 at 4°C. They were then transferred to a 15% sucrose PBS solution where they were stored overnight at 4°C (Furness et al., 1978). The brains were then mounted in a cryostat, frozen and cut into 40 micron sections. These sections were floated in 0.05 M PBS and mounted on slides and allowed to air dry. The slides were then placed in air-tight slide boxes and kept at 0°C until processed (approximately 48 hrs). Sections were then washed and incubated in immunocytochemistry
(ICC) buffer consisting of phosphosaline buffer (PBS), 1.0% normal goat serum (from second antibody host species), 0.1% gelatin, 0.01% thimerosal, 0 - 0.002% neomycin, and 0 - 0.2% triton X-100. Sections were incubated consecutively in the primary antibody, rabbit anti-bovine adrenal medullary dopamine β-hydroxylase (DBH, 1:1000 dilution) for 12 hours in humidity-controlled chambers at 4°C. The DBH antibody was obtained from two sources: (1) Eugene-Tech, Allendale, NJ and (2) Dr. Eric Schweitzer, Madison, WI. The two DBH antibodies gave nearly identical results when tested on control rats. This incubation was followed by addition of a 1:50 dilution of goat anti-rabbit IgG followed by an incubation with 1:200 peroxidase-antiperoxidase (rabbit PAP) solution for 30 minutes at room temperature. Sections were washed between each immune incubation with 0.02 M PBS. Bound peroxidase was visualized by incubating sections in 25 mg/100 ml diaminobenzidine tetrahydrochloride (DAB-4HCl) and 0.01% hydrogen peroxide for 5-7 minutes. To increase sensitivity this solution also included 2.5% nickel ammonium sulfate. Sections were washed, mounted with cover slips, and viewed on a Leitz Ortholux photomicroscope.

J. Stress (CER) Procedure

In each of the experiments, the rats had at least a two-week post-operative recovery period before being subjected to the conditioned emotional response (CER) paradigm. The CER was performed in a ten-inch square, clear Plexiglass chamber with a grid floor composed of stainless steel rods (0.3 inches in diameter) spaced 0.5 inches apart. The chamber was illuminated by a 7.5 W incandescent lamp. The chamber was located inside a larger, rectangular sound-attenuating box equipped with a two-
way mirror (13" x 9") so that the rat's behavior could be observed. The sound-proof box also had a small fan in the back wall for ventilation. Scrambled current (0.8 mA D.C.) shock for 10 seconds was delivered through the grid floor by a Grayson-Stadler shock generator.

The rats were assigned at random before testing to a particular treatment group (control or stress) such that cage mates were members of the same group. Cage mates were tested sequentially and at the same time each day (between 10:00 and 15:00 hours) on four consecutive days.

On each day, the rats were transported ten feet from their living quarters to the stress chamber. Ten minutes following placement in the chamber, the experimental animals received an inescapable foot shock, then were returned to their home cage. This procedure was repeated for three consecutive days. The behavior of the rats was observed on days 3 and 4 of the CER paradigm. It was apparent from these observations that the stressed rats had learned that their placement in the chamber would be followed by a foot shock. By the third day, in contrast to the control animals, the stressed rats defecated, urinated and demonstrated freezing (no movement) behavior. On the fourth day, after 10 minutes in the test chamber, instead of receiving shock the rats were removed, transported to a third area outside the stress room and immediately sacrificed by decapitation. The control rats were treated identically except that shock was not administered at any time.

The stress chambers were cleaned carefully after each rat had been tested. Fecal boli and urine were removed, as well as washing the grid floor and sides of the plexiglass chamber. This was to prevent the analgesic effect described by Fanselow (1985) that can occur when an
unstressed rat is placed in a chamber containing the odor of a rat that has received an electric shock. Rats have been shown to release an innately recognized odor in response to many types of aversive events (Brown, 1979). In particular, aversive electric shock elicits an odor that conspecifics can discriminate from the odors of unstressed rats (Valenta and Rigby, 1968). These odors have been shown to interfere with the acquisition of one-way shock avoidance (Dua and Dobson, 1974).

K. Biochemical Determinations

1. Plasma Renin Activity (PRA)

PRA was measured by radioimmunoassay for generated angiotensin I (Haber et al., 1969; Stockigt et al., 1971). The pH of the samples was reduced to pH 6.5 by addition of 0.5 ml of 0.5 M phosphate buffer pH 6.0. The conversion of angiotensin I to angiotensin II was prevented by addition of 0.025 ml of 8-hydroxyquinoline (8-HQ; 10% suspension in 0.3 M EDTA; 3.4 mM final concentration; Mallinckrodt, St. Louis, MO) and 0.020 ml of phenylmethylsulfonyl fluoride (PMSF; 5 g in 150 ml ethanol; 2.5 mM final concentration; Sigma, St. Louis, MO). Plasma samples were incubated for three hours at 37°C to generate angiotensin I (AI). The incubation was stopped by immersion of the samples in boiling water for three minutes. The radioimmunoassay of AI was performed with an antiserum against AI at a dilution of 1:30,000, and a total binding of 35%. The antibody was added to the samples in a volume of 0.3 ml (AI antiserum in a 0.1 M Tris buffer, pH 8.0, containing 0.1% gelatin) and incubated overnight at 4°C. The tracer (125I labelled AI) was added to the samples the following morning, in a volume of 0.4 ml per tube, approximately 10,000 counts per
minute per tube. On the next day, the antibody-bound (iodinated AI) tracer was separated from the free tracer by addition of 0.5 ml of a suspension containing activated charcoal and dextran T500 (Pharmacia, Piscataway, NJ) and centrifugation at 2000 x g for 15 minutes. The pellet containing the unbound tracer was counted on a micromedic 4/200 plus automatic gamma counter which was connected via a buffer (Quadram microfazer) to an IBM PC-AT computer. The data were analyzed by a program (RIA-AID) that was purchased from Robert Maciel Associates (Arlington, MA). The sensitivity limit of the RIA is 10 pg AI per tube and the intra-assay variability was 4.0%. The inter-assay variability was 7.4% (Van de Kar et al., 1984). The tracer (AI; Beckman, Arlington Heights, IL) was iodinated via the chloramine T (Sigma, St. Louis, MO) method (Greenwood et al., 1963). Phosphate buffer (0.05 M; 0.02 ml), 12 µl AI (0.5 µg/µl) and 20 µl chloramine T (3.5 mg/ml phosphate buffer) were added to the vial containing the 125I and mixed for three seconds. This was immediately followed by addition of 25 µl of sodium metabisulfite (4.5 mg/ml phosphate buffer; J.T. Baker Chemical Co., Phillipsburg, NJ) to terminate the reaction. Chromatography of the iodinated AI was performed first by ion exchange (Biorad AG-1X4, 200-400 mesh) chromatography. The column consisted of a siliconized Pasteur pipette (5 3/4"; Scientific Products) with a small amount of glass wool placed in the tip of the pipette to support the anion exchange resin. Fractions (12 drops per tube) were collected from the column and the radioactivity in each tube was measured. The radioactive peak fractions eluted off this column were then placed on a Sephadex G-15 column for gel filtration and eluted with 0.05 N acetic acid containing 0.1% bovine serum albumin (Sigma, St. Louis, MO). Two ml
fractions were collected off the Sephadex column and measured for radioactivity. The tubes containing the radioactive AI were pooled and stored at -10°C.

2. Plasma Renin Concentration (PRC)

Renin is the enzyme responsible for the conversion of the substrate angiotensinogen, produced by the liver, to Angiotensin I. The measurement of plasma renin activity reflects the concentration of renin as well as renin substrate (angiotensinogen) in plasma. The normal angiotensinogen concentration is only about or less than Km (Reid et al., 1978) and thus much less than the concentration required to generate AI at maximum velocity (Vmax). Studies by Van de Kar et al. (1981) have demonstrated that 5-HT dependent changes in plasma renin activity are not accompanied by changes in plasma angiotensinogen levels. However, since electrolytic lesions in the PVN could cause a reduction in corticosterone levels, this could lead to decreased secretion of angiotensinogen from the liver. This would cause plasma renin activity values to be low even when renin levels were unaffected. The assay for plasma renin concentration involves the addition of a saturating concentration of exogenous renin substrate to the samples such that renin is working at Vmax. The measurement of plasma renin concentration eliminates the possibility that decreased substrate levels would be misinterpreted as decreased levels of renin.

The renin substrate was plasma from nephrectomized rats which received dexamethasone (0.2 mg/rat) 24 hours before sacrifice. The plasma samples (0.2 ml) received 0.1 ml of nephrectomized plasma, 0.1 ml of 0.5
M phosphate buffer pH 6.0, and 5 µl each of PMSF and 8-hydroxyquinoline (to a final concentration of 2.5 mM and 3.4 mM, respectively). The mixture was incubated at 37°C for one hour. The reaction was stopped by immersion of the tubes in a boiling water bath for three minutes. The radioimmunoassay of AI is as described for PRA.

3. Plasma Angiotensinogen

This radioimmunoassay is similar to that for PRC except that a saturating amount of the enzyme renin (from kidney homogenates) is added to the plasma samples before incubation to allow conversion of endogenous renin substrate to AI (Menard and Catt, 1972). Therefore any differences in the production of AI will be attributed to differences in substrate, not enzyme levels.

The plasma samples (0.1 ml) received 0.1 ml of 0.5 M phosphate buffer pH 6.0, 0.1 ml renin (from rat kidneys homogenized in cold water 1 g/ml at a 1:1,000 dilution), and 5 µl each of PMSF and 8-HQ. The mixture was incubated at 37°C for one hour. The reaction was stopped by immersion of the tubes in a boiling water bath for three minutes. The remainder of the radioimmunoassay for AI is as described for PRA.

4. Plasma Corticosterone

Corticosterone radioimmunoassays on unextracted plasma (5 and 10 µl) were performed using procedures and antiserum from Radioassay Systems Laboratories (Carson, CA). The binding proteins were denatured by incubation of the sample tubes and assay buffer in a hot water (80°C) bath for twenty minutes. The standards were made of serial dilutions of
corticosterone (Steraloids, Wilton, NH) in assay buffer. The antiserum (catalog # 1472, lot # 3R3-38) was used at a final dilution of 1:10,500 and total binding of 40%. The incubation of the samples (or standard) with the antiserum and tracer (corticosterone 1,2,6,7-3H(N)-; NET-399, New England Nuclear) occurred overnight at 4°C. The antibody-bound tracer was separated from the unbound tracer by addition of a charcoal-Dextran T70 suspension, centrifugation, and decanting of the supernatant into scintillation vials containing 5 ml of a scintillation cocktail (4 g of Omnifluor in 1 liter of toluene). The samples containing the supernatant and scintillation cocktail were counted in a Packard liquid scintillation counter. The data were stored on a diskette by a Diskstore (United Technologies, Packard) and were analyzed using the RIA-AID software (Robert Maciel Associates, Arlington, MA). The intra- and inter-assay variability were 4.5% and 11.9% respectively (Van de Kar et al., 1985).

L. Statistics

The data are reported as mean ± S.E.M. (standard error of the mean). The sample mean was the average of the number of animals in each group (n). The standard error of the mean was calculated as described below. Statistical analyses of the data was performed by a two-way analysis of variance (ANOVA). Individual group means were compared by Student Newman-Keul’s multiple range test (Steel and Torrie, 1960).

The most common measurement of central tendency for given samples of a population is the arithmetic average or mean. In addition to the measure of central tendency, it is important to be aware of how the samples differ in variability. This measure is the variance, or its
square root, the **standard deviation** (S.D.). This variance will provide information as to whether an observation is an ordinary or an unusual value in a specified population, giving a small value when the observations cluster closely about a central value, and a larger value when they are spaced widely. The **sum of squares** is the sum of the squared deviations from the arithmetic mean. The quantity \( (n-1) \) is known as degrees of freedom. Sum of squares is divided by degrees of freedom to give unbiased estimates of the population variances. Since sample means are also subject to variation, the **standard error of the mean** is also reported. It is calculated by dividing the S.D. by the square root of the number of observations in the mean.

1. Analysis of Variance (ANOVA)

Analysis of variance is defined by Steel and Torrie (1960) as "...an arithmetic process for partitioning a total sum of squares into components associated with recognized sources of variation." Using this type of test, the data are classified according to their treatment group. With respect to the present studies, a two-way analysis of variance was the appropriate test, since the animals were subjected to two separate treatments: (1) surgical (or drug) and (2) behavioral (stress).

The error mean square is an average of the components contributed by the treatments. It is an estimate of a common variation among observations of the same treatment group. Treatment mean square is an independent estimate of the variance when the null hypothesis is true. \( F \) is defined as the ratio of two independent estimates of the same variance, thus, the \( F \) value is obtained by dividing the treatment mean
square by the error mean square. Once $F$ has been calculated, it is compared with the tabular $F$ at the appropriate degrees of freedom at the .05 (5%) probability level. If the calculated $F$ is greater than the tabulated $F$, then the null hypothesis (no difference between population means) can be rejected and it can be concluded that there are real differences among treatment means. With respect to the two-way ANOVAS of the present studies, three $F$ values were obtained: $F_a$ for the surgical or drug treatment, $F_b$ for the stress treatment, and $F_{a \times b}$ for the interaction between the drug and stress treatments. The $F$ value reported was the one that accounts for interaction between both the stress and surgical/drug treatment groups.

2. Student-Newman-Keuls' Test

Multiple comparison tests can be used to compare each treatment mean with every other treatment mean. Student-Newman-Keuls' test is a multiple range test "...for judging the significance of a set of differences; it permits decisions as to which differences are significant and which are not...Error rate is seen to apply neither on an experiment-wise nor a per-comparison basis since a particular range test may be made for one or more comparisons in a particular experiment" (Steel and Torrie, 1960). This test also takes into account the number of treatments in the experiment. To perform the Newman-Keuls' test, the mean square error from the ANOVA is divided by the harmonic mean (average number of $n$ per group). The square root of this number is $S_x$, the standard error of the mean. Next a tabular value, $q$, is selected. This value is based on a .05 level of probability, and the appropriate $p$ (number of treatment means) and degrees
of freedom. $Q$ is multiplied by $S_x$ to give a range of significance. If the distance between two means is greater than the range of significance, then the difference between the two means is significant at the 0.05 probability level.
CHAPTER IV

RESULTS

A. Electrolytic Lesions in the PVN

Figure 1 is a coronal section of an intact PVN, stained with cresyl violet. Figure 2 shows an electrolytic lesion in the PVN. These lesions did not extend caudally beyond the PVN, and the dorsomedial nucleus was intact. There was some damage to the caudal aspect of the anterior hypothalamic area, as in Figure 2. This was not evident in all cases. The lesions did not extend laterally to the fornix. Only lesions which destroyed 90% or more of the PVN and did not extend dorsally into the thalamus were considered in the statistical evaluation of hormonal data. Following histological verification, the lesion control/group consisted of five rats, while the lesion/stress group consisted of seven rats with accurate and complete PVN lesions. One rat did not survive the anesthesia in the sham/control group, making the total for this group seven rats.

Electrolytic lesions in the PVN prevented the stress-induced increases in plasma renin activity (PRA) (Figure 3), plasma renin concentration (PRC) (Figure 4), and in plasma corticosterone levels (Figure 5). By comparison, rats with lesions which missed the PVN (n=5), but destroyed areas rostral to the PVN, showed no inhibition of the effect of stress on PRA (24.0 ± 3.2), PRC (33.7 ± 6.2) and corticosterone (29.3 ± 6.1) levels.
FIGURE 1

CORONAL SECTION OF RAT BRAIN WITH AN INTACT PARAVENTRICULAR NUCLEUS

FIGURE 2

CORONAL SECTION OF RAT BRAIN WITH AN ELECTROLYTIC LESION IN THE PARAVENTRICULAR NUCLEUS
The effect of stress on plasma renin activity in rats with electrolytic PVN lesions. The data represent mean ± S.E.M., n=7 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/control group, and n=7 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 5.7 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the lesion F(1,21) = 10.789; stress F(1,21) = 14.617; and lesion*stress interaction F(1,21) = 9.031.
The effect of stress on plasma renin concentration in rats with electrolytic PVN lesions. The data represent mean ± S.E.M., n=7 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/control group, and n=7 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 4.5 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a highly significant effect of the lesion F(1,21) = 16.713; stress F(1,21) = 26.858; and lesion*stress interaction F(1,21) = 17.588.
The effect of stress on plasma corticosterone levels in rats with electrolytic PVN lesions. The data represent mean ± S.E.M., n=7 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/-control group, and n=7 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 7.0 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress $F(1,21) = 11.06$; and lesion*stress interaction $F(1,21) = 14.245$. The lesion did not produce a significant effect $F(1,21) = 0.64$. 
B. Electrolyic Lesions in the Nucleus Reuniens

In a control experiment, lesions in the thalamic nucleus reuniens were made, dorsal and caudal to the PVN. These lesions were small, discrete and did not extend to the region of the third ventricle and the PVN (Figure 6). Following histological verification of placement in the nucleus reuniens, five rats were included in the lesion/control and six rats included in the lesion/stress group. There were six rats in the sham/stress group, and eight rats in the sham/control group.

Electrolytic lesions in the nucleus reuniens did not prevent the stress-induced increase in plasma renin activity (Figure 7) or plasma renin concentration (Figure 8). The stress-induced increase in plasma corticosterone levels was attenuated but not completely blocked by nucleus reuniens lesions (Figure 9). It is possible that this lesion interrupted afferent fibers to the PVN that are involved in stimulating CRF neurons in the PVN. Stressed rats in which the lesions missed both the nucleus reuniens and the PVN (n=5), had hormonal values similar to the sham/stress group and the group in which the nucleus reuniens was destroyed (PRA 14.3 ± 3.6; PRC 13.5 ± 1.8; and corticosterone 18.2 ± 1.7).
CORONAL SECTION OF RAT BRAIN WITH AN ELECTROLYTIC LESION IN THE THALAMIC NUCLEUS REUNIENS
The effect of stress on plasma renin activity in rats with electrolytic lesions in the nucleus reuniens. The data represent mean ± S.E.M., n=8 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/control group and n=6 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 6.4 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress $F(1,21) = 11.58$; but not of the lesion $F(1,21) = 0.761$ or lesion*stress interaction $F(1,21) = 0.92$. 
The effect of stress on plasma renin concentration in rats with electrolytic lesions in the nucleus reuniens. The data represent mean ± S.E.M., n=8 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/control group and n=6 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 7.4 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress F(1,21) = 10.291; but not of the lesion F(1,21) = 0.042 or lesion*stress interaction F(1,21) = 1.109.
The effect of stress on plasma corticosterone levels in rats with electrolytic lesions in the nucleus reuniens. The data represent mean ± S.E.M., n=7 for the sham/control group, n=6 for the sham/stress group, n=5 for the lesion/control group and n=6 for the lesion/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 6.5 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress F(1,20) = 10.29; but not of the lesion F(1,20) = 0.601 or lesion*stress interaction F(1,20) = 1.83.
C. Injection of Ibotenic Acid into the PVN

Figure 10 is a section of an intact PVN stained with cresyl violet. Figure 11 shows the needle tracks and destruction of neurons in the PVN following an injection of ibotenic acid. Acceptable lesions were restricted to the PVN and, compared with control sections, showed greater than 50% cell loss. Following histological verification of the injection site and evaluation of the hormonal data, there were seven rats included in both the vehicle/control and vehicle/stress group. Four rats in the ibotenic acid/control and six rats in the ibotenic acid/stress group were considered accurate PVN lesions.

Ibotenic acid-induced lesions in the PVN prevented the stress-induced increase in plasma renin activity (Figure 12) and plasma renin concentration (Figure 13). Stress-induced increases in corticosterone levels also were prevented by ibotenic acid lesions of neurons in the PVN (Figure 14). By comparison, stressed rats with ibotenic acid injections which missed or caused unnoticeable damage to neurons in the PVN (n=6) had PRA (28.9 ± 3.8), PRC (35.5 ± 4.5) and corticosterone levels (16.9 ± 1.9) which were indistinguishable from the vehicle/stress group. Thus far, the data suggested that cell bodies in the PVN are involved in stress-induced renin and corticosterone secretion.
FIGURE 10

INTACT PARAVENTRICULAR NUCLEUS
FIGURE 11

PARAVENTRICULAR NUCLEUS FOLLOWING BILATERAL INJECTION OF IBOTENIC ACID
The effect of stress on plasma renin activity in rats with ibotenic acid lesions in the PVN. The data represent mean ± S.E.M., n=7 for both vehicle groups, n=4 for the ibotenic acid/control group, and n=6 for the ibotenic acid/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 10.5 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the drug $F(1,20) = 4.959$; stress $F(1,20) = 9.488$; and drug*stress interaction $F(1,20) = 6.063$. 
The effect of stress on plasma renin concentration in rats with ibotenic acid lesions in the PVN. The data represent mean ± S.E.M., n=7 for both vehicle/control and vehicle/stress groups, n=4 for the ibotenic acid/control group, and n=6 for the ibotenic acid/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 13.5 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the drug $F(1,20) = 5.063$; and stress $F(1,20) = 8.069$. The drug*stress interaction $F(1,20) = 3.619$ was not significant.
The effect of stress on plasma corticosterone levels in rats with ibotenic acid lesions in the PVN. The data represent mean ± S.E.M., n=7 for both vehicle/control and vehicle/stress, n=4 for the ibotenic acid/control group, and n=6 for the ibotenic acid/stress group.

* Significant difference from the corresponding control group, p < 0.05. A minimal difference of 4.3 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the drug $F(1,21) = 12.273$; and stress $F(1,21) = 19.645$. The drug*stress interaction was not significant $F(1,21) = 3.198$. 
D. Injection of 6-OHDA into the PVN

Figure 15A shows a coronal section of the PVN following vehicle injection and staining with dopamine \( \beta \)-hydroxylase (DBH) antiserum. Figure 15B shows a coronal section of the PVN following 6-OHDA injection and staining with DBH antiserum, showing loss of DBH immunoreactivity. In general, there was a 60-80% reduction in DBH staining (as compared to vehicle rats) in a 500-1000 micron area that encompassed the PVN and anterior hypothalamus, but did not spread as far as the mediobasal hypothalamus. Following immunocytochemical and histological verification, the vehicle/control and 6-OHDA/control groups consisted of seven rats each, the vehicle/stress group consisted of six rats, and the 6-OHDA/stress group had five rats.

The stress (GER) procedure produced a significant increase in plasma renin activity and concentration as well as increasing plasma corticosterone levels in rats which received an injection of vehicle or non-injected animals. Injection of 6-OHDA into the PVN prevented the effect of stress on plasma renin activity (Figure 16) and plasma renin concentration (Figure 17). In addition the effect of stress on plasma corticosterone levels was also blocked (Figure 18). Since 6-OHDA injections into the PVN prevented the stress-induced increase in corticosterone levels, it is possible that this caused decreased secretion of the renin substrate (angiotensinogen) from the liver. This would cause PRA values to be low even though renin levels were unaffected. To test this, in
addition to measurements of PRC, plasma angiotensinogen levels were determined (Figure 19). There were no differences observed between the groups. This suggested that neither stress nor 6-OHDA injections affected plasma levels of renin substrate. Thus far the data suggest that catecholaminergic neurons or nerve terminals in the PVN play a role in the stress-induced increase in renin and corticosterone secretion.
**FIGURE 15A**

Coronal section from rat injected with vehicle and stained with DBH antiserum.

**FIGURE 15B**

Coronal section from rat injected with 6-OHDA and stained with DBH antiserum, showing loss of DBH immunoreactivity.
The effect of stress on plasma renin activity in rats with 6-OHDA lesions. The data represent mean ± S.E.M.; n=7 for vehicle/control, n=6 for vehicle/stress, n=7 for 6-OHDA/control and n=5 for 6-OHDA/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 5.48 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the drug F(1,21) = 13.576; stress F(1,21) = 35.595; and drug*stress interaction F(1,21) = 12.427.
The effect of stress on plasma renin concentration in rats with 6-OHDA lesions. The data represent mean ± S.E.M.; n=7 for vehicle/control, n=6 for vehicle/stress, n=7 for 6-OHDA/control and n=5 for 6-OHDA/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 12.9 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of the drug $F(1,21) = 6.939$; stress $F(1,21) = 24.623$; and drug*stress interaction $F(1,21) = 6.059$. 
The effect of stress on plasma corticosterone levels in rats with 6-OHDA lesions. The data represent mean ± S.E.M.; n=7 for vehicle/control, n=6 for vehicle/stress, n=7 for 6-OHDA/control and n=5 for 6-OHDA/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 10.0 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress $F(1,21) = 9.422$. There were no significant effects of either the drug $F(1,21) = 0.194$ or drug*stress interaction $F(1,21) = 3.371$. 
The effect of stress on plasma angiotensinogen levels in rats with 6-OHDA lesions. The data represent mean ± S.E.M.; n=7 for vehicle/control, n=6 for vehicle/stress, n=7 for 6-OHDA/control and n=5 for 6-OHDA/stress.

No significant differences found among the groups, p < 0.05. A minimal difference of 33.1 would be required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates no significant effects of the drug F(1,21) = 1.395; stress F(1,21) = 0.072; and drug*stress interaction F(1,21) = 0.721.
E. Injection of Sotalol into the PVN

Figure 20 shows a coronal section of the PVN stained with cresyl violet following a bilateral injection of sotalol into the PVN. Only rats in which the tip of the cannula was immediately dorsal to the border of the PVN, or inside the dorsal PVN, were acceptable. In several rats, the cannulae ended inside the PVN, producing large mechanical lesions. These rats were excluded from the statistical analysis of the hormonal data. Following histological verification of the injection site, eight rats were used in the vehicle/control, five rats in vehicle/stress and non-injected/stress, and four rats in the non-injected/control group. There were six rats used in the sotalol/control group and eight rats in the sotalol/stress group.

Figures 21 and 22 show the effect of stress on plasma renin activity and plasma renin concentration, respectively, in rats injected with either vehicle or sotalol, or in non-injected rats. Stress-induced increases in PRA and PRC were not prevented by injection of sotalol. However, the stress-induced increase in corticosterone levels was prevented by injections of sotalol into the PVN (Figure 23).
FIGURE 20

CORONAL SECTION OF THE PARAVENTRICULAR NUCLEUS FOLLOWING BILATERAL INJECTION OF SOTALOL
The effect of stress on plasma renin activity in rats injected with either vehicle or sotalol, or in non-injected rats. The data represent mean ± S.E.M.; n=8 for vehicle/control, n=4 for vehicle/stress, n=6 for sotalol/control, n=7 for sotalol/stress, n=4 for non-injected/control and n=5 for non-injected/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 8.42 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress F(1,28) = 32.526; but not of the drug F(2,28) = 0.871 or drug*stress interaction F(2,28) = 0.528.
The effect of stress on plasma renin concentration in rats injected with either vehicle or sotalol, or in non-injected rats. The data represent mean ± S.E.M.; n=8 for vehicle/control, n=4 for vehicle/stress, n=6 for sotalol/control, n=7 for sotalol/stress, n=4 for non-injected/control and n=5 for non-injected/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 9.6 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress F(1,28) = 17.357; but not of the drug F(2,28) = 0.249 or drug*stress interaction F(2,28) = 0.553.
The effect of stress on plasma corticosterone levels in rats injected with either vehicle or sotalol, or in non-injected rats. The data represent mean ± S.E.M.; n=8 for vehicle/control, n=4 for vehicle/stress, n=6 for sotalol/control, n=7 for sotalol/stress, n=4 for non-injected/control and n=5 for non-injected/stress.

*Significant difference from the corresponding control group, p < 0.05. A minimal difference of 8.6 is required between the means by the Student-Newman-Keuls' test.

Two-way ANOVA indicates a significant effect of stress F(1,28) = 9.823. No significant effect is seen with the drug F(2,28) = 1.724 or drug*stress interaction F(2,28) = 1.411.
F. Comparison of Normal Plasma Pools to Experimental Controls

Pooled plasma samples for "normal" and "high" PRA or "normal" and "high" corticosterone levels are run routinely with each respective assay. The pooled plasma samples are compiled from rat plasma that has been previously assayed and classified within the range of "normal" or "high" based on its corticosterone or PRA value. A random sample of these values, taken from assays run between 1986 and 1989, gave the following values (mean ± standard error of the mean):

- "normal" PRA = 7.6 ± 0.5 (n = 23)
- "high" PRA = 23.6 ± 2.8 (n = 23)
- "normal" corticosterone = 8.9 ± 1.1 (n = 13)
- "high" corticosterone = 20.9 ± 2.0 (n = 10)

The pooled values for "normal" PRA and corticosterone are very similar to previously published values for PRA and corticosterone in untested control animals, shown below.

Untested Control Rats

PRA = 7.1 ± 1.2 (Van de Kar et al., 1985)
Corticosterone = 8.9 ± 2.1 (Van de Kar et al., 1985)

The normal plasma pool values were compared to the means of the sham- or vehicle-treated groups in each of the experiments of this dissertation. ANOVA and Newman-Keuls' Multiple Range Test were run to determine if significant differences between the groups exist. With respect to plasma renin activity, the data are shown on the following page:
Table 1.
Comparison of Mean Values: Normal PRA Pool versus Experimental Sham- or Vehicle-Treated Groups

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Description</th>
<th>Mean ± Std.Error</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6-OHDA Expt. Vehicle/Control</td>
<td>3.6 ± 0.8</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>Electrolytic PVN Sham/Control</td>
<td>4.5 ± 0.6</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Sotalol Expt. Vehicle/Control + Non-injected/Control</td>
<td>6.3 ± 0.5</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Electrolyt. N. Reun. Sham/Cont.</td>
<td>6.4 ± 0.4</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Normal PRA Pool</td>
<td>7.6 ± 0.5</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Ibotenic Expt. Vehicle/Control</td>
<td>9.2 ± 0.9</td>
<td>7</td>
</tr>
</tbody>
</table>

Standard Error of Treatment Means = 0.74
Table 2
Newman-Keuls' Multiple Range Test for PRA

<table>
<thead>
<tr>
<th>Treatment vs. Treatment</th>
<th>Difference</th>
<th>Sig .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 4 vs. Group 1</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Group 4 vs. Group 5</td>
<td>2.71</td>
<td>*</td>
</tr>
<tr>
<td>Group 4 vs. Group 2</td>
<td>2.82</td>
<td>*</td>
</tr>
<tr>
<td>Group 4 vs. Group 6</td>
<td>4.06</td>
<td>*</td>
</tr>
<tr>
<td>Group 4 vs. Group 3</td>
<td>5.62</td>
<td>*</td>
</tr>
<tr>
<td>Group 1 vs. Group 5</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>Group 1 vs. Group 2</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>Group 1 vs. Group 6</td>
<td>3.11</td>
<td>*</td>
</tr>
<tr>
<td>Group 1 vs. Group 3</td>
<td>4.68</td>
<td>*</td>
</tr>
<tr>
<td>Group 5 vs. Group 2</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Group 5 vs. Group 6</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Group 5 vs. Group 3</td>
<td>2.91</td>
<td>*</td>
</tr>
<tr>
<td>Group 2 vs. Group 6</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>Group 2 vs. Group 3</td>
<td>2.80</td>
<td>*</td>
</tr>
<tr>
<td>Group 6 vs. Group 3</td>
<td>1.56</td>
<td></td>
</tr>
</tbody>
</table>
The means of the experimental vehicle- or sham-treated groups were not significantly elevated above the normal PRA plasma pool. However, two experimental groups had PRA values lower than the normal PRA pool levels.

With respect to corticosterone, the data are as follows:

Table 3
Comparison of Mean Values:
Normal Corticosterone Pool versus Experimental Sham- or Vehicle-Treated Groups

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Description</th>
<th>Mean ± Std.Error</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Ibotenic Expt. Vehicle /Control</td>
<td>6.5 ± 0.5</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>6-OHDA Expt. Vehicle/Control</td>
<td>7.1 ± 0.5</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Normal Corts Pool</td>
<td>8.9 ± 1.1</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>Electrolyt. PVN Sham/Control</td>
<td>9.3 ± 2.3</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Electrolyt. N. Reun. Sham/Cont.</td>
<td>12.8 ± 0.9</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Sotalol Expt. Vehicle/Control  + Non-injected Control</td>
<td>12.9 ± 1.2</td>
<td>12</td>
</tr>
</tbody>
</table>

Standard Error of Treatment Means = 0.74
Table 4

Newman-Keuls' Multiple Range Test for Corticosterone

<table>
<thead>
<tr>
<th>Treatment vs. Treatment</th>
<th>Difference</th>
<th>Sig .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3 vs. Group 4</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>Group 3 vs. Group 6</td>
<td>2.36</td>
<td>-</td>
</tr>
<tr>
<td>Group 3 vs. Group 1</td>
<td>2.75</td>
<td>-</td>
</tr>
<tr>
<td>Group 3 vs. Group 2</td>
<td>6.31</td>
<td>*</td>
</tr>
<tr>
<td>Group 3 vs. Group 5</td>
<td>6.34</td>
<td>*</td>
</tr>
<tr>
<td>Group 4 vs. Group 6</td>
<td>1.86</td>
<td>-</td>
</tr>
<tr>
<td>Group 4 vs. Group 1</td>
<td>2.26</td>
<td>-</td>
</tr>
<tr>
<td>Group 4 vs. Group 2</td>
<td>5.81</td>
<td>*</td>
</tr>
<tr>
<td>Group 4 vs. Group 5</td>
<td>5.85</td>
<td>*</td>
</tr>
<tr>
<td>Group 6 vs. Group 1</td>
<td>0.39</td>
<td>-</td>
</tr>
<tr>
<td>Group 6 vs. Group 2</td>
<td>3.95</td>
<td>-</td>
</tr>
<tr>
<td>Group 6 vs. Group 5</td>
<td>3.98</td>
<td>-</td>
</tr>
<tr>
<td>Group 1 vs. Group 2</td>
<td>3.55</td>
<td>-</td>
</tr>
<tr>
<td>Group 1 vs. Group 5</td>
<td>3.58</td>
<td>-</td>
</tr>
<tr>
<td>Group 2 vs. Group 5</td>
<td>0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

None of the vehicle- or sham-treated experimental groups were significantly different from the normal corticosterone plasma pool.
A. Overview

The following conclusions can be drawn from the results presented:

1. Neurons in the PVN mediate the stress-induced increase in renin and corticosterone secretion.
2. Catecholaminergic nerve terminals in the PVN mediate the effect of stress on renin and corticosterone secretion.
3. Stress-induced corticosterone secretion appears to be mediated by $\beta$ receptors in the PVN, while 4. stress-induced renin secretion is mediated by a receptor that is not $\beta$-adrenergic. Stress-induced renin secretion may be mediated by a different catecholamine receptor, a neuropeptide or other substance(s) colocalized in catecholaminergic nerve terminals.

B. Justification of the Stress Paradigm

Abundant evidence was presented in the literature review to demonstrate that renin and corticosterone secretion can be elevated by many different stressors. The stress paradigm used in these experiments was a conditioned fear paradigm that involved exposing the rats to an initially neutral stimulus (the stress chamber) which was followed after ten minutes by an aversive stimulus (footshock). The latter unconditioned stimulus elicited a range of unconditioned responses, including neuroendocrine (increased renin and corticosterone levels) and behavioral (defecation, urination, freezing) responses. As the pairings of stimuli
were continued, the originally neutral stimulus became a conditioned stimulus, capable of eliciting the same responses as the unconditioned stimulus.

1. Classical Conditioning of Fear Stimuli

Classical conditioning can account for the wide range of stimuli that can produce fear in animals and humans (Watson, 1924). An experiment by Hunt and Otis (1953) demonstrated that one symptom of fear, defecation, can be classically conditioned. They presented a rat with a flashing light for three minutes, at the end of which time the animal received an electric foot shock. Eight conditioning trials were run. At first, defecation occurred primarily when the shock was delivered, but it increasingly began to occur during the presentation of the flashing light.

A similar phenomenon was observed with the conditioned fear paradigm used in the experiments of this dissertation. Behavioral observations were made throughout the testing procedure, and recorded on the third and fourth (final) days. Rats were monitored for defecation and urination, as well as exploratory, freezing or grooming behaviors. On the first day, the rats were placed in the stress chamber for a ten minute interval, at the end of which time they received a mild foot shock. The rats were tested in this manner for four consecutive days, the only exception being that on the final day, no foot shock was given. On the first day in the stress chamber the rats did not defecate until the time of the foot shock. However by the third day (and in some cases the second), as soon as the rats were placed in the stress chamber they began
to defecate. The defecation suggested that the rats had paired the placement in the chamber with the impending foot shock, supporting the hypothesis that classical conditioning is an integral part of the ability of previously neutral stimuli to elicit fear. Lorens et al. (1989) reported the number of fecal boli as a defecation score, a quantitative measurement of a behavioral response to conditioned fear.

In the experiments of this dissertation, the control animals explored the test chamber and groomed themselves. The stressed animals, after the first or second day of conditioning, would sometimes explore, and sometimes groom, but for the most part would defecate, urinate and exhibit freezing behavior. Freezing behavior consists of tense and silent immobility, and is seen in many species in times of danger. The observation of freezing behavior in response to the CS represents another similarity between my experiments and those presented by Hunt and Otis (1953). Normally a rat responds to an electric foot shock with a great increase in activity - running around frantically, jumping, and trying to escape the shock. However, in my experiments and the experiments of Hunt and Otis (1953), the stimulus followed by shock caused a radically different behavior - immobility. This is quite an exception to the usual pattern of the CS (in my case, the stress chamber) eliciting a similar (though not exact) reaction to that of the UCS (the footshock). Gray (1971) suggested the possibility that freezing and more active forms of escape and avoidance behavior are controlled by separate brain systems, and represent a combination of learning and innate behavior.

The process of producing electrolytic lesions or injecting neurotoxins is an invasive procedure that could, theoretically, interrupt
fiber pathways that are involved in the learning/conditioning process. Because this surgical intervention occurs two weeks prior to the behavioral conditioning paradigm, it is important to look for potential differences in the behavior of sham or vehicle-injected rats compared with rats with electrolytic or chemical lesions in the PVN. Behavioral observations during the conditioning process indicated that the lesions did not prevent defecation, urination and freezing in the stressed rats. This suggests that the lesions did not interfere with the conditioning process.

C. Electrolytic and Ibotenic Acid Lesions in the PVN

The data suggest that neurons in the PVN mediate the effect of stress on renin secretion and confirm results by others (Rivier and Vale, 1983; Dohanics et al., 1986) that neurons in the PVN mediate the effect of stress on corticosterone secretion. This conclusion is based on the finding that both complete electrolytic lesions in the PVN and selective destruction of cells in the PVN prevented the stress-induced elevation in plasma renin activity, plasma renin concentration and plasma corticosterone levels, whereas electrolytic lesions in the thalamic nucleus reuniens were ineffective. Ibotenic acid was selected for its ability to selectively destroy cell bodies while leaving fibers of passage intact (Markowska et al., 1985; Winn et al., 1984). Magnocellular neurons in the PVN have been reported to be resistant to the neurotoxic effects of kainic acid (Zhang and Ciriello, 1985). Herman and Wiegand (1986) have reported that ibotenic acid selectively destroyed parvocellular neurons in a dose-dependent manner while sparing magnocellular neurons. They reported that
loss of parvocellular neurons occurred at 0.5, 0.75, 1.0 and 2.0 µg doses of ibotenic acid. Loss of magnocellular neurons (mostly vasopressinergic) was observed only after the 2.0 µg dose. Their protocol involved unilateral injections (compared to our protocol of bilateral injections) directed towards the central portion of the PVN at the level of the posterior magnocellular division. In our study, the drug volume was higher (0.3 µl compared to 0.1 µl) and the injection site had to be at least 0.5 mm lateral to the midsagittal suture. Injections of ibotenic acid closer to the midline led to diffusion of the drug into the third ventricle, resulting in death of the rats. We did not observe selective sparing of the magnocellular neurons after injections of ibotenic acid.

The effect of stress on corticosterone secretion is most likely propagated by stimulation of CRF neurons in the PVN. The majority of the diencephalic CRF-neurons are located in the parvocellular neurons in the dorsal and medial subnuclei of the PVN (Liposits et al., 1987; Liposits and Paull, 1985). A small number of CRF-neurons also are present in the magnocellular divisions of the nucleus (Swanson and Sawchenko, 1983; Piekut and Joseph, 1986). Dohanics et al. (1986) reported that the full corticosterone and ACTH response to ether or ether plus laparotomy stress requires not only an intact PVN, but also an intact neurointermediate lobe. Therefore, our data are consistent with the hypothesis that CRF neurons in the parvocellular PVN are activated during the CER procedure to stimulate ACTH and thus corticosterone secretion. Furthermore, the data with corticosterone support the histological verification of the accuracy of the lesions in the PVN. The finding that basal corticosterone concentration was not altered by the PVN lesions is in agreement with
previous studies by Meyerhoff et al. (1987) and Makara et al. (1986), who reasoned that in the absence of CRF input, the cells in the pituitary can maintain a basal level of ACTH but cannot respond to stimuli which would increase ACTH secretion. Similar findings were also reported using CRF antisera (Nakani et al., 1985; Ono et al., 1985; Rivier et al., 1982).

Nucleus reuniens lesions inhibited the effect of stress on corticosterone but not renin secretion. This suggests that fibers which course through the nucleus reuniens and innervate the CRF neurons in the PVN may have been disconnected by this lesion. Since no inhibition of stress-induced renin secretion occurred in these rats, the data suggest that the fibers, and possibly cells, which mediate the effect of stress on renin secretion are different from the fibers and cells which mediate stress-induced corticosterone secretion.

The nature of the PVN neurons which mediate the effect of stress on renin secretion is not known. It is clear, both from the present results and from studies by Porter (1988), that cells in the PVN play an important role in the regulation of renin secretion. Electrolytic lesions in the PVN prevent the increase in plasma renin activity after injection of the serotonin releasing drug p-chloroamphetamine (Gotoh et al., 1987). Furthermore, these lesions also prevent the increase in plasma renin activity that follows immobilization stress, although a reduction in plasma renin substrate (angiotensinogen) levels could account for this effect (Gotoh et al., 1987). It is important to note that, in the present study, a saturating concentration of exogenous renin substrate was added in the plasma renin concentration assay to control for possible variation of renin substrate in the plasma samples from the experimental rats. In
contrast, the plasma renin activity assay was a measure of the production of angiotensin I from endogenous renin, combined with endogenous renin substrate. Since plasma renin activity and concentration were equally affected by the lesions, the changes were clearly due to changes in renin, not renin substrate concentration in plasma.

D. Injection of 6-OHDA into the PVN

The main conclusion of the 6-OHDA experiment is that catecholaminergic nerve terminals in the PVN mediate the effect of stress on both renin and corticosterone secretion. This conclusion is based on the observation that injection of 6-OHDA into the PVN inhibited the stress-induced increase in renin and corticosterone secretion. 6-OHDA is a neurotoxin that is taken up by the catecholaminergic nerve endings and produces degeneration of the nerve terminals (Breese and Traylor, 1970; Uretsky and Iversen, 1970). 6-OHDA is considered to be selective for catecholaminergic neurons. Uretsky and Iversen (1970) reported that doses of 6-OHDA that decreased brain norepinephrine and dopamine levels did not affect brain serotonin or GABA. Shor-Posner et al. (1986) have reported that injection of 6-OHDA into the PVN, in the same dose as in my experiment, produced a 75% reduction in norepinephrine, 56% reduction of epinephrine and 47% reduction in dopamine concentration in the PVN. The catecholamines were determined by HPLC and electrochemical detection. Histochemical analysis also revealed a significant decrease in catecholamine fluorescence in the PVN. This study suggests that 6-OHDA can cause a degeneration of noradrenergic, adrenergic and dopaminergic elements in the PVN. Further support was provided by Fety et al. (1984), who injected 6-OHDA into the fourth ventricle of rats to study the biochemical responses
of the adrenergic (C1, C2) cell groups versus the noradrenergic (A1, A2 and A6) cell groups. Their results indicated that the adrenergic cell groups are sensitive to the neurotoxic effects of 6-OHDA since they exhibited changes in tyrosine hydroxylase and dopamine-ß-hydroxylase in a similar manner to the noradrenergic cell groups.

In the 6-OHDA experiment of this dissertation, norepinephrine depletion was verified immunocytochemically by using an antibody for dopamine-ß-hydroxylase. No attempt was made to differentiate between loss of norepinephrine-, dopamine- or epinephrine-containing nerve terminals. Consequently, these results cannot indicate which catecholamine mediates the effects of stress on renin or corticosterone secretion.

The suggestion that catecholaminergic nerve terminals in the PVN mediate stress-induced corticosterone secretion is supported by studies involving the ventral noradrenergic bundle (VNAB), which conveys most of the noradrenergic (Moore and Bloom, 1979; Palkovits, 1981; Swanson and Sawchenko, 1980) and adrenergic (Ungerstedt, 1971) afferents to the PVN. Injections of 6-OHDA into the VNAB inhibited the ACTH stress response (Szafarczyk et al., 1985), prevented the increase in corticosterone secretion following photic, acoustic and sciatic nerve stimulation (Feldman et al., 1988), and significantly reduced CRF levels in hypophysial portal blood (Guillaume et al., 1987). In addition, Feldman et al. (1986) demonstrated that 6-OHDA injections into the PVN significantly inhibit the adrenal response to photic, acoustic and sciatic nerve stimulation. Together, these results suggest a role for catecholaminergic afferents to the PVN in the activation of adrenocortical responses to stress.
The dorsal noradrenergic bundle (DNAB) may play a role as a mediator of stress-induced renin secretion. Some afferent fibers from the locus coeruleus travel to the PVN in the DNAB (Kobayashi et al., 1975; Loizou, 1969). Lightman et al. (1984) demonstrated that injections of 6-OHDA into the DNAB markedly attenuate the increase in plasma renin activity in response to hemorrhage, while injections of 6-OHDA into the VNAB had no effect. This study, in combination with my results, suggests that catecholaminergic afferents to the PVN mediate the renin response to stress.

E. Injection of Sotalol into the PVN

Since the data suggest that catecholaminergic nerve terminals in the PVN mediate the stress-induced increase in renin and corticosterone secretion, the next question to address was whether or not β-adrenergic receptors mediate this effect. Previous studies demonstrated that the β-adrenoceptor antagonist propranolol (1 mg/kg, i.p.) significantly attenuated the stress-induced increase in plasma renin activity (Van de Kar et al., 1985). Since propranolol is known to cross the blood-brain barrier (Garvey and Ram, 1975; Lemmer et al., 1985), and the PVN contains a high concentration of β receptors (Wanaka et al., 1989), it is possible that a β-receptor, located in the PVN, could be involved in mediating the effect of stress on renin and corticosterone secretion. In the present experiment, sotalol was selected instead of propranolol because it is devoid of local anesthetic activity and is both a β₁ and β₂ antagonist (Fitzgerald, 1984). Injection of sotalol into the PVN prevented the stress-induced increase in corticosterone secretion but did not prevent
the stress-induced increase in renin secretion. This suggests that a β-adrenergic receptor in the PVN plays a role in the corticosterone response to stress. However, many β-adrenoceptor antagonists also bind to serotonin receptors in the brain (Conway et al., 1978). Since only one dose of sotalol was administered, it is possible that this dose (15 nmol) could affect serotonergic as well as β-adrenergic receptors. Other investigators have injected much higher doses of sotalol and propranolol in the brain. Injections of sotalol (400 nmol) or propranolol (120 nmol) into the perifornical region of the anterior hypothalamus did not prevent the feeding response induced by either epinephrine (20 nmol) or norepinephrine (20 nmol). This feeding response was prevented by injections of the α-adrenoceptor antagonists phentolamine (30 or 60 nmol), tolazoline (100 nmol) or phenoxybenzamine (15 nmol) in the same brain region (Leibowitz, 1975). Injection of propranolol (116 nmol) or timolol (95 nmol) into the cerebral ventricles prevented the effect of air stress on renal sympathetic nerve activity in spontaneously hypertensive rats. In addition, icv injection of propranolol (4 or 25 nmol) produced an inhibition of epinephrine-stimulated ACTH release (Szafarczyk et al., 1987). In my study, the fact that sotalol inhibited the stress-induced increase in corticosterone secretion but not the stress-induced increase in renin secretion argues for a relative selectivity in receptor binding.

Other investigators have provided evidence for the role of β-receptors in the regulation of corticosterone secretion. Szafarczyk et al. (1987) demonstrated a stimulatory role for epinephrine and norepinephrine on corticosterone release. Injection (icv) of epinephrine or norepinephrine induced ACTH release comparable to that occurring under
(ether) stress conditions. Epinephrine was more efficacious than norepinephrine on an equimolar basis. The stimulating effect of norepinephrine was reversed by icv pretreatment with prazosin, an α₁ antagonist, whereas the effect of epinephrine was inhibited by either prazosin or propranolol. These results suggest that norepinephrine stimulates ACTH release via α₁ receptors, whereas epinephrine acts via both α₁- and β-adrenoceptors. In vitro studies support the role of β-adrenergic receptors in CRF release. Norepinephrine stimulated the release of immunoreactive CRF from cultured neonatal rat hypothalamic cells. This effect was blocked by propranolol but not by prazosin. In addition, the β-adrenoceptor agonist isoproterenol significantly increased CRF while the α-adrenoceptor agonist phenylephrine was ineffective, except at high concentrations (Widmaier et al., 1989). Together, these data strongly suggest a stimulatory role for β-receptors in the regulation of corticosterone secretion.

To speculate as to which neurotransmitter could be involved in mediating the effect of stress on corticosterone secretion, several studies should be considered. Leibowitz et al. (1986) demonstrated that norepinephrine injections into the PVN dose-dependently increased serum corticosterone levels, while dopamine had no effect. Epinephrine in the PVN was even more efficacious than norepinephrine in increasing serum corticosterone levels. Further, a study by Spinedi et al. (1988) demonstrated that central inhibition of phenylethanolamine-N-methyltransferase (PNMT), producing a selective decrease in hypothalamic epinephrine levels, significantly decreased the plasma ACTH response to ether stress, and at later times also caused a selective decrease in CRF content. These
results support a stimulatory role of central epinephrine-containing neurons in CRF release. Sawchenko et al. (1987) estimated that at least 40% of the retrogradely-labeled catecholaminergic cell groups that project to the PVN could be adrenergic (PNMT-immunoreactive). These results suggest that stress-induced increases in corticosterone levels could be mediated via adrenergic nerve terminals activating β-receptors in the PVN.

Stress-induced increases in plasma renin activity and concentration are mediated by a different receptor. It is possible that an α-adrenergic receptor could mediate the stress-induced increase in renin secretion. However, pharmacological studies suggest that activation of brain α2-adrenoceptors lowers renin secretion by inhibiting sympathetic outflow to the kidneys. Injection of clonidine (icv) prevented the air jet stress-induced increase in renal sympathetic nerve activity in spontaneously hypertensive rats. This effect was reversed by icv injection of the α2-adrenergic antagonists yohimbine and rauwolscine (Koepke and DiBona, 1986). ICV injection of clonidine lowered plasma renin activity at a dose which is ineffective when given intravenously (Reid et al., 1975). Blair et al. (1977) demonstrated that administration of L-DOPA to dogs pretreated with the peripheral decarboxylase inhibitor carbidopa results in a drop in blood pressure and an inhibition of renin secretion. Since this treatment increases brain catecholamine content, the results suggest that catecholamines formed from L-DOPA can act within the central nervous system to cause a decrease in renin secretion. In this study the decrease in plasma renin activity was dependent upon the presence of the renal nerves. These studies suggest that central α2-adrenoceptors are involved in decreasing renin secretion. However, it is possible that central α1-
adrenoceptors could mediate the stress-induced increase in renin secretion. Ganong et al. (1982) suggested that the activation of central $\alpha_1$ receptors increases renin secretion in anesthetized dogs. However, these data are not very convincing since they only reported renin values following icv injection of the $\alpha_1$-agonists methoxamine and phenylephrine, without the appropriate vehicle controls.

The PVN has been shown to project to those segments of the thoracic cord containing sympathetic preganglionic neurons innervating the kidney (Swanson and McKellar, 1979). Other studies have suggested that the sympathetic nervous system mediates the stress response from the brain to the kidney (Golin et al., 1988). However, in our laboratory, (see STRESS: Effect of Peripheral Sympathectomy) sympathectomized-adrenal enucleated rats showed no inhibition of the stress-induced increase in plasma renin activity/concentration or plasma corticosterone levels (Richardson Morton et al., 1988). This suggests that the sympathetic nervous system and adrenal catecholamines are not the only mediators of the stress-induced increase in renin secretion. An alternate theory is that cell bodies in the PVN are stimulated to secrete a renin-releasing factor to the bloodstream to promote renin release from the kidneys (Van de Kar et al., 1987).

If $\alpha$ and $\beta$ receptors in the PVN do not mediate stress-induced renin secretion, another possibility is that a neuropeptide, coreleased at the catecholaminergic nerve terminals in the PVN, mediates stress-induced renin secretion. A likely candidate is neuropeptide Y, which is coexpressed in noradrenergic and adrenergic brain stem cell groups and is present in noradrenergic and adrenergic nerve terminals in the PVN.
Intraventricular injection of NPY has been shown to increase plasma ACTH and corticosterone secretion (Harfstrand et al., 1987) and plasma angiotensin II levels (Harfstrand et al., 1986). However, Fuxe et al. (1983) reported that intracisternal injection of NPY causes hypotension, which could also increase renin secretion by activation of the intra-renal stretch receptor (Fahri et al., 1982).

Yet another neuropeptide of interest is galanin, due to its preferential coexistence with noradrenergic cell groups that project to the PVN. Combined immunohistochemical-retrograde transport studies have demonstrated galanin-immunoreactivity coexisting with dopamine-ß-hydroxylase immunoreactivity in the A1 and A6 cell groups, with no evidence for galanin coexistence in adrenergic (PNMT-immunoreactive) neurons that project to the PVN. Galanin inputs were confined to the anterior, periventricular, dorsal and ventral subdivisions of the parvocellular PVN, with only a sparse projection to the oxytocinergic neurons of the magnocellular division of the PVN (Levin et al, 1987).

F. Hypothetical Organization of Brain Pathways Mediating Stress-Induced Renin and Corticosterone Secretion

The neural pathways mediating stress-induced renin and corticosterone secretion are likely to be complex and multi-synaptic, due to the many inputs which must be integrated. First, there is a sensory component that arises during behavioral conditioning. When the rat receives the foot shock as part of the conditioned fear paradigm, the sensory message is transmitted from the nerve fibers in the skin to the spinal cord, where it travels in the lateral spinothalamic tract to the
posteroventral nuclei of the thalamus. The spinothalamic fibers are accompanied by fibers that terminate in the reticular formation (spino-reticular) and the periaqueductal gray and tectum (spinotectal). The periaqueductal grey has been shown to play a role in flight or defensive behavior (de Molina and Hunsperger, 1962).

The pain input route most likely continues from the midbrain reticular formation, through the thalamus, to reach the septum and hippocampus. The septo-hippocampal system plays a major role in pain reception. The hippocampus emits a theta wave (4-9 cps rhythm) as a response to a painful stimulus in the rat, and in the emotional states of disappointment and frustration in humans. Lesions in the medial nuclei of the septum and the thalamic nucleus centrum medianum abolish the theta wave emitted by the hippocampus in response to a painful stimulus (Gray, 1971).

Damage to the hippocampus has been shown to facilitate acquisition of shuttle box avoidance (Green et al., 1967; Van Hoesen et al., 1972) possibly due to a deficit in a behavioral inhibitory system (Douglas, 1967; Gray, 1982). These studies, and others (Squire and Zola-Morgan, 1983), suggest a critical role of the hippocampal formation in learning and memory. The hippocampal formation and septal region share extensive bidirectional connections. The lateral, medial and posterior divisions of the septal regions are associated with the hippocampal formation while the ventral division (bed nuclei) is primarily related to the amygdala (Swanson et al., 1987). The subicular complex and the entorhinal area of the hippocampus receive an extensive sensory input from the cortex, including olfactory, visual, auditory and somatosensory. Additional
sensory information is relayed through two routes, one via the thalamus, extrahippocampal cortex and amygdala and the other via ascending pathways from the brainstem and basal forebrain. The hippocampus also receives considerable serotonergic input from the raphe nuclei (Azmitia, 1978; Lorens and Guldberg, 1974; Steinbusch, 1981). Immunoreactive-CRF neurons have been demonstrated in the hippocampus (Swanson, 1983). However, the axonal projections of these cells are relatively short, suggesting that they might function as interneurons (Kohler, 1986).

The hippocampus has many interconnections to another limbic structure, the amygdala (Krettek and Price, 1978). The amygdaloid complex has been shown to be involved in the regulation of fear-motivated behavior (Blanchard and Blanchard, 1972; Spevack et al., 1975). The amygdala appears to play a role in the stress response via the pituitary-adrenal cortical axis. The central nucleus of the amygdala, in particular, contains large numbers of CRF-containing cell bodies and terminals (Swanson et al., 1983; Moga and Gray, 1985). Both the central and medial amygdaloid nuclei project to the thalamic nucleus reuniens (Aggleton and Mishkin, 1984). This helps to explain why lesions in the nucleus reuniens prevented the effect of stress on corticosterone secretion.

Most amygdaloid nuclei project to the bed nucleus of the stria terminalis (BNST) and the hypothalamus. Since the hypothalamus also receives a substantial projection from the BNST, there exists a disynaptic route for the amygdala to modulate hypothalamic function (Krettek and Price, 1978). The BNST also receives fibers from the dorsal and median raphe nucleus (Azmitia and Segal, 1978), as well as a massive projection from the hippocampus (Swanson and Cowan, 1977). To summarize,
the incoming sensory information and "learning"/associative processes that accompany the conditioned fear paradigm are likely conveyed to the PVN via limbic regions of the telencephalon since direct neocortical projections to the hypothalamus cannot be demonstrated (Swanson et al., 1987). The hippocampus, amygdala and ventral septal region project heavily to the BNST, which in turn projects to the CRF regions of the PVN. The question that remains is: how does this input to the PVN become integrated with the direct noradrenergic (A2) and adrenergic (Cl-C3) input via the VNAB (brain stem to the CRF neurons in the PVN)?

Swanson et al. (1983) suggested that the nucleus of the solitary tract, which receives direct vagal and glossopharyngeal afferents (Beckstead and Norgren, 1979) could serve as a major relay point of visceral sensory information to CRF cell bodies in the basal forebrain. Likewise the parabrachial nucleus, which receives a large input from the NTS (Norgren, 1978), and the locus coeruleus also project to many of the sites that contain CRF-immunoreactive cell bodies, including the parvocellular PVN, the bed nucleus of the stria terminalis and the central nucleus of the amygdala. As mentioned above, these nuclei may also be involved in the relay of visceral sensory information to CRF cell bodies (Swanson et al., 1983). This hypothetical pathway could provide a direct stimulus for corticosterone secretion.

Another possibility is that the noradrenergic input to the PVN is serving as a neuromodulator to "set a bias" to amplify or buffer incoming stimuli to the cells. Even though norepinephrine might have little effect on the resting excitability of the CRF neurons, it could have profound
effects on the amount of CRF released following an action potential (Nicoll, 1982). For example, pretreatment with norepinephrine (5 µM) before iontophoretically-applied glutamate (50 nA) in a pyramidal (hippocampal) cell has been shown to evoke a higher frequency and longer duration train of action potentials than following glutamate alone (Madison and Nicoll, 1986).

Wallace et al. (1989) demonstrated that the amygdala innervates the locus coeruleus (A6) and NTS (A2) noradrenergic cells, and the C2 adrenergic cells. Most of the rostral A6 and A2 neurons had amygdaloid terminal contacts. This pathway could be involved in integrating the sensory/associative processes with the stimulatory catecholaminergic afferents to the PVN.

The possibility exists that serotonergic neurons play a role in transmitting the stress stimulus. The dorsal raphe nucleus (B7) is one of the nine 5-HT cell groups originally described by Dahlstrom and Fuxe (1965), which innervates the forebrain. Studies from our laboratory have shown that electrolytic lesions in the dorsal raphe nucleus prevent the stress-induced increase in plasma renin activity using a three-minute CER (Van de Kar et al., 1984). This experiment was repeated, extending the CER to ten minutes so that corticosterone levels could be evaluated. Confirming the previous results, we found that electrolytic lesions in the dorsal raphe nucleus prevented the stress-induced increase in plasma renin activity and concentration and corticosterone levels (Richardson Morton et al., 1986). There could be a connection between the fact that both dorsal raphe lesions and PVN lesions prevent the stress-induced increase in plasma renin activity, plasma renin concentration and corticosterone
levels. Sawchenko et al. (1983) have demonstrated that serotonergic neurons in the midbrain (especially the dorsal raphe) innervate the PVN. Although the serotonergic fiber density in the PVN is low compared with immediately surrounding areas, there is a distinct projection to the medial parts of the parvocellular division. Liposits et al. (1987) have demonstrated by electron microscopic methods that serotonergic nerve terminals innervate CRF-immunoreactive parvocellular cells in the PVN.

Injections (i.p.) of the 5-HT\textsubscript{2} antagonist, LY53857, did not prevent the stress-induced increase in renin or corticosterone secretion (Richardson Morton et al., 1986). However, injection of the 5-HT\textsubscript{1A} agonists buspirone and ipsapirone significantly attenuated the stress-induced increase in plasma renin activity, plasma renin concentration and corticosterone levels (Urban et al., 1986; Van de Kar et al., 1985; Lorens et al., 1989). Both buspirone and ipsapirone inhibit the firing of serotonergic neurons in the dorsal raphe (Basse-Tomsk, 1986; VanderMaelen et al., 1986). Thus, serotonergic neurons in the dorsal raphe nucleus could be involved in stress-induced renin and corticosterone secretion. There are fiber projections from the locus coeruleus to the dorsal raphe nucleus (Cowan and Park, 1986), with direct noradrenergic nerve terminal innervation of serotonin nerve cells in the dorsal raphe (Baraban and Aghajanian, 1981), and projections from the dorsal raphe to the locus coeruleus (Conrad et al., 1974). These interconnections could provide a relay for the noradrenergic bundles to influence hypothalamic neuroendocrine responses.

Another possibility is that the dorsal raphe serotonergic neurons, which also project to the hippocampus (Swanson et al., 1987), provide a
tonic inhibition of the "behavioral inhibition" system of the hippocampus. In this case, administration of 5-HT$_{1A}$ anxiolytic agents or electrolytic lesions in the dorsal raphe nucleus would decrease the input to the septo-hippocampal system, thus reducing the activity of the septo-hippocampal behavioral system, ultimately decreasing the behavioral and possibly neuroendocrine stress response (Kuhar, 1986).

Clearly, the neural pathways mediating stress-induced renin and corticosterone secretion are complex and multi-synaptic. From this dissertation, it can be concluded that stress-induced corticosterone secretion appears to be mediated by $\beta$ receptors in the PVN, while stress-induced renin secretion is mediated by a receptor that is not $\beta$-adrenergic. Stress-induced renin secretion may be mediated by a different catecholamine receptor, a neuropeptide or other substance(s) colocalized in catecholaminergic nerve terminals.

The following figure is a schematic diagram of the hypothetical brain pathways mediating stress-induced renin and corticosterone secretion.
FIGURE 24

Hypothetical Brain Pathways Mediating Stress-Induced Renin and Corticosterone Secretion
LITERATURE CITED


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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Date 9/25/89  Director's Signature